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Review

Treatment of malignant gliomas with ketogenic or caloric restricted diets: A systematic review of preclinical and early clinical studies

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SUMMARY

Background & aims: Patients with malignant gliomas have a poor prognosis. Diets that lower blood glucose, such as ketogenic or caloric restricted diets (KCRDs), are hypothesized to reduce tumor growth and improve survival. In this systematic review, we summarize preclinical and clinical data on KCRDs in gliomas.

Methods: We searched PubMed and Embase for preclinical and clinical studies on KCRDs in gliomas, and extracted data on surrogate and clinically relevant endpoints, in accordance with PRISMA statement. Quality assessment of clinical studies was performed with use of Cochrane Collaboration's tool. We performed Fisher's exact test to examine associations between surrogate and clinically relevant endpoints.

Results: We included 24 preclinical studies, seven clinical studies and one mixed study. Both preclinical and clinical studies were highly heterogeneous. Preclinically, KCRDs reduced tumor growth, but only a small majority of the *in vivo* studies found improved survival. These effects were stronger in groups with decreased blood glucose than in those with increased ketones, and also when other therapies were used concomitantly. Finally, KCRDs influence multiple molecular-biological pathways, including the PTEN/Akt/TSC2 and NF-kB pathway. In clinical studies, KCRDs seem to be safe and feasible in glioma patients. Clinical data were insufficient to draw conclusions regarding efficacy.

Conclusions: KCRDs have positive effects on malignant gliomas in published preclinical studies. Preliminary clinical data suggest that KCRDs are safe and feasible. However, because of the paucity of clinical data, the efficacy of KCRDs for improving survival and quality of life of glioma patients remains to be proven in prospective studies.

1. Introduction

Malignant gliomas are primary brain tumors and comprise anaplastic gliomas (WHO grade III) and glioblastomas (grade IV) [1]. The standard treatment for glioblastomas, the most common and lethal subtype [2], is maximal surgical resection, followed by radiotherapy with concomitant and adjuvant temozolomide chemotherapy [3,4]. Despite this aggressive policy, practically all patients develop disease progression or recurrence with limited second-line treatment options [3], resulting in a poor 5-year overall

Abbreviations: AMPK, AMP-activated protein kinase; BTIC, brain tumor initiating cell; CR, caloric restriction (or caloric restricted conditions *in vitro*); HIF-1α, hypoxia inducible factor-1α; IGF-I, insulin-like growth factor I; IGF-IR, insulin-like growth factor I receptor; KCRD, ketogenic or caloric restricted diet; KD, ketogenic diet; KD-R, restricted ketogenic diet; KD-UR, unrestricted ketogenic diet; KS, ketone supplementation; mTOR, mechanistic target of rapamycin; PFS, progression-free survival; PTEN, phosphatase and tensin homologue; STS, short-term starvation (or STS-mimicking conditions *in vitro*); TIN, treatment-induced necrosis; TSC2, tuberous sclerosis complex 2; VEGF, vascular endothelial growth factor; VEGFR2, VEGF-receptor-2.

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survival of 5–10% [2,5,6]. The prognosis for anaplastic gliomas is better, but these tumors are still invariably fatal.

Insights in glucose metabolism may offer new treatment options. In non-tumor cells, ATP is mainly produced by the citric acid cycle and oxidative phosphorylation in mitochondria. A common characteristic of tumor cells – including glioblastoma cells – is, however, their supposed dependence on glycolysis for the production of ATP, regardless of the presence of oxygen (the 'Warburg effect') [7]. This led to the hypothesis that tumor cells have dysfunctional mitochondria, and since these organelles are involved in apoptosis, their dysfunction could lead to tumor cells' survival [8,9]. Moreover, tumor cells seem to be less able to use ketone bodies as their primary energy source in glucose restricted conditions [8]. This hypothesis is supported by the *in vitro* observation of reduced expression of ketolytic enzymes in gliomas [10]. Another contributing factor to the Warburg effect is altered cell signaling, which is reflected in gliomas, for example, mutations in the mechanistic target of rapamycin (mTOR) pathway [11].

These insights, the finding that hyperglycemia is associated with a worse prognosis [12], and the finding that ketones may have direct antitumor effects through the inhibition of histone deacetylation (HDAC) [13] led to the development of dietary interventions to reduce blood glucose and thus induce ketosis, such as a ketogenic diet (KD), caloric restriction (CR) and short-term starvation (STS). A KD is a diet with a fat to carbohydrate and protein ratio of 4:1 (a standard diet has a ratio of less than 1), and can be used with and without CR. Both the unrestricted and restricted KD, CR and STS result in lower blood glucose and higher blood ketones [14,15], and are here collectively termed 'ketogenic or caloric restricted diets' (KCRDs). The effects of KCRDs can be simulated preclinically by ketone supplementation (KS).

The aim of this review is to summarize all preclinical and early clinical data on KCRDs for the treatment of malignant gliomas. We address the following research questions:

1. What are the effects of KCRDs on malignant gliomas *in vitro* and *in vivo*, with regard to endpoints including blood glucose and ketone concentrations, tumor growth, cell death, and survival?
2. Which molecular-biological pathways and mechanisms are influenced by KCRDs that may contribute to the effects of KCRDs?
3. What are the effects of KCRDs as (adjunct) treatment modalities in glioma patients, with regard to endpoints including blood glucose and ketone concentrations, tumor growth, survival, safety, feasibility, and quality of life?

2. Methods

We conducted a systematic search in PubMed and Embase (July 1, 2017) that combined synonyms for gliomas and KCRDs (see Supplemental Tables 1 and 2).

This paper is performed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [16], which is primarily intended for systematic reviews and meta-analyses of clinical intervention studies. This paper mainly focuses on preclinical studies, but we adhered to the guidelines as closely as possible. The review protocol was reviewed internally (among the authors) before completion of the search and data extraction. No formal review protocol was submitted to the public domain.

After removing duplicate records, a step-by-step selection process based on titles, abstracts and full texts was performed (LN and FYV). We checked the completeness of the search by a 'golden hit' list. This list of landmark papers on KCRDs was made prior to and independent of the search (AK). We hand-searched reference lists of five relevant reviews [17–21], and contacted authors of papers that were not available through our institution to identify additional papers. LN performed and TJS reviewed the data extraction.

For all studies, we applied the following inclusion criteria: 1) articles had to be written in English; 2) articles concerned original research (including observational clinical studies, case reports and clinical abstracts). Reviews, editorials and comments were excluded.

Additional inclusion criteria for preclinical studies were: 1) the domain had to be a glioma cell line or model; 2) the study included a KCRD intervention; 3) data on one of the endpoints of interest were reported.

Additional inclusion criteria for clinical studies were: 1) patients (either adults or children) were diagnosed with a malignant glioma (WHO grade III or IV) [1]; 2) patients were treated with a KCRD (before, during or after standard treatments).

Results are mainly presented in a descriptive manner. We categorized results based on the study design (*in vitro*, *in vivo* or clinical); Supplemental Tables 3 and 4 contain study characteristics of the preclinical and clinical studies, respectively. We also subdivided outcome measures in surrogate (laboratory) and clinically relevant endpoints. Surrogate endpoints include blood glucose and ketone concentration, since these are used to monitor the effects of KCRDs in the clinical practice (reference values in the treatment of refractory epilepsy with a KD are 2–5 mmol/L for blood ketones and 8–16 mmol/L for urine ketones [22]), and antiangiogenic effects, since tumor growth is partly dependent on angiogenesis [23]. Clinically relevant endpoints include tumor growth, survival, and quality of life (the latter in clinical studies). Other (secondary) endpoints are described in the Results section or presented in Supplemental Table 5.

Quality assessment of clinical studies was performed with use of the Cochrane Collaboration's tool for assessing risk of bias [24]. We considered laboratory effects, tumor growth and survival as essential parameters, and they should have been reported in all clinical studies. Therefore, if one of the essential parameters was not reported, incomplete outcome data for that essential parameters was scored as unclear risk of bias. Safety and feasibility and quality of life were considered as non-essential parameters, and if one of these was not reported, this was scored as not applicable. We performed a Fisher's exact test (VassarStats) to examine whether laboratory effects of KCRDs are associated with clinically relevant effects in the preclinical studies. We used this simplified method, since a formal meta-regression was not possible due to the heterogeneity of the preclinical studies.

3. Results

After the step-by-step selection process, 29 records remained. The hand-searching of reference lists and contacting of authors resulted in three additional records [25–27]. Adding these resulted in a final selection of 32 records (Fig. 1), consisting of 24 preclinical studies, seven clinical studies and one study that contained both clinical and preclinical data.

3.1. In vitro preclinical studies

Ten studies implemented KCRDs *in vitro* [26,28–36]; seven reported on endpoints as listed in Table 1 [26,29–33,36], and three reported on molecular-biological pathways [28,34,35]. One study supplied ketones to glioma cell lines [30], four studies lowered glucose concentrations in media to mimic CR or STS [29,31–33], and two studies used both [26,36].

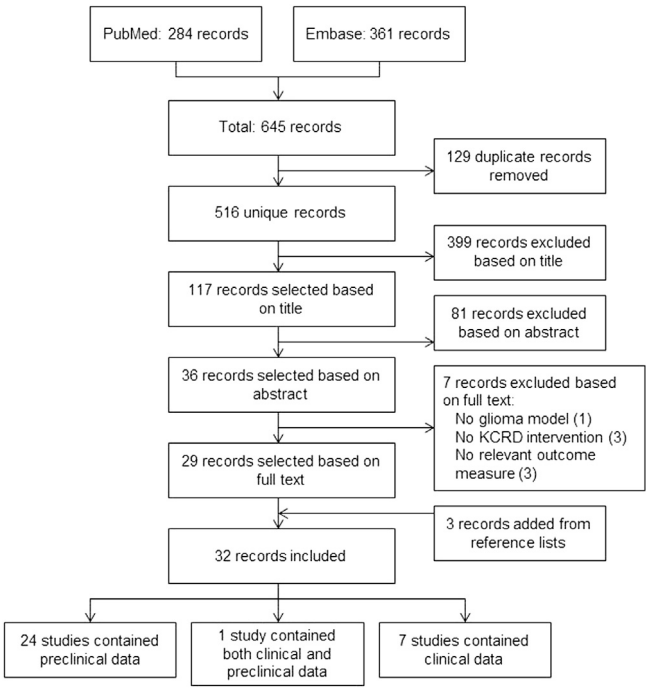

Fig. 1. Flowchart of the step-by-step selection process.

Table 1
In vitro studies: summary of results for clinically relevant endpoints.[a]

	Significant?	KS	CR or STS	Total
Decreased tumor growth	Yes	2	3	5
	No	1		1
Increased stress resistance of normal brain cells	Yes	1		1
Increased chemotoxicity resistance of normal brain cells	Yes		1	1
Sensitization of tumor cells to chemotherapy	Yes		3	3
	No	1		1
Increased death rates of tumor cells				
KCRD intervention only	Yes	1	3	4
Combined with chemotherapy	Yes		1	1

Abbreviations: CR, caloric restriction (or caloric restricted conditions *in vitro*); KCRD, ketogenic of caloric restricted diet; KS, ketone supplementation; STS, short-term starvation (or STS-mimicking conditions *in vitro*).

[a] Some studies used more than one glioma cell line or KCRD intervention, or reported more than one endpoint, and are thus listed more than once in the table. The numbers behind 'Yes' indicate the number of studies that reported a statistically significant effect on this endpoint ($P \leq 0.05$). 'No' indicates that the reported effect was not significant.

Maurer et al. found no differences in tumor growth, motility and invasiveness after KS, compared with a standard diet [30]. Interestingly, the authors observed that ketone bodies rescued hippocampal neurons from cell death induced by glucose withdrawal, but did not sensitize tumor cells to temozolomide.

In one study, the death rate of tumor cells increased synergistically when doxorubicin was added to low glucose concentrations [29].

3.2. *In vivo preclinical studies*

Twenty-four studies had an *in vivo* study design, and were highly heterogeneous with regard to the KCRDs used and the endpoints reported. Therefore, these studies are categorized based on the KCRD used, as presented in Table 2 (clinically relevant endpoints) and Table 3 (surrogate endpoints). All studies compared results in animals on a KCDR with those on a standard diet.

3.2.1. *Ketogenic diet*

Twelve studies implemented a KD [25,30,34–43], with ketogenic ratios (grams of fat to grams of carbohydrate and protein) varying from 2.7:1 [30] to 6:1 [34,36]. Eight studies used a caloric unrestricted KD (KD-UR) [25,30,34,36,37,39,41,43], and two a caloric restricted KD (KD-R) [35,38]. One was designed to reduce body weight by 20% in ten days [38], while the other eased CR when body weight was reduced by 20% or more [35]. Two studies implemented a KD-UR in one group and a KD-R in another, wherein the caloric intake was restricted by 35–40% compared with the recommended daily allowance [40,42].

Zhou et al. used a subcutaneous and intracerebral glioma model [42]. They used tumor growth to 2.5 cm^3 as a proxy for survival in the subcutaneous model, and tumor growth differences in the intracerebral model. Survival increased and tumor growth decreased with the KD-R, but not with the KD-UR. Seyfried et al. found the same with an intracerebral model: tumor growth was only reduced with the KD-R [40].

Poff et al. used a subcutaneous model and reported no decrease in tumor growth in the KD-UR group, but nevertheless found improved survival compared with the control group [25].

Four studies added the KD to another treatment modality. When the KD was used concomitantly with radiotherapy, hyperbaric oxygen therapy, 2-deoxy-D-glucose, or bevacizumab, synergistic effects with regard to reduced tumor growth and improved survival were found [25,37–39].

Blood glucose concentrations decreased significantly with the KD-R, but not with the KD-UR in two studies [40,42]. Blood ketone concentrations, however, increased with both interventions. Zhou et al. found no antiangiogenic effects of the KD-UR, but the KD-R reduced tumor microvessel density [42]. The KD-UR in the study of Woolf et al. did not reduce the VEGF concentration, but the expressions of VEGFR2, CD31 and the VEGF-B gene were reduced, which seems to indicate reduced angiogenesis [41]. In addition, the

Table 2
In vivo studies: summary of results for clinically relevant endpoints.[a]

	Significant?	KD-UR	KD-R	KS	CR	STS	Total
Decreased tumor growth							
KCRD intervention only	Yes	3	3		9	2	17
	No	4	1		1	1	7
Combined with chemotherapy	Yes					3	3
Combined with radiotherapy	Yes	1					1
Combined with HBO_2T	Yes	1					1
Combined with 2-DG	Yes	1					1
Increased death rates of tumor cells	Yes				3		3
Increased survival							
KCRD intervention only	Yes	5	1	1	2	1	10
	No	3	1		1	1	6
Combined with chemotherapy	Yes					2	2
Combined with radiotherapy	Yes	1				1	2
Combined with bevacizumab	Yes	1					1
Combined with HBO_2T	Yes	1					1

Abbreviations: 2-DG, 2-deoxy-D-glucose; CR, caloric restriction; HBO_2T, hyperbaric oxygen therapy; KCRD, ketogenic or caloric restricted diet; KD-R, restricted ketogenic diet; KD-UR, unrestricted ketogenic diet; KS, ketone supplementation; STS, short-term starvation.

[a] Some studies used more than one glioma model or KCRD intervention, or reported more than one endpoint, and are thus listed more than once in the table. The numbers behind 'Yes' indicate the number of studies that reported a statistically significant effect on this endpoint ($P \leq 0.05$). 'No' indicates that the reported effect was not significant.

Table 3
In vivo studies: summary of results for surrogate endpoints.[a]

	Significant?	KD-UR	KD-R	KS	CR	STS	Total
Decreased glucose	Yes	5	3	1	8	2	19
	No	4		1			5
Increased ketones	Yes	9	3	1	5		18
	No	1		1			2
Decreased IGF-I	Yes		1		4	1	6
	No	2					2
Antiangiogenic effects[b]	Yes	1	1		5		7
	No	1					1

Abbreviations: CR, caloric restriction; IGF-I, insulin-like growth factor I; KD-R, restricted ketogenic diet; KD-UR, unrestricted ketogenic diet; KS, ketone supplementation; STS, short-term starvation.

[a] Some studies used more than one glioma model or KCRD intervention, or reported more than one endpoint, and are thus listed more than once in the table. The numbers behind 'Yes' indicate the number of studies that reported a statistically significant effect on this endpoint ($P \leq 0.05$). 'No' indicates that the reported effect was not significant.

[b] Antiangiogenic effects include a decrease in the vascular endothelial growth factor (VEGF) concentration or the VEGF-receptor-2 (VEGFR2) expression, a lower tumor microvessel density, and the shift of immature tumor vessels to a more mature and less leaky state.

authors reported an increased expression of the tight junction protein zona occludens-1 and a decreased expression of aquaporin-4, which (together with the reduced angiogenesis) seemed to cause a reduction of peritumoral edema. Finally, the concentrations of matrix metalloproteinase-2 and vimentin were decreased. These proteins are hypothesized to contribute to tumor invasiveness [44,45], and therefore the authors suggest this could be reduced by KDs.

Eleven studies with a KD recorded body weight. Seven studies with a KD-UR found no significant decrease in body weight [30,34,37,40–43]. Two studies, however, found weight loss in the KD-UR groups compared with the control groups [25,36]. As expected, the KD-R mice had weight loss of 10–23% in 7–13 days [35,38,40,42].

Martuscello et al. also studied a protein enriched, supplemented high-fat low-carbohydrate diet, which had similar effects on surrogate and clinically relevant endpoints (decreased glucose, increased ketones, reduced tumor growth, and improved survival), but to a lesser extent than the KD-UR [36]. The only difference was the weight gain in the mice on this new diet, which the authors attributed to its better palatability, compared with the weight loss in the KD-UR mice.

3.2.2. Ketone supplementation

Poff et al. supplied ketones to mice with a subcutaneous model, by adding 1,3-butanediol or a ketone ester to a standard diet [26]. Survival improved in both groups compared with the control group. Only in the ketone ester-group, blood glucose decreased and ketones increased. In addition, only the ketone ester-mice lost body weight, approximately 20%. This was significantly associated with increased survival. Because of this finding, Poff et al. discussed the possibility that the improved survival in the ketone ester-group could partly be due to (indirect) CR and not only to KS.

3.2.3. Caloric restriction

Nine studies used 30–40% CR [26,31,40,46–51], and one study alternated days of fasting with 25% CR [52].

Marsh et al. used a subcutaneous and an intracerebral model [52], and in both models, tumors grew slower in the CR-group. The study examined survival differences with the subcutaneous model, and found improved survival in the CR-group, compared with the control group.

Three studies reported other endpoints in addition to tumor growth; CR led to a decrease in peritumoral edema (possibly due to an increase in tight junctions) [46], in inflammation [49], and in tumor invasiveness from the hemisphere with tumor cells to the contralateral hemisphere [50].

Five studies reported antiangiogenic effects, the main ones being a decrease in the VEGF concentration [46,47,51], a lower tumor microvessel density [47,48,50,51], and a shift of immature tumor vessels to a more mature and less leaky state [46,51].

Body weight was recorded in nine studies [26,40,46–52]. As expected, body weight decreased during periods of CR in all, with weight loss of 10–22% in 7–15 days compared with baseline body weight in five [26,40,47,48,51], and weight loss of 23.9–30% in 14–18 days compared with controls in three [46,49,52]. One study did not report the percentage of weight loss [50].

3.2.4. Short-term starvation (STS)

Two studies implemented STS [29,33]. STS induced significant weight loss in both [29,33], but the mice rapidly regained their initial body weight.

Lee et al. used a subcutaneous model, and starved mice for 48 h, in one group concomitantly with administration of doxorubicin [29]. Tumor growth decreased synergistically when both STS and doxorubicin were used.

Safdie et al. studied STS in a subcutaneous and an intracerebral model [33]. The animals with the subcutaneous model were withdrawn of food for two 48 h cycles, and those with the intracerebral model all fasted for one 48 h cycle and partly for an additional 24 h. In the subcutaneous model, STS alone reduced tumor growth and improved survival, but temozolomide and radiotherapy alone did not. Combining STS with each of these standard treatments had synergistic effects on tumor growth and survival. In the intracerebral model mice that fasted for 48 h, significant effects on tumor growth and survival were found in the group with both STS and temozolomide, but not with STS or temozolomide alone. In the intracerebral model mice that fasted the additional 24 h, decreased tumor growth and improved survival were reported in the group with temozolomide alone and the group with both STS and temozolomide, but not in the group with STS alone.

3.3. Associations between endpoints

Thirteen of the sixteen studies reporting a significant decrease in blood glucose found positive effects on tumor growth or survival. Two studies did not report a decrease in glucose and did not find reduced tumor growth or improved survival. We found a trend towards an association between decreased glucose and the clinically relevant endpoints tumor growth and survival ($P = 0.07$, Phi = 0.57, Fisher's exact test).

Thirteen studies reported a significant increase in blood ketones, and nine of these reported decreased tumor growth or increased survival. One study reported no increase in ketones, but nevertheless found improved survival. No association was found between ketones and tumor growth or survival ($P = 1.0$).

3.4. Molecular-biological pathways and mechanisms

Ten studies described molecular-biological effects of KCRDs on gliomas. Three studies examined the hypothesis that glioma cells are less able to use ketones as their primary energy source than normal brain cells [30,35,42]. One study found a decreased expression of ketolytic enzymes in glioma tissue (CT-2A and U87-MG) compared with normal brain tissue [42]. In contrast, another study found that these enzymes were expressed at mRNA and

protein levels in the studied glioma cell lines (T98G, U87MG, A172, LNT-229, and U251MG), and that the expressions did not change with KS [30]. However, these enzymes were not able to metabolize the ketone body β-hydroxybutyrate. Finally, De Feyter et al. studied tumor metabolism both *in vitro* and *in vivo* and found conflicting results [35]. *In vitro*, β-hydroxybutyrate uptake and oxidation in the glioma cells (RG2 and 9L) were low to undetectable in a low-glucose high-ketone body medium. *In vivo*, however, β-hydroxybutyrate uptake and oxidation were similar in glioma tissue and contralateral brain when this ketone body was infused, with increased uptake in glioma tissue of mice on a KD, leading to comparable oxidation in glioma tissue and contralateral brain. The authors could not find an explanation for the difference between the *in vitro* and *in vivo* results.

One study described the proapoptotic effects of CR, with use of a CT-2A glioma model [52]. This model is characterized by constitutive phosphorylation of Akt due to decreased expression of phosphatase and tensin homologue (PTEN) tumor suppressor, and is deficient for tuberous sclerosis complex 2 (TSC2). In addition, the expression of insulin-like growth factor I (IGF-I) and its receptor (IGF-IR) is significantly higher than in normal brain cells, which results in a dependency of CT-2A gliomas on glycolysis. These mutations in the PTEN/Akt/TSC2 pathway cause the antiapoptotic phenotype of CT-2A gliomas, but make tumors unable to adapt to ketosis. Moreover, CR lowers the expression of IGF-I and IGF-IR, and thus downregulates glycolysis. These effects subsequently result in increased cell death of CT-2A gliomas.

Mukherjee et al. found that glucose withdrawal (*in vitro*) and CR (*in vivo*) cause an increase in phosphorylation of AMP-activated protein kinase (AMPK) in CT-2A glioma cell lines and models, respectively, compared with normal brain cells [31]. Increased AMPK phosphorylation leads to inefficient cell signaling in the downstream TSC2-mTOR pathway, and subsequently to increased cell death.

Mulrooney et al. describe the effects of CR on the constitutive activation of the NF-kB pathway in a CT-2A glioma model [49]. CR reduces phosphorylation and activation of genes dependent on this pathway, and expression of downstream proinflammatory markers. By this, the authors conclude that CR reduces the inflammatory response of CT-2A gliomas, and thus inhibits angiogenesis, peritumoral edema and tumor progression, since these processes are stimulated by inflammation.

Woolf et al. used a GL261 glioma model and found no difference in total NF-kB expression between the KD-UR group and control group [41]. However, phosphorylated NF-kB was significantly reduced, and this suggests reduced activation of the NF-kB pathway. In addition, the authors studied the effect of a KD on hypoxia in tumors, by measuring hypoxia inducible factor-1α (HIF-1α) and carbonic anhydrase IX. Carbonic anhydrase IX is controlled by HIF-1α, and HIF-1α stimulates angiogenesis and tumor invasiveness. Both expressions were significantly reduced in tumors of the KD-group, and this led to the hypothesis that hypoxia in tumors could be reduced with a KD.

The same research group studied the effects of a KD-UR on the glioma-mediated immune suppression and tumor-reactive immune response in the GL261 glioma model [43]. On the one hand, KD-UR mice expressed significantly less immune inhibitory receptors and ligands in tumors. On the other hand, an increase in CD4+ T cells, in cytokine production (such as interferon gamma and tumor necrosis factor) of CD8+ T cells and natural killer cells, and in cytotoxic capability of CD8+ T cells was found in KD-UR mice. This study thus suggests an effect of a KD on the immune response against gliomas.

Flavahan et al. studied the effects of glucose restriction on brain tumor initiating cells (BTICs) [28]. The authors found that BTICs preferentially survive *in vitro* when glucose is restricted, compared with non-BTICs. In addition, non-BTICs that survive low glucose conditions acquire characteristics of BTICs. Moreover, when glioblastoma cells are cultured in low glucose conditions, and subsequently implanted *in vivo*, the resulting tumors grow significantly faster than those cultured in normal glucose conditions. This resulted in lower median survival. Because of these findings, the authors conclude that lowering glucose through KCRDs could lead to tumor progression instead of regression.

Stafford et al. found that a KD results in improved survival, as well as in an overall change in gene expression patterns of GL261 glioma cells, with a shift towards the patterns found in normal brain cells, particularly of genes involved in oxidative stress responses [34]. Reactive oxygen species significantly decreased *in vitro* with KS and *in vivo* with a KD (Supplemental Table 4), and the authors suggested that this may have caused the improved survival.

3.5. Clinical studies

We included eight clinical studies, consisting of three cohort studies [39,53,54], four case reports [27,55–57] and one clinical abstract [58]. For study characteristics, see Supplemental Table 3.

3.5.1. Quality assessment

The results of the quality assessment of the clinical studies are shown in Fig. 2. We used all components of the Cochrane Collaboration's tool for assessing risk of bias [24]. Blinding of outcome assessment was not done for any of the endpoints. As described previously, we assessed incomplete outcome data separately for laboratory effects, tumor growth, survival (essential parameters), safety and feasibility and quality of life (non-essential parameters). We found a high risk of attrition bias due to incomplete outcome assessment for survival and laboratory effects in multiple studies. Because of the uncontrolled study designs, the lack of blinding in all studies and the high risk of attrition bias, we consider the overall

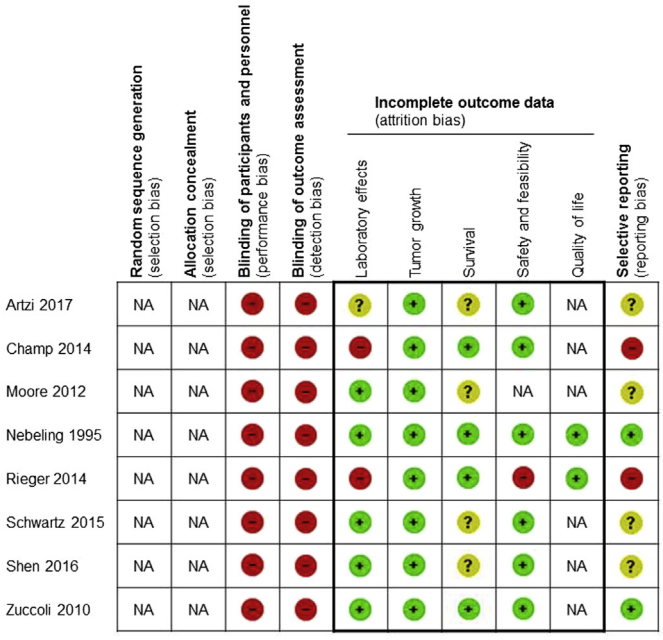

Fig. 2. Quality assessment of clinical studies was performed with use of the Cochrane Collaboration's tool for assessing risk of bias [24]. *Legend*: +, low risk of bias; −, high risk of bias; ?, unclear risk of bias; NA, not applicable. Of note, incomplete outcome data was assessed separately for 5 endpoints.

risk of bias to be high. Many of these sources of bias are inherent to the study designs (two small prospective cohort studies, but mainly case reports).

3.5.2. Outcome

Categorization and statistical analyses were not possible due to the small sample sizes and the heterogeneity of the clinical studies. Therefore, a short description of each study is given below.

In a prospective cohort study, a KD-UR was implemented in 20 patients with recurrent glioblastomas [39]. Data collection was completed in thirteen patients, and eight of them (62%) reached ketosis, defined as urine ketones >0.5 mmol/L in >50% of the urine samples, and these patients had a modest, non-significant increase of progression-free survival (PFS) ($P = 0.07$), compared with those without ketosis (6 weeks and 3 weeks, respectively). The mean blood glucose concentration did not decrease significantly. All patients progressed while they were on the KD-UR, and nine stopped the diet. Seven continued the diet concomitantly with bevacizumab. In six of them (85%), the tumor regressed, and they had a median PFS of 20.1 weeks. These results did not differ significantly from a cohort of historic controls, treated with bevacizumab alone in whom 65% responded ($P = 0.4$), with a median PFS of 16.1 weeks ($P = 0.38$).

Another prospective cohort study implemented a KD in 4 recurrent glioblastoma patients, who were concomitantly treated with bevacizumab and corticosteroids [54]. They had a PFS of 2.5 months (2, 4, 0 and 2 months), which was not superior to 4 recurrent glioblastoma patients on a standard diet and temozolomide, bevacizumab or rindopepimut (PFS of 4 months in two patients, and ongoing at 14 and 24 months in two patients). Another patient with gliomatosis cerebri (with low grade glioma histology) had been stable for 31 months with the KD as monotherapy at the time of publication. Ketosis, based on urine ketones, was only achieved in this patient and one recurrent glioblastoma patient.

A retrospective cohort study compared 53 patients with malignant gliomas, of whom six were on a KD and the others on an unspecified standard diet [53]. The KD was initiated by the patients, at different time points during standard treatments. All patients were treated with concomitant chemoradiation and adjuvant chemotherapy. The KD significantly reduced the mean blood glucose concentration ($P = 0.02$), even in three patients with high dose corticosteroids. Ketosis was confirmed in all patients on the KD. Four were alive at a median follow-up of 14 months, with a median time to disease progression or recurrence of 10 months. These results have not been compared with the control group, nor to historic controls.

The first case report described two pediatric patients with advanced malignant gliomas, who were treated for eight weeks with a diet consisting of 60% medium chain triglyceride oil, 10% carbohydrate, 20% protein and 10% fat [55]. After eight weeks, PET scans showed a mean reduction of 21.8% in the tumors' glucose uptake. Blood glucose concentrations were low-normal and ketones increased 20 to 30 times. When the case report was published, the patients remained without disease recurrence and with good quality of life for 5 and 4 years.

The second case report described regression of a glioblastoma within 2.5 months in a 65-year old female, who was treated with fasting and caloric restricted (ketogenic) diets before, during and after chemoradiation [56]. Blood glucose decreased and urine ketones were 2.5 mmol/L. The tumor recurred ten weeks after discontinuing CR.

Another case report described a 40-year old male with a progressive glioblastoma after standard and antiangiogenic treatments, in whom a KD-R was implemented concomitantly with bevacizumab [27]. The tumor remained stable for four months, with blood glucose and ketones of respectively 55–70 mg/dL and 4 mmol/L.

The last published case report described the effects of a 12-week KD-R (after a 48-h fast to initiate ketosis) in two adult males with recurrent glioblastomas [57]. The protocol defined optimal blood glucose as 50–70 mg/dL and optimal blood ketones as 3–8 mmol/L. A 55-year-old male initially reached these concentrations, until he changed to a regular foods KD-R due to the low palatability of the original KD-R (KetoCal®). He withdrew from the study when tumor progression was diagnosed after four weeks. A 52-year-old male maintained ketosis during the 12 weeks, with glucose less than 100 mg/dL most of the time. His tumor was stable at six weeks, but progressed at 12 weeks.

Finally, a clinical abstract described a 7-year-old girl with a glioblastoma, who was treated with a KD during and after chemoradiation [58]. Glucose decreased and ketosis was reached (blood ketones 2.75–10.16 mmol/L), and tumor size was reduced at 10 and 16 weeks.

3.5.3. Safety, feasibility and adverse effects

KCRDs seem to be safe and feasible in the preliminary clinical data to date, even when standard treatments are used concomitantly. No serious adverse effects were reported, except for grade II fatigue in a patient who was on a KD with 30% CR [53]. In the same study, weight loss was described as minimal, except in the above mentioned patient and another who intentionally lost weight. Four patients in the prospective cohort studies discontinued the KCRD [39,54], because they felt it reduced their quality of life [39]. In the other 17 patients on the KD-UR, Rieger et al. reported a statistically but not clinically significant weight loss of 2.2%. The body weight of the two pediatric patients stabilized, and quality of life improved in one of them, based on the development of skills and an improved mood [55]. The patient of Zuccoli et al. lost 20% of her body weight, but nevertheless maintained a Karnofsky performance status of 100% [56]. Schwartz et al. reported that their patients lost 6% and 7% of their body weight [57]. Three studies did not specifically mention the effect of the KCRD on body weight [27,54,58].

4. Discussion

4.1. Preclinical studies

In vitro, KCRDs improve stress and chemotoxicity resistance of normal brain cells [30,32], and sensitize glioma cells to chemotherapeutics [29,32,33]. Published *in vivo* studies suggest that KCRDs (and particularly caloric restricted forms) reduce tumor growth. This is in line with two meta-analyses of preclinical studies on effects of KCRDs in brain and non-brain tumors [59,60]. However, survival improved in only a small majority of studies included in our review. Positive effects increased when KCRDs were used concomitantly with other treatment modalities [25,29,33,37–39]. Because of these synergistic effects, we recommend to use KCRDs concomitantly with chemoradiation in future studies. Although bevacizumab is commonly prescribed in the treatment of malignant gliomas, recent randomized controlled trials have shown no benefit for overall survival [61,62]; given this absence of proof for efficacy, the role of bevacizumab, combined with KCRDs or other emerging therapies, remains to be determined. Regarding the combination of a KCRD and radiotherapy [37], it is unclear whether the synergistic effect is based on the metabolic effects of the KCRD, or on the radiosensitizing inhibition of HDAC by the KCRD [13]. Future studies that combine these interventions should include measurements of appropriate markers (e.g., histone acetylation).

In contrast to these mainly positive results, Flavahan et al. reported shorter median survival *in vivo* if tumor cells were

previously exposed to low glucose conditions *in vitro* [28], possibly due to the preferential survival of BTICs and the acquisition of BTIC characteristics by non-BTICs. BTICs are hypothesized to be responsible for tumor recurrence [63], and are relatively resistant to radiotherapy [64] and chemotherapy [65]. This is in line with a study on epithelial cancer cells that found that the ketone body β-hydroxybutyrate stimulates tumor growth [66]. It is unclear why Flavahan et al.'s findings of shortened survival are so contradictory to most other preclinical studies (showing improved survival). Nevertheless, because of these findings, we recommend to carefully monitor patients on KCRDs in future studies for accelerated tumor growth or the development of a pattern of tumor recurrence with a strong resistance to therapies.

We found a trend ($P = 0.07$, $Phi = 0.57$) towards an association between decreased glucose concentrations on the one hand, and reduced tumor growth and improved survival on the other. Decreased glucose seems to be more important than increased ketones in generating antitumor effects *in vivo*.

The preclinical studies had multiple limitations. Firstly, uncommon chemotherapeutic agents were used [29,32]. To increase the validity of findings, we recommend that the selection of chemotherapeutics for future studies are based on proven efficacy for gliomas [3,4,67]. Secondly, multiple studies used subcutaneous glioma models [25,26,29,33,42,52]. However, brain tumors are located in a specific microenvironment, isolated from the normal circulation by the blood–brain barrier. Therefore, we recommend to use only intracerebral models in future studies. In the study of Safdie et al., tumor growth was not reduced and survival not improved with temozolomide or radiotherapy alone [33], even though these therapies have proven efficacy [3,4]. Therefore, one could doubt the validity of these findings. Moreover, translation of preclinical findings into the clinical practice could be difficult, particularly for STS, given its questionable applicability. This is not addressed in the specific studies. In general, neutral and negative animal studies may be more prone to publication bias [68], which may cause an overestimation of the positive effects of KCRDs. We cannot exclude such an overestimation in our review, although two meta-analyses of preclinical studies in broader oncological fields converge with our findings [59,60].

4.2. Molecular-biological pathways and mechanisms

Glioma cells seemed to have a lesser expression and function of ketolytic enzymes *in vitro* and *in vivo* [30,35,42], although this was contradicted by the *in vivo* results of one study [35]. Use of other glioma cell lines and models may explain these differences. In the future, screening for the presence of ketolytic enzymes may be useful to select patients will benefit most from KCRDs.

Multiple pathways are influenced by KCRDs, such as the PTEN/Akt/TSC2 pathway, AMPK-TSC2-mTOR pathway and NF-kB pathway [31,41,49,52]. These findings provide insights in the molecular-biological effects of KCRDs. The study of Lussier et al. [43] suggests that KCRDs may have an effect on the glioma-mediated immune suppression, which warrants future studies and confirmation in humans.

4.3. Clinical studies

Overall, KCRDs seem to be safe and feasible in the preliminary clinical data to date, but efficacy data are scarce and of low methodological quality with high risk of bias. Causality cannot be examined with clinical abstracts and case reports, and their positive results were not reproduced in two small prospective cohort studies [39,54]. In the retrospective cohort study, however, the PFS seemed to be improved with the KD (10 months) [53], compared with standard treatments (6.9 months) [3]. However, no statistical analysis was performed on these two groups, and therefore no formal conclusion can be drawn.

Another source of bias is the possibility of treatment-induced necrosis (TIN, also often referred to as pseudoprogression or radiation necrosis), a phenomenon that mimics progressive or recurrent tumor on imaging and is commonly mistaken to be tumor recurrence [69]. Almost all patients in the clinical studies underwent radiotherapy, either before or concomitantly with a KCRD, which may have led to inclusion of recurrent gliomas that actually were TIN. This was not discussed by any of the studies. Also, KCRDs may alter the incidence of TIN due to alterations in the VEGF signaling pathway, which complicates the comparison of PFS data with historic controls.

In addition, five clinical studies administered KCRDs concomitantly with chemotherapy [53,55,56,58] or bevacizumab [54]. Therefore, the respective effects of both treatment modalities cannot be assessed accurately from these uncontrolled studies.

In the majority of the clinical studies, blood and urine ketone concentrations did not reach the reference values recommended by a Dutch guideline for use of a KD in pediatric refractory epilepsy [22], possibly due to the unrestricted nature of the KCRDs [39,53–55,58], administration of corticosteroids [39,53,54,58], and the lack of treatment by a registered dietician [27,39,53,55,56].

The classic KD, as used in pediatric refractory epilepsy, is poorly tolerated, due to unpalatability and gastrointestinal adverse effects [14]. In two studies with advanced malignancies (not gliomas), however, KCRDs was well-tolerated in the majority of the patients [70] and did not cause serious adverse effects [70,71], besides weight loss of on average 4% [71] or less [70], which was considered harmless [71]. In a case report of a boy with a grade I astrocytoma (which is not a high-grade glioma [1], and therefore not included in this review) on diverse KCRDs, quality of life, neurological and endocrine functions improved while his tumor remained stable [72,73]. Except for grade II fatigue in one patient [53], no serious adverse effects were reported in the discussed studies. Weight loss due to KCRDs was absent [55] or considered minimal [39,53,57], except for one patient with a 20% body weight reduction, but nevertheless a Karnofsky performance status of 100% [56]. Four patients found the KD intolerable [39,54], and felt it reduced their quality of life [39].

The selection of the optimal KCRD should not only be based on efficacy, but also on safety, feasibility, and the effect on quality of life. In addition, body weight should be maintained, since weight loss and malnutrition in cancer patients are associated with a poor response to therapies, more adverse effects and complications, short median survival and poor quality of life [74]. Therefore, it is recommended not to use caloric restricted diets in cancer patients at risk for malnutrition [75]. The selection of the optimal patient, however, is equally important.

5. Conclusions

The role of KCRDs as (adjunct) treatment modalities for malignant gliomas is supported by the majority of published preclinical studies to date. However, because of the paucity of clinical data, conclusions on efficacy and quality of life cannot be drawn. Data on adverse effects, such as weight loss, suggest that KCRDs are safe and feasible in cancer patients, but these data are preliminary. Effects on nutritional status, body composition and physical functioning are largely unknown. Therefore, KCRDs in gliomas should be regarded as experimental, and should preferably only be implemented in the context of clinical studies.

The time is right for multidisciplinary prospective clinical studies that will examine whether KCRDs have clinically relevant

effects for glioma patients. As of February 18, 2018, 11 prospective clinical studies are registered in ClinicalTrials.gov and recruiting glioma patients for (adjunct) treatment with KCRDs. In addition to the above recommendations, new clinical studies should systematically report effects on blood glucose and ketone concentrations, body weight, nutritional status, body composition, physical functioning, tumor growth, survival and quality of life. The latter should be a leading factor, since the treatment of gliomas to date is palliative in nature. Inclusion of patients should be based on a modern classification of gliomas that comprises both histology and molecular-biological markers [76]. The possibility of TIN should be considered at inclusion and during (radiological) follow-up. Finally, biomarker data should be systematically collected, for analysis on tumor metabolism and the discussed molecular-biological pathways. This could form the basis for future translational research on optimal use of KCRDs, combinations of KCRDs with (future) targeted therapies, and the selection of patients who will benefit most from KCRDs. This could lead to a better understanding of the role of KCRDs in the personalized treatment of gliomas.

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Statement of authorship

The authors' contributions were as follows – All authors contributed to the conception and/or the analysis of the paper; All authors contributed to the writing of the manuscript and approved the final version; LN performed the data extraction from the selected papers and wrote the first draft; AK made the 'golden hit' list; LN and FYV independently performed the selection of abstracts; MLB and PAR reviewed the preclinical data; TJS reviewed the data extraction, co-wrote the first draft and supervised the project.

Conflict of interest

The authors reported no conflicts of interest.

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Appendix A. Supplementary data

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