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Original Article

Anti-tumor effects of ketogenic diets and their synergism with other treatments in mice: Bayesian evidence synthesis of 1755 individual mouse survival data

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ABSTRACT

Background: Ketogenic diets (KDs) are high-fat diets with putative anti-tumor effects. The aim of this study was to synthesize the evidence for the anti-tumor effects of KDs in mice, with a focus on their possible synergism with chemotherapy (CT), radiotherapy (RT), or targeted therapies (TT).
Methods: Relevant studies were retrieved from a literature search. A total of 43 articles reporting on 65 mouse experiments fulfilled the inclusion criteria, and 1755 individual mouse survival times were collated from the study authors or the publications. The restricted mean survival time ratio (RMSTR) between the KD and control groups served as the effect size. Bayesian evidence synthesis models were used to estimate pooled effect sizes and to assess the impact of putative confounders and synergism between KD and other therapies.
Results: Overall, there was a significant survival-prolonging effect of KD monotherapy (RMSTR = 1.161 ± 0.040), which was confirmed in meta-regression accounting for syngeneic versus xenogeneic models, early versus late KD start and subcutaneous versus other organ growth. Combining the KD with RT or TT, but not CT, was associated with a further 30% (RT) or 21% (TT) prolongation of survival. An analysis accounting for 15 individual tumor entities showed that KDs exerted significant survival-prolonging effects in pancreatic cancer (all treatment combinations), gliomas (KD + RT and KD + TT), head and neck cancer (KD + RT), and stomach cancer (KD+RT and KD + TT).
Conclusions: This analytical study confirmed the overall anti-tumor effects of KDs in a large number of mouse experiments and provides evidence for synergistic effects with RT and TT.

Background

Since the 1920s, it has been well established that cancer is characterized by an abnormal metabolism, with tumor cells heavily relying on substrate-level phosphorylation, mainly the fermentation of glucose to lactate [1–4]. This phenomenon has become known as the Warburg effect after Otto Warburg, who was among the first to systematically study it [5]. A large body of evidence now points towards mitochondrial dysfunction as the putative root cause for developing a Warburg phenotype, with genetic mutations in the nucleus following secondarily as an adaption to compensate for diminished energy and increased reactive oxygen species production in the mitochondria [6–9]. The central role of the mitochondria is consistent with the view that cancer is an evolutionary back-transition where an ancient set of nuclear genes which is actively silenced in multicellular organisms is turned on again in order to ensure the survival of the whole cell when the cooperation between the mitochondria and the nucleus that emerged during eukaryogenesis breaks down. This readily explains all hallmarks of cancer since a tumor cell behaves not too differently from a unicellular organism predating the emergence of multicellularity.

Already in 1928, the famous couple Carl Ferdinand and Gerty Theresa Cori observed that higher blood glucose concentrations increased the rate of glycolysis in tumor tissue *in vivo* and hypothesized that the Warburg effect may be exploited therapeutically through dietary manipulation ([4], 311–312):

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The influence of the blood sugar concentration on the activity of the tumor raises the question as to whether any benefit would be derived from a low carbohydrate diet in the treatment of patients suffering from malignancy. The fact, that aerobic glycolysis is one of the fundamental characteristics of the malignant cell, should be sufficient reason to keep the glycolytic activity of the tumor at a low level by preventing an undue rise in the blood sugar concentration.

A diet which is able to keep blood glucose levels constantly low without the need for caloric restriction is known as a ketogenic diet (KD). A KD is characterized by a proportionately high fat content comprising at least 60% of energy intake with concurrently very low carbohydrate intake which promotes the production of ketone bodies from stored and dietary fatty acids.

Until about 2010, only a few clinical or preclinical studies on the KD as a cancer treatment were conducted, but then research interest increased sharply [10]. Meta-analyses of controlled clinical trials published between 2018 and 2022 have shown that short-term application of a KD is safe for cancer patients and exerts some beneficial effects on metabolic health and quality of life when compared to a standard diet [11,12], although strong evidence for anti-tumor effects could not yet be established [13,14]. Meta-analyses of animal studies, however, have provided strong evidence for the overall anti-tumor effects of KDs [15–17]. In addition, animal studies provided mechanistic evidence for the hypothesis that a KD is especially helpful when combined with standard-of-care therapy such as chemotherapy (CT), radiotherapy (RT), or targeted therapy (TT) [18–21]. This is because tumor cells have high levels of intrinsic ROS production, and they utilize glycolysis for their own anti-oxidative protection [18,19,21]; by limiting the supply of glucose to tumor cells and inhibiting glycolysis, a KD effectively reduces the anti-oxidative defence of tumor cells so that they get sensitized to additional ROS induced by CT, RT or TT through primary or secondary mechanisms.

The synergism between a KD and standard-of-care therapies is of high translational relevance as cancer patients are likely to combine both, but meta-analyses of mouse studies thus far have focussed on experiments in which KD was applied as a monotherapy [15–17]. Therefore, the aim of this study was to undertake a new meta-analysis of mouse experiments to estimate the effects on mouse survival attributed to a KD *per se* and its synergism with standard-of-care therapies. Besides providing an updated estimate of the anti-tumor effects of a KD in mice based on a larger number of experiments than used in previous meta-analyses, the results of this study may be helpful for identifying the types of therapy and cancers which may profit, especially from a combination with a KD.

Methods

Data

The inclusion criteria for this study were defined as follows.

(i) Studies investigating tumor growth in a mouse cancer model.
(ii) Studies testing the effects of an **unrestricted** KD with a ketogenic ratio of at least 1.5:1 on tumor growth in comparison to a non-ketogenic control diet (CD) with or without one of the following additional treatments: a) CT, b) RT, c) TT.
(iii) Endpoint defined as reaching a pre-defined tumor volume or other sign of disease progression or termination of the experiment at a pre-defined time interval.
(iv) Measurement of animal survival times and inclusion of a Kaplan-Meier plot in the publication.

Studies not fulfilling all of the above four inclusion criteria were excluded from the analysis. In case that two different tumor cell lines belonging to the same organ were studied separately using the same experimental procedures, the outcomes were considered as belonging to one experiment and survival times were pooled. If two experimental procedures only differed in the KD composition, their results were also pooled. These choices reflect the fact that in human patients, individual tumors from the same organ may vary with respect to their cell biology, and patients might consume KDs with different food compositions.

A primary search for articles published since 2010 was performed in PubMed on December 4, 2022, and repeated on March 24, 2023, using the search term (“animal” OR “xenograft” OR “allograft” OR “syngeneic” OR “mice” OR “mouse”) AND “cancer” AND (“ketogenic diet” OR “ketosis” OR “ketone bodies”) AND 2010/01/01:2022/12/01 [dp]. Additional studies were searched in a personal library of this author who collects papers on KD treatment for cancer since 2011 through regular searches in PubMed and other sources such as ResearchGate (https://www.researchgate.net/). Individual mouse survival times were obtained by requesting them from the corresponding study authors or, in case of no reply, by retrieving them from Kaplan-Meier plots using the software DigitizeIt version 2.5.9 (https://www.digitizeit.xyz/de/). In the latter case, the correctness of the extracted survival times was checked by creating their own Kaplan-Meier plots and comparing them to the original ones. The software DigitizeIt was also used to extract mean or median serum glucose and ketone body concentrations of mice on a KD, which most papers displayed in boxplots, and from these values, the glucose-ketone-index was estimated [22].

Bayesian evidence synthesis

As in our previous work [14], the restricted mean survival time (RMST) ratio (henceforth denoted as RMSTR) between the intervention and control group in each experiment was chosen as the outcome of interest measuring the anti-tumor effect of a KD intervention. The RMST is a measure of average survival up to the specified follow-up time, which distinguishes it from the mean survival time μ:

$$\mu = \int_0^\infty S(t)dt \tag{1}$$

$$\mathrm{RMST}(t^*) = \mathrm{E}[\min(T, t^*)] = \int_0^{t^*} S(t)dt \tag{2}$$

The RMST thus measures the area under the survival curve up to a specified time point. For the specified time point, I adopted the last recorded time point in each study group, so that in cases in which all animals experienced the event of interest (as in most experiments), RMST $= \mu$. In these cases, time parameters such as mean survival times provide a more useful description of group survival statistics than the hazard ratio [23]. Another advantage of the RMST is that it is valid under any distribution of the time to event in the treatment groups, of which proportional hazards models are only a (small) sub-class; it is, therefore, an alternative to the hazard ratio whenever the proportional hazards assumption is doubtful [24,25].

The amalgamation of RMSTRs was performed in three separate analyses addressing three different questions.

(i) To what extent does a KD *per se* prolong survival in mouse tumor models?
(ii) To what extent does combining a KD with standard-of-care therapy boost its anti-tumor effects?
(iii) What are the predictions for combining a KD with standard-of-care therapy for individual tumor types?

To address the first question, I conducted a Bayesian meta-analysis of individual study results for all studies which applied a KD as monotherapy using the methodology already applied in our previous meta-analysis from 2016 [16]. First, the RMSTR was transformed to the log scale by taking the natural logarithm of it. Then a random effects model

was fit, assuming a normal likelihood for the individual log-transformed study outcomes [26–28]:

$$y_i \sim N\left(\theta_i, s_i^2\right) \tag{3}$$

Here y_i and s_i denote the log-transformed outcome, ln(RMSTR), and its standard error in the i th study, and the true study effects θ_i are assumed to be exchangeable [26] and drawn from an underlying distribution given by

$$\theta_i \sim N\left(\mu, \tau^2\right) \tag{4}$$

Heterogeneity was assessed by the between-study variance τ^2 which was modelled using three different prior distributions [27,28]: (i) a prior for τ uniform on [0,1]; (ii) a half-normal prior for τ with standard deviation 0.25, corresponding to an anticipated "upper" value for τ of 0.49; (iii) DuMouchel's prior $P(\tau) = \frac{s_0}{(s_0+\tau)^2}$ with $s_0^2 = K/(\sum_{i=1}^{K} s_i^{-2})$ being the harmonic mean of the K individual study variances s_i^2. Using three different priors for τ probes the sensitivity of the results to different *a priori* assumptions about the between-study heterogeneity. To determine possible sources of heterogeneity, Bayesian meta-regression [27,28] was conducted using the tumor location (subcutaneous versus non-subcutaneous), the timing of diet initiation ($\geq$1 day after versus $\geq$0 days prior to tumor implantation), and tumor model type (syngeneic versus xenogeneic).

$$\theta_i \sim N\left(\mu + \beta_{subcut} \cdot \mathrm{x}_{1i} + \beta_{diet.start} \cdot \mathrm{x}_{2i} + \beta_{syngeneic} \cdot \mathrm{x}_{3i}, \tau^2\right) \tag{5}$$

To address the second question, a meta-regression analysis was conducted, which included also those experiments in which a KD had been combined with another therapy:

$$\theta_i \sim N\left(\mu + \beta_{KD+chemo} \cdot \mathrm{x}_{1i} + \beta_{KD+RT} \cdot \mathrm{x}_{2i} + \beta_{KD+targeted} \cdot \mathrm{x}_{3i}, \tau^2\right) \tag{6}$$

Here, $\mathrm{x}_{ji} = 0, j \in \{1,2,3\}$, if KD was applied as monotherapy in an experiment i or $\mathrm{x}_{ji} = 1$ if it was combined with CT ($j = 1$), RT ($j = 2$) , or TT ($j = 3$), respectively. Thus, the β_{KD+} coefficients denote the corresponding gain in the RMSTR through additional treatment.

Finally, to address the third research question, a Bayesian model originally developed by DuMouchel and Harris [29], DuMouchel and Groër [30], and Jones et al. [31] was adopted. The model differentiates between different tumor types specified by the experimental cell line origin and different treatments (KD monotherapy, KD + CT, KD + RT, KD + TT). Its underlying assumption is that all experiments are "*related through some unifying biological hypothesis*" and that "*the results of each experiment are summarized by a single number …*" [29].

The unifying biological hypothesis mentioned in the quote consists in assuming an "equal relative potency" of the interventions, meaning that if one treatment (e.g., KD + RT) prolongs survival by a factor of x compared to the control group in one tumor entity, the same factor should apply to another tumor entity treated with the same treatment. The deviation from this assumption can be estimated in the model.

The Bayesian model was specified using the notation given by Jones et al. [31]. I assume a normal likelihood for the ln (RMSTR) in the ith row (tumor type) and jth column (intervention) in Supplementary [Table 1]:

$$y_{ij} \sim N\left(\theta_{ij}, c_{ij}^2\right) \tag{7}$$

where

$$\theta_{ij} \sim N\left(\alpha_i + \gamma_j, \sigma^2\right),$$

$$\alpha_i \sim N\left(\mu_{\alpha_i}, \sigma_{\alpha_i}^2\right),$$

$$\gamma_j \sim N\left(\mu_{\gamma_j}, \sigma_{\gamma_j}^2\right) \tag{8}$$

Thus, θ_{ij} is the true ln (RMSTR) and c_{ij}^2 the variance of y_{ij} for tumor type i and intervention j. The variables α_i and γ_j represent the tumor type and intervention effects, respectively, and σ^2 measures the deviation from the assumption of equal relative potency of interventions across tumor types [31].

The basic input data structure for the model specified by Equation (7) is shown in [Supplementary Table 1]for a total of 15 tumor entities. Each entry in this table denotes the logarithm of an RMSTR estimate for a given tumor type and intervention. Because the model required exactly one datum per intervention and tumor type, in case that for a given tumor type and intervention, several study estimates were available, the estimates were pooled by a Bayesian meta-analysis as described above (Equation (3) and Equation (4)); thereby all three priors on τ were utilized and an overall posterior probability distribution obtained by combining the corresponding Markov chain results of the three priors.

Prior distributions

Prior distributions on the hyperparameters α_i, γ_j , and σ^2 allow us to incorporate eventual prior knowledge or beliefs about disease mechanisms or relationships between tumor entities and treatments. The relationship between the 15 different tumor entities is specified through the 15 × 15 covariance matrix Ralpha, and the relationship between the 4 different treatments by the 4 × 4 covariance matrix Rgamma [14,31].

Prior for $\boldsymbol{\sigma}$

The parameter σ gauges the accuracy of the equal relative potency assumption by measuring the deviations of the $\alpha_i + \gamma_j$ from the true effects θ_{ij}. Thus, the assumption that the ratio between the RMSTRs of two interventions is preserved across tumor types is only accurate to within a deviation of $\exp(\pm\sigma)$ with 68% probability [29]. I use a uniform prior distribution $\sigma \sim U(0,1)$, implying the assumption that with 68% probability, the constant relative potency assumption would be accurate to within a factor ranging from $\exp(0) = 1$ to $\exp(1) \approx 2.72$.

Priors for $\boldsymbol{\alpha_i}$ *and* $\boldsymbol{\gamma_j}$

I assume weakly informative priors $\alpha_i \sim N(0,1)$ and $\gamma_j \sim N(0,1)$, implying a variance of the true study effects of $1 + \sigma^2$. I further assumed that each tumor entity would yield no prior information about the other tumor entities, so that

$$\mathrm{Ralpha} = \begin{pmatrix} 1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & 1 \end{pmatrix} \tag{9}$$

Furthermore, I assume strong correlations between the three types of treatment combined with a KD. These are expressed by off-diagonal elements in the 4x4 matrix Rgamma:

$$\mathrm{Rgamma} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0.95 & 0.95 \\ 0 & 0.95 & 1 & 0.95 \\ 0 & 0.95 & 0.95 & 1 \end{pmatrix} \tag{10}$$

The sensitivity of the results towards the assumption of a strong correlation was tested by conducting an additional analysis assuming no such correlations:

$$\mathrm{Rgamma} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \tag{11}$$

In addition, a sensitivity analysis was conducted in which a correlation of 0.9 between tumors of the gastrointestinal tract (colon, pancreas,

stomach) was assumed. This was specified by assigning a value of 0.9 to the corresponding off-diagonal elements in the matrix Ralpha (Equation (9)).

Model inference

Models were run in OpenBUGS version 3.2.3. For each model, two separate Markov chains were initialized, and the first 100,000 iterations from each chain were considered as burn-in samples and discarded. Then, the next 100,000 iterations from each chain were sampled for inference using a thinning of 20 in order to reduce auto-correlation. From the resulting 10,000 samples, the mean or median was taken as point estimates for normally and non-normally distributed variables, respectively, and 95% highest posterior density intervals (HPDIs) were derived.

Plausibility of prior assumptions

The plausibility of the various priors was checked using the Deviance Information Criterion (DIC) [32,33]. DIC is defined as the sum of the posterior mean deviance $\overline{D}$ (a measure of model adequacy) and the effective number of parameters p_D (a measure of model complexity). Compared to Bayes factors, DIC is more appropriate for comparing hierarchical models; it measures what the data tell us about the predictive accuracy rather than the truth of a model and can easily be monitored in OpenBUGS [34].

Results

Characteristics of the included studies

The primary PubMed search resulted in 184 hits of which 156 were discarded after title, abstract, or full-text screening, resulting in 28 articles for inclusion [35–62]. An additional 15 articles were retrieved from my personal library [20,63–76]. Thus, a total of 43 articles reporting on 65 experiments fulfilled the inclusion criteria [Supplementary Figure S1]. Their characteristics are given in [Table 1]. For 33 of the experiments, individual survival times were provided by study authors, while for 32 experiments, they were extracted from the Kaplan-Meier plots. In total, 875 mice had been treated with a KD, while 880 had received a CD. A total of 35 tumor entities were studied using syngeneic tumor models, while 30 tumor entities were studied in xenogeneic tumor models. Of the 65 experiments, five experiments had combined KD with CT, four with RT, and ten with a TT. Glucose-ketone-index values were available for 30 of the 65 experiments, and they were significantly correlated with the ketogenic ratio (Spearman's $\rho = -0.44$, $p = 0.0081$).

Results for ketogenic diet(KD) monotherapy

A total of 46 experiments had tested a KD as monotherapy in a mouse tumor model. Of these, 21 (45.7%) used a subcutaneous tumor transplant, 25 (54.3%) a syngeneic tumor model, and 36 (78.3%) started the KD treatment at least one day after tumor transplantation.

The results of pooling the experimental outcomes through meta-analysis are given in [Table 2]. Overall, a KD was found to significantly prolong the RMST by around 16%, with the 95% HPDI supporting survival advantages between 8% and 24%. The prior specification for the true effect standard deviation τ had a negligible influence on this result, and the DIC values provided no strong evidence that one prior specification resulted in a better model fit than the other two.

When including only studies with sample sizes $\geq$10 in both the KD and CD groups (n = 24), the posterior estimate for the RMST slightly increased to 1.176 ± 0.070, HPDI = [1.046,1.323], and heterogeneity also increased to $\tau = 0.268$, 95% HPDI = [0.196,0.382].

Subgroup analysis and meta-regression of experimental variables

[Fig. 1] shows the results of subgroup analysis according to different experimental variables. Models with an early start of the KD appeared to have somewhat higher efficacy. This was confirmed in meta-regression [Table 3]: experiments in which the KD had been initiated at least one day after tumor initiation were associated with less anti-tumor efficacy. However, compared to the results displayed in [Table 2], inclusion of the three possible confounders had basically no impact on the between-study variance, and their effects were not statistically significant. A meta-regression using the ketogenic ratio as a continuous variable to predict the RMSTs yielded no significant effect ($\beta_{ratio} = 0.004 \pm 0.023$). Also, no significant effect was found for the glucose-ketone-index (available for 27 experiments with KD monotherapy) as a predictor ($\beta_{GKI} = 0.004 \pm 0.006$).

Subgroup analysis for individual tumor entities

I performed a subgroup analysis for different tumor entities which were used in at least 6 experiments. These were colon cancer (n = 7), glioma (n = 21), lung cancer (n = 6) and pancreatic cancer cell lines (n = 9). [Fig. 2] shows a forest plot of the overall RMSTR estimates for these tumor models for experiments with a KD monotherapy and in addition a KD combination therapy (not differentiating between type of additional treatment due to the small number of studies). In colon cancer ($\widehat{RMSRT} = 1.236$, 95% HPDI = [1.114,1.381]) and glioma models ($\widehat{RMSRT} = 1.188$, 95% HPDI = [1.047,1.353]), there were significantly longer survival times in mice treated with KD monotherapy compared to mice on a CD, but not in lung cancer models ($\widehat{RMSRT} = 1.022$, 95% HPDI = [0.792,1.404]); for pancreatic cancer the mean point estimates indicated strong anti-tumor effects of KD mono- and combination therapy, but uncertainties were large.

Results for synergism with additional treatment

[Table 4] displays the results of meta-regression (Equation (6)) for estimating the effects of additional treatment by CT, RT or TT. The sole effect of a KD was compatible with the estimate for the effects of KD monotherapy shown in[Table 2]. The regression model yielded no synergistic effect for CT, while RT and TT were estimated to more than double the survival-prolonging effect of a KD. Although the 95% HPDIs for the TT regression coefficient just included 0, a synergistic effect between KD and TT was supported with 97% probability.

In a sensitivity analysis, only the 19 studies from [Table 1] which had actually studied combination treatments were included in the meta-regression model. Except for Aminzadeh-Gohari et al. [51], all of them had also performed an experiment with KD monotherapy, so that the total number of experiments in the meta-regression was 37. The results were consistent with the full analysis shown in [Table 4], with posterior estimates of $\widehat{RMSRT} = 1.20 \pm 0.08$, $\beta_{KD+CT} = -0.07 \pm 0.13$, $\beta_{KD+RT} = 0.27 \pm 0.16$ and $\beta_{KD+TT} = 0.17 \pm 0.15$.

Prediction of individual treatment effects in different tumor entities

This analysis addressed the third research question posed above: *What are the predictions for combining a KD with standard-of-care therapy for individual tumor types?* [Supplementary Table 3] displays the input data for this analysis, and the results for using the priors specified in Equations (9) and (10) are shown in [Fig. 3]. Several outcomes are visible from this forest plot: First, when taking into account individual tumor types, RT had the strongest synergistic effects when combined with a KD, followed by TT, while CT did not synergize with a KD. Second, significant effects (the 95% HPDIs excluding 1) were obtained for pancreatic cancer (all treatments), gliomas (KD + RT and KD + TT), and stomach cancer (KD + RT and KD + TT). Third, the worst response was

Table 1
Characteristics of the included studies.

Author	Year	Cell line origin	Model	Intervention	Organ	KD start	KD ratio	GKI	N_{KD}	N_{CD}	RMSTR	$RMSTR_{low}$	$RMSTR_{up}$
Hopkins	2019	Akute myeloid leukemia	syngeneic	KD	Bone marrow	day0	6	NA	5	6	1.082	0.995	1.176
Hopkins	2019	Akute myeloid leukemia	syngeneic	KD + Targeted	Bone marrow	day0	6	NA	5	7	1.189	1.043	1.356
Zou	2020	Breast	syngeneic	KD	Breast	before	4	3.05	9	9	1.272	1.177	1.374
Zou	2020	Breast	syngeneic	KD + Targeted	Breast	before	4	3.1	8	8	1.231	1.119	1.354
Zhang	2018	Cervix	xenogeneic	KD	Subcutaneous	after	3	1.5	8	8	0.731	0.589	0.908
Dai	2021	Colon	syngeneic	KD	Subcutaneous	after	6	NA	12	12	1.307	1.201	1.422
Dai	2021	Colon	syngeneic	KD + Targeted	Subcutaneous	after	6	NA	11	12	1.200	1.01	1.426
Dmitrieva-Posocco	2022	Colon	syngeneic	KD	Colon	before	4	NA	15	15	1.226	0.988	1.52
Hao	2015	Colon	xenogeneic	KD	Subcutaneous	day0	3	3.3	12	12	1.364	1.201	1.548
Kasumi	2019	Colon	syngeneic	KD	Peritoneum	after	4	1.2	9	9	1.197	1.085	1.32
Wei[a]	2022	Colon	syngeneic	KD	Subcutaneous	after	6	NA	20	20	1.139	1.075	1.207
Wei[a]	2022	Colon	syngeneic	KD + Targeted	Subcutaneous	after	6	NA	20	20	1.262	1.058	1.505
Abdelwahab	2012	Glioma	syngeneic	KD	Brain	after	4	6.8	20	19	1.454	1.067	1.981
Abdelwahab	2012	Glioma	syngeneic	KD + RT	Brain	after	4	3.4	11	11	1.933	1.499	2.494
Akgoc	2022	Glioma	syngeneic	KD	Multiple (metastases)	after	4.2	8.5	10	9	1.205	1.06	1.37
Ciusani	2020	Glioma	syngeneic	KD	Brain	after	6	1.9	10	8	1.187	1.103	1.278
Javier	2021	Glioma	syngeneic	KD	Brain	after	4	6.4	17	24	1.014	0.911	1.129
Kesarwani	2022	Glioma	syngeneic	KD	Brain	after	4	5.9	17	16	1.204	1.098	1.32
Kesarwani	2022	Glioma	syngeneic	KD + Targeted	Brain	after	4	5.9	11	5	1.381	1.115	1.712
Lussier	2016	Glioma	syngeneic	KD	Brain	after	4	3.1	12	11	1.274	1.071	1.516
Maeyama	2021	Glioma	xenogeneic	KD	Brain	after	4	NA	7	7	1.106	1.015	1.206
Maeyama	2021	Glioma	xenogeneic	KD + Targeted	Brain	after	4	NA	7	7	1.254	1.061	1.483
Martuscello[b]	2015	Glioma	xenogeneic	KD	Brain	after	6	1.85	15	17	2.755	2.111	3.597
Maurer	2011	Glioma	xenogeneic	KD	Brain	after	2.7	NA	12	12	0.906	0.8	1.027
Poff	2013	Glioma	syngeneic	KD	Multiple (metastases)	day0	3.7	10.5	8	13	1.569	1.208	2.037
Poff	2015	Glioma	syngeneic	KD	Multiple (metastases)	day0	1.5	39.8	7	13	1.561	1.156	2.107
Rieger	2014	Glioma	xenogeneic	KD	Brain	after	3.1	NA	8	8	1.052	0.956	1.157
Rieger	2014	Glioma	xenogeneic	KD + Targeted	Brain	after	3.1	NA	8	8	1.144	1.018	1.286
Sperry	2020	Glioma	syngeneic	KD	Brain	after	6	NA	10	10	0.887	0.713	1.103
Sperry[c]	2020	Glioma	xenogeneic	KD	Brain	after	6	5.65	20	19	0.916	0.825	1.017
Stafford	2010	Glioma	syngeneic	KD	Brain	after	6	NA	5	5	1.263	1.142	1.397
Zhou	2007	Glioma	syngeneic	KD	Subcutaneous	after	4	21.8	9	7	1.117	0.977	1.276
Zhou	2007	Glioma	xenogeneic	KD	Subcutaneous	after	4	9.6	7	11	0.927	0.769	1.116
Ma	2021	HNC	xenogeneic	KD	Subcutaneous	after	4	NA	12	6	1.339	1.117	1.605
Ma	2021	HNC	xenogeneic	KD + RT	Subcutaneous	after	4	NA	7	7	1.422	0.815	2.48
Vidali	2017	Kidney	xenogeneic	KD	Subcutaneous	after	7	4.8	5	6	0.837	0.68	1.031
Allen[d]	2013	Lung	xenogeneic	KD + RT	Subcutaneous	after	4	NA	29	30	1.298	1.14	1.477
Allen[e]	2013	Lung	xenogeneic	KD	Subcutaneous	after	4	NA	11	13	0.961	0.784	1.178
Hsieh	2019	Lung	syngeneic	KD	Lung	after	4	NA	13	19	0.973	0.79	1.199
Langer	2022	Lung	syngeneic	KD	Lung	after	6	3.9	12	11	0.900	0.812	0.999
Zhang	2022	Lung	xenogeneic	KD	Subcutaneous	after	4	NA	5	5	1.580	1.101	2.268
Zhang	2022	Lung	xenogeneic	KD + Chemo	Subcutaneous	after	4	NA	5	5	0.955	0.82	1.111
Dang	2015	Medulloblastoma	syngeneic	KD	Brain	after	4	NA	4	4	1.092	0.856	1.393
Ferrere	2021	Melanoma	syngeneic	KD	Subcutaneous	day0	4	NA	30	30	0.947	0.828	1.083
Ferrere	2021	Melanoma	syngeneic	KD + Targeted	Subcutaneous	day0	4	NA	30	30	1.126	1	1.268
Weber	2022	Melanoma	syngeneic	KD	Multiple (metastases)	after	8	1.1	8	9	1.202	1.002	1.441
Weber[f]	2022	Melanoma	xenogeneic	KD	Subcutaneous	after	8	2.25	21	25	1.109	0.993	1.238
Aminzadeh-Gohari[g]	2017	Neuroblastoma	xenogeneic	KD + Chemo	Subcutaneous	after	7	2.9	22	22	1.119	1.034	1.21
He	2020	Neuroblastoma	xenogeneic	KD	Subcutaneous	after	4	3.1	8	8	1.222	1.138	1.313
Morscher[h]	2015	Neuroblastoma	xenogeneic	KD	Subcutaneous	after	2	9.6	21	21	1.232	1.047	1.45
Morscher[i]	2016	Neuroblastoma	xenogeneic	KD + Chemo	Subcutaneous	after	2	9.6	22	20	1.018	0.997	1.04
Cortez[j]	2022	Pancreas	syngeneic	KD	Pancreas	after	1.9	12.2	23	18	1.122	0.885	1.422
Cortez[j]	2022	Pancreas	syngeneic	KD + Chemo	Pancreas	after	1.9	10.1	18	16	1.207	0.983	1.482
Hopkins	2019	Pancreas	syngeneic	KD	Subcutaneous	after	6	NA	10	10	2.483	2.114	2.916
Hopkins	2019	Pancreas	syngeneic	KD + Targeted	Subcutaneous	after	6	NA	10	10	3.315	3.079	3.568
Yang	2022	Pancreas	syngeneic	KD	Subcutaneous	after	6	5.1	14	14	0.951	0.778	1.163
Yang[k]	2022	Pancreas	syngeneic	KD + Chemo	Subcutaneous	after	6	5.1	52	48	1.315	1.157	1.492

(*continued on next page*)

Table 1 (*continued*)

Author	Year	Cell line origin	Model	Intervention	Organ	KD start	KD ratio	GKI	N_{KD}	N_{CD}	RMSTR	$RMSTR_{low}$	$RMSTR_{up}$
Zahra	2017	Pancreas	xenogeneic	KD	Subcutaneous	after	4	NA	7	9	1.000	0.682	1.465
Zahra	2017	Pancreas	xenogeneic	KD + RT	Subcutaneous	after	4	NA	6	6	1.685	1.209	2.347
Zhang	2018	Pancreas	xenogeneic	KD	Subcutaneous	after	3	1.1	8	8	1.308	1.016	1.683
Freedland	2008	Prostate	xenogeneic	KD	Subcutaneous	before	2.1	NA	25	25	1.209	1.077	1.356
Mavropoulos	2009	Prostate	xenogeneic	KD	Subcutaneous	before	2.1	NA	48	41	0.934	0.817	1.068
Otto	2008	Stomach	xenogeneic	KD	Subcutaneous	day0	2.7	4.2	12	12	1.464	1.721	1.246
Zhao	2022	Thyroid	xenogeneic	KD	Thyroid	after	4	4.2	8	9	0.999	0.839	1.189
Zhao	2022	Thyroid	xenogeneic	KD + Targeted	Thyroid	after	4	4.2	8	8	1.097	0.976	1.233

Abbreviations: GKI: Glucose-ketone-index; RMSTR: Restricted mean survival time ratio; $RMSTR_{low}$: lower 95% confidence limit of RMSTR; $RMSTR_{up}$: upper 95% confidence limit of RMSTR; N_{CD}: Number of animals in control diet group; N_{KD}: Number of animals in ketogenic diet group; RT: radiotherapy. [a] Data for Balb/cJ mice with CT26 tumor and C57BL/6J mice with MC38 tumor were pooled (Fig. 6I+6J); [b] Data for L0 and L2 cell lines were pooled; [c] data for U87 and HK408 cell lines (Fig. 3D+4H) were pooled; [d] Data for various RT protocols (Fig. 1B+2B+3B) were pooled; [e] data from Figures 2B+3B were pooled; [f] data for A375 and WM3000 cell lines (Fig.1M+1P) were pooled; [g] data for SH-SY5Y and SK-NBE(2) (Fig. 1C+1D) were pooled; [h] data for SH-SY5Y and SK-NBE(2) (Fig. 1C+1D) were pooled; [i] data for SH-SY5Y and SK-NBE(2) (Fig. 1A2+1B2) were pooled; [j] data for male and female mice were pooled; [k] data for C57BL/6 + NSG mice (Fig. 3E+6C) were pooled.

Table 2
Results of the meta-analysis of experiments having used a KD as monotherapy.

Parameter	Uniform prior		Half-normal prior		DuMouchel prior		Combined	
	$\widehat{RMSTR}$	τ	$\widehat{RMSTR}$	τ	$\widehat{RMSTR}$	τ	$\widehat{RMSTR}$	τ
Prior distribution	$\mu \sim N(0,10)$	$\tau \sim U(0,1)$	$\mu \sim N(0,10)$	$\tau \sim HN(0,0.25)$	$\mu \sim N(0,10)$	$P(\tau) = \frac{s_0}{(s_0+\tau)^2}$		
Posterior median	1.161	0.219	1.161	0.216	1.161	0.213	1.161	0.214
Standard deviation	0.041	0.029	0.040	0.028	0.0399	0.028	0.040	0.028
95% HPDI	[1.082,1.243]	[0.168,0.282]	[1.085,1.245]	[0.165,0.277]	[1.086,1.242]	[0.163,0.274]	[1.084,1.243]	[0.165,0.278]
DIC	−62.61		−61.08		−62.3			

The results are for 46 experiments. $\widehat{RMSTR}$ denotes the estimated true factor by which a KD monotherapy prolongs survival in tumor-bearing mice (the restricted mean survival time ratio) and τ the standard deviation between studies.

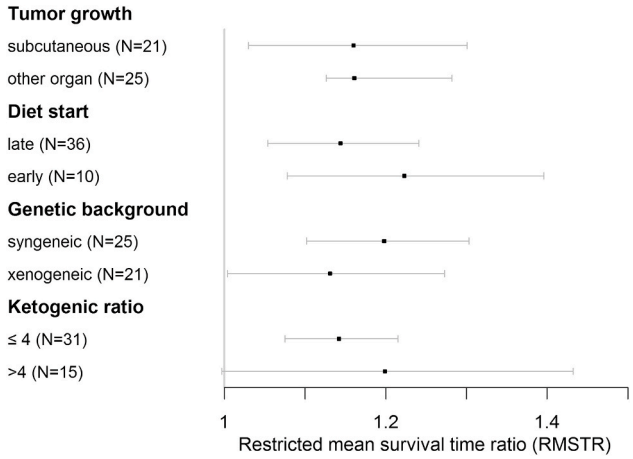

Fig. 1. Subgroup analysis of different experimental variables. Late diet start refers to switching mice to the KD at least one day after tumor initiation.

obtained for cervical and kidney cancer where KD monotherapy or its combination with CT even yielded (weak) evidence for a survival-reducing effect.

The DIC was −26.48. The median posterior estimate for σ which gauges the accuracy of the equal relative potency assumption was 0.203 (95% HPDI = [0.122,0.325]). This indicates that with 68% probability, the $\alpha_i + \gamma_j$ deviated from the true effects θ_{ij} by a factor of 0.82–1.23, so that the equal relative potency assumption appeared to hold approximately.

Assuming a correlation of 0.9 between tumors of the gastrointestinal tract did only marginally improve model fit (DIC = −26.99), but it affected the RMSTR posterior estimates for the correlated tumor entities so that synergistic effects between KD and RT or TT in colon and stomach cancer cell lines became more evident(Supplementary Figure S2).

The results o a sensitivity analysis assuming no correlation between the three standard-of-care treatments (Equation (11)) are shown in [Fig. 4]. The combination with CT was now estimated to reduce survival times compared to KD monotherapy in all tumor entities, while TT and RT exhibited clear synergistic effects. Significant survival-prolonging effects were found in glioma models (KD + RT and KD + TT), HNC (KD + RT), pancreatic cancer (KD monotherapy, KD + RT and KD + TT), and stomach cancer (KD + RT and KD + TT). The DIC of this model was −26.37 and thus slightly larger than the DIC of the model assuming strong correlations. The median posterior estimate for σ was 0.197 (95% HPDI = [0.119,0.324]).

Discussion

Main results

The aim of this study was to assess the evidence for anti-tumor effects of KDs in mice when applied as a monotherapy or combined with standard-of-care treatments (i.e., CT, RT, and TT). The main results can be summarized as follows:

First, there was strong evidence that a KD applied as a monotherapy extended mouse survival by 16–18% across all tumor types. This estimate was robust against excluding studies with small sample sizes <10 and was confirmed in subgroup analysis for colon cancer and glioma models, but not in lung cancer models [Fig. 2]. Restricting the analysis to studies in which a KD was implemented after tumors became detectable (n = 36) still yielded a significant survival-prolonging effect of KD monotherapy ($\widehat{RMSRT}$ = 1.14, 95% HPDI = [1.05,1.24]; Fig. 1). These studies have much higher translational relevance than those

Table 3
Results of meta-regression of experiments having used a KD as monotherapy.

Parameter	$\widehat{RMSTR}$	τ	β_{subcut}	$\beta_{diet.start}$	$\beta_{syngeneic}$
Prior distribution	$\mu \sim \mathrm{N}(0, 10)$	$\tau \sim \mathrm{U}(0, 1)$ $\tau \sim \mathrm{HN}(0, 0.25)$ $P(\tau) = \frac{s_0}{(s_0 + \tau)^2}$	$\beta_{subcut} \sim \mathrm{N}(0, 10)$	$\beta_{diet.start} \sim \mathrm{N}(0, 10)$	$\beta_{syngeneic} \sim \mathrm{N}(0, 10)$
Posterior median	1.192	0.221	0.018	−0.066	0.018
Standard deviation	0.123	0.031	0.080	0.087	0.102
95% HPDI	[0.971,1.451]	[0.168,0.289]	[-0.144,0.173]	[-0.237,0.105]	[-0.191,0.204]

Results are for 46 experiments. $\widehat{RMSTR}$ denotes the estimated true factor by which a KD monotherapy prolongs survival in tumor-bearing rodents (the restricted mean survival time ratio) and τ the standard deviation between studies. The regression coefficients β estimate the effects of subcutaneous versus non-subcutaneous tumors, diet initiation at least one day after versus before tumor implantation/initiation and syngeneic versus xenogeneic tumors.

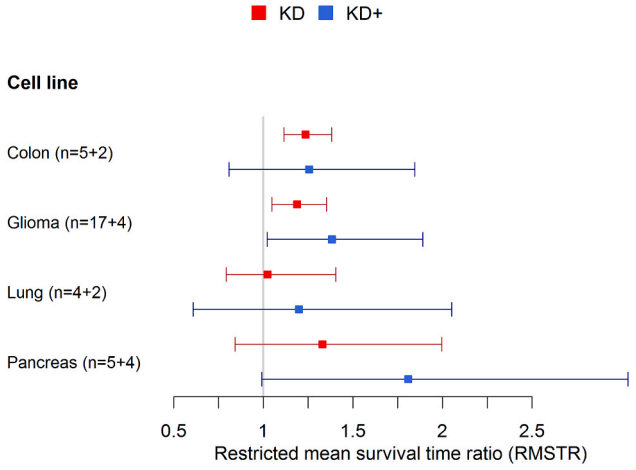

Fig. 2. Subgroup analysis of experiments utilizing glioma, colon cancer, lung cancer and pancreatic cancer models. The numbers in parenthesis are the number of experiments with KD monotherapy and KD combination therapy. Due to small sample sizes, no distinction between additional treatments was made. These were TT for all colon cancer experiments, 3 × TT and 1 × radiotherapy for glioma experiments, 1 × CT and 1 × RT for lung cancer experiments and 2 × CT, 1 × RT and 1 × TT for pancreatic cancer experiments.

which initiated the KD before tumors became detectable, because patients will only adopt a KD after having been diagnosed with cancer. Second, the combination between a KD and CT did not result in an overall synergistic effect, while its combination with RT and TT yielded synergistic effects with a very high (≥97%) probability. Of the 19 studies assessing synergistic effects, 16 had initiated the KD after tumors became detectable, mimicking the clinical setting. Third, a Bayesian evidence synthesis model accounting for differences between tumor entities qualitatively confirmed these findings. Significant anti-tumor effects of a KD combined with RT and TT could be established for glioma, pancreatic, and stomach cancer models [Figs. 3 and 4].

Evidence for synergistic effects

That KDs exert synergistic effects with other treatment modalities is understandable from an ecological concept known as "press-pulse" [77]. Thereby, a KD serves as a constant "press" on tumor cells which makes them more vulnerable when particular treatment "pulses" such as CT, RT, or TT are applied. Mechanistically, it has been shown that a KD downregulates several signalling pathways in tumor cells which are causally connected to the Warburg phenotype, so that glycolysis is

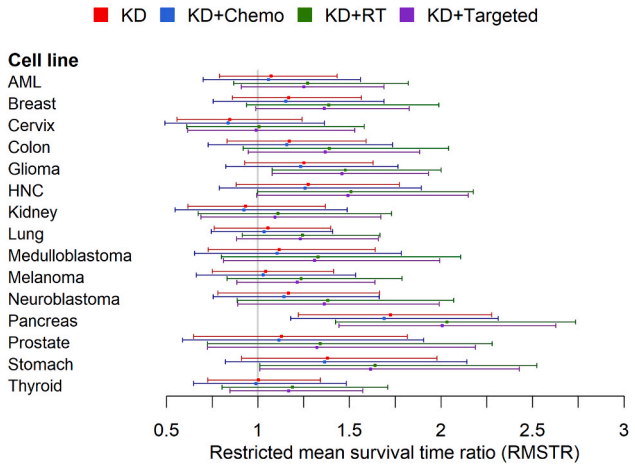

Fig. 3. Results of Bayesian evidence synthesis for assessing the effects of a KD monotherapy or its combination with CT, radiotherapy (RT) and TT in the different tumor entities. The model allowed estimating results also for treatment combinations for which no input data were available. A correlation of 0.95 between the treatment effects of CT, RT, and targeted therapies was assumed.

Table 4
Results of meta-regression for assessing synergistic effects between a KD and standard-of-care therapies.

Parameter	$\widehat{RMSTR}$	τ	β_{KD+CT}	β_{KD+RT}	β_{KD+TT}
Prior distribution	$\mu \sim \mathrm{N}(0, 10)$	$\tau \sim \mathrm{U}(0, 1)$ $\tau \sim \mathrm{HN}(0, 0.25)$ $P(\tau) = \frac{s_0}{(s_0 + \tau)^2}$	$\beta_{KD+CT} \sim \mathrm{N}(0, 10)$	$\beta_{KD+RT} \sim \mathrm{N}(0, 10)$	$\beta_{KD+TT} \sim \mathrm{N}(0, 10)$
Posterior median	1.171	0.237	−0.040	0.298	0.210
Standard deviation	0.044	0.025	0.115	0.146	0.129
95% HPDI	[1.085,1.26]	[0.192,0.292]	[-0.267,0.192]	[0.007,0.584]	[-0.009,0.514]

Results are for 64 experiments. $\widehat{RMSTR}$ denotes the estimated true factor by which a KD monotherapy prolongs survival in tumor-bearing rodents (the restricted mean survival time ratio) and τ the standard deviation between studies. The regression coefficients β estimate the effects of combining a KD with chemotherapy (CT), radiotherapy (RT) and targeted therapy (TT), respectively.

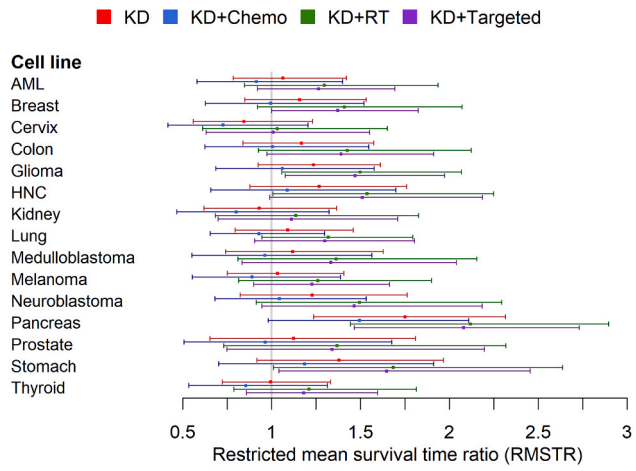

Fig. 4. Same as [Fig. 2] but now without assuming any correlation between the treatment effects of CT, RT and targeted therapies.

reduced. Because tumor cells utilize glycolysis not only for energy production, but also for producing anti-oxidative substrates such as lactate, pyruvate and (via the pentose phosphate pathway) NADPH [78,79], a KD acts as a constant pro-oxidative stressor for these cells. In addition, some tumor cells may be forced to oxidize the ketone body β-hydroxybutyrate which elevates their NADH levels, inhibits glycolysis and leads to the additional production of ROS [21]; this was shown by Yang et al. [40] in a pancreatic cancer model also considered in these meta-analyses. Normal cells in contrast are able to adopt a defensive state with more efficient DNA repair and increased production of glutathione and other anti-oxidants when glucose and insulin levels fall and ketone body levels rise 79.

From these mechanistic insights, it would be predicted that a KD may be an especially potent "press" mechanism when combined with "pulses" exerting oxidative stress. RT produces large amounts of free radicals through biophysical interactions between photons and molecules (mainly water). Monoclonal antibodies such as CTLA-4 or PD-1 inhibitors which have been utilized in several of the experiments included in this meta-analysis have also been shown to induce large amounts of ROS in tumor cells [80]. This would explain the large synergistic effects when combining a KD with RT and TT [Table 4]. However, there was no evidence for a general synergistic effect of a KD with CT, although some individual studies had evidence for synergism; these were Aminzadeh-Gohari et al. who combined a KD with the alkylating agent cyclophosphamide in a neuroblastoma model [51] and Yang et al. who used Paclitaxel + Gemcitabine + Cisplatin in a pancreatic cancer model [40]. The other studies yielded smaller effect sizes and larger uncertainties so that, overall, no synergistic effect of a KD with CT resulted. It is tempting to speculate that a KD may not necessarily synergize with CT if the mechanism of action of the drug is not dependent on free radical generation. While some chemotherapeutic drugs act directly via increasing free radicals in tumor cells (e.g., anthracyclines, alkylating agents and platinum coordination complexes), others do so only indirectly to a much lesser extent and do not depend on free radical formation for their anti-tumor activity; for example, antifolates and nucleoside and nucleotide analogues impact DNA synthesis, the vinca alkaloids and taxanes interfere with microtubule function, the epipodophyllotoxins interfere with topoisomerase II activity, and the camptothecins (topotecan, irinotecan) interfere with topoisomerase I activity [81]. Hence, the possibility of synergism between a KD and different chemotherapeutics needs to be studied more thoroughly in future experiments.

Comparison with previous meta-analyses

A comparison of the pooled effect of KD monotherapy which is based on 46 experiments (RMST = 1.161, [Table 2]) with the estimate of an earlier meta-analysis by Klement et al. [16] that was based on 13 experiments (RMSTR = 1.176) shows excellent agreement.

The evidence that a KD synergizes with RT and TT found in this meta-analysis is extremely relevant from a translational point of view because most cancer patients will undergo one or the other (or both) of these therapies. In this case, the treatment period may be an especially important period for using a KD as a complementary, adjuvant therapy [18,19]. Previous meta-analyses of mouse studies were conducted in a time when too few experiments studying the synergism between a KD and other therapies had been published [15–17]. The most recent meta-analysis by Li et al. from 2021 [17] included only 12 experiments with survival times as the outcome of interest and included experiments in which the KD was administered as a calorically restricted diet. In contrast, my meta-analysis has included survival outcomes from a total of 65 experiments which uniformly supplied a KD in unrestricted amounts. Application of restricted KDs to mice has mostly been studied for central nervous system tumors where it was found to be more effective against tumor growth than feeding an unrestricted KD [57,63,68]. The group of Thomas Seyfried and Purna Mukherjee has shown that calorie-restricted KDs are highly effective in slowing down the growth of high-grade gliomas in mice, especially when combined with additional metabolic treatments such as 2-deoxy-D-glucose [82] or 6-diazo-5-oxo-L-norleucine (DON) which antagonizes glutamine, the second fermentable fuel used by cancer cells besides glucose [83]. The superiority of unrestricted KDs for slowing tumor growth in mice relates to the fact that the additional energy intake reduction is a potent stimulus for lowering glucose, insulin, and IGF-1 levels and enhancing ketogenesis [84]. Furthermore, in some mouse strains, feeding a KD in unrestricted amounts may induce hyperglycemia and hyperlipidemia [85], which is, however, contrary to what is observed in cancer patients [11]. Meidenbauer et al. [85] also pointed out the possibility that mice tend to self-restrict their energy intake when switched to an unfavourable KD despite *ad libitum* feeding. In humans, KDs frequently suppress appetite or hunger [86] so that many patients also involuntarily self-restrict their energy intake; in this case, however, ketosis and sufficient protein intake may help to preserve muscle mass [87,88].

Study limitations

In contrast to previous meta-analysis, this study was able to estimate effects for different tumor types separately [Figs. 3 and 4]. It has now become clear that different tumors may respond differently to KD therapy, partly depending on their expression of ketolytic enzymes and genetic mutations [50,89]. I was not able to take into account that even within the same organ, there may be large genetic heterogeneity between tumor cells. The results of the Bayesian meta-regression model could reflect this since uncertainties were quite large for all estimates, so the results should be considered in light of this limitation. Sensitivity analysis showed that the model was not very sensitive towards different prior assumptions. As more data become available, the possibility exists to extend this model to individual tumor cell lines and eventually derive more precise estimates.

As is always the case with meta-analytical approaches, the results crucially depend on the quality of the included studies and the underlying model assumptions. Many studies had only small sample sizes, which is understandable from an ethical point of view, but increases the uncertainty of input data. Furthermore, many individual survival times had to be read off the Kaplan-Meier plots because the summary statistics (mean survival time with uncertainty estimate) were not given in the publication. Most often, only p-values for survival curve comparison were reported. This limits utilization of the full information produced in animal experiments for meta-analytical research; unfortunately, such

poor reporting of results aligns with the frequent poor reporting of methodology of mouse studies, even in highly ranked journals [90]. Nevertheless, the endpoints of all experiments were sufficiently described and I was able to overcome this limitation by retrieving most individual mouse survival times from the study authors or using software to extract them from Kaplan-Meier plots.

A limitation in estimating synergistic effects between a KD and other therapies was that the number of corresponding experiments is still small. Finally, the site of tumor cell injection, initiation time of the KD and species-specific background of the tumors were not found to be able to explain the heterogeneity between individual study outcomes. I was not able to take into account other cell-line specific factors, such as metabolic enzyme expression which can co-determine the effects of KD treatment [50].

Conclusions

Based on 1755 individual survival times of tumor-bearing mice, the results of this analytical study imply that a KD exerts an overall anti-tumor effect in mice when applied as a monotherapy, but much more potently when combined with RT or TT. Future studies should particularly focus on the possibility of synergism with standard-of-care as well as other treatments, of which hyperbaric oxygen therapy and hyperthermia are promising candidates [59,91].

Declarations

Ethics approval and consent to participate

Since this is an analysis of already published studies, no ethical approval was needed.

Consent for publication

Not applicable.

Availability of data and materials

All data used in this analysis can be found in the Tables provided herein and the original studies from which the data were extracted.

Conflicts of interest

The author occasionally follows a KD. No other potential conflicts of interest related to this work exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2023.100609.

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