Reviewer's report

Title: Ketogenic Diets as an Adjuvant Therapy in Glioblastoma (The KEATING Trial): Study Protocol for a Randomised Pilot Study

Version: 0 Date: 05 Jun 2017

Reviewer: Thomas Seyfried

Reviewer's report:

The authors describe a randomized pilot protocol to treat newly diagnosed GBM patients with two different ketogenic diets: the modified ketogenic diet (MKD) and the medium chain triglyceride ketogenic diet (MCT). The goal of this study is to inform the feasibility, methodological design, and power calculations of future phase III clinical trials investigating the effectiveness of KD as an adjuvant therapy in the management of GBM. The protocol, as presently designed, is not likely to provide much new information over what has already been observed in previous studies that have used the KD for GBM management. There are a number of issues that should be considered to improve their study design.

Major Comments

1. The authors state that the rationale for treating GBM patients with KD is due to an increasing clinical interest in ketogenic diet (KD) therapy, and to a growing popularity of alternative treatment options for patients with GBM. No mention is made for a scientific rationale of using the KD for GBM management. The protocol could be strengthened with inclusion of a scientific rational for using the KD as a therapy for GBM management. The authors are advised to consider the information in a recent review on the subject (Klement, bioRxiv; doi: http://dx.doi.org/10.1101/137950).

2. The use of two different ketogenic diets is unnecessary at this time. If the more restrictive MCT diet is not effective, it is unlikely that the MKD diet would be effective. It would therefore be best to focus on the MCT diet before initiating further studies with the MKD.

3. The absence of a stand-alone KD group is the most serious flaw with the protocol design. A randomized study, by necessity, would require a stand-alone KD group. The absence of this group significantly weakens the proposed study and will complicate data interpretation. How will the authors separate the possible adverse effects of the KD (lines 372-374) from the known adverse effects of radiation, TMZ, and steroids? Radiotherapy increases glutamine and glutamate in the tumor microenvironment that will enhance GBM growth (Dahlberg et al., 80:917-924, 2017; Neurosurgery 2017; Seyfried et al., Cancer letters 2015, 356:289-300; Takano et al., Nat Med,7:1010-1015, 2001); whereas TMZ is known to increase GBM driver...
mutations (Johnson et al., Science 343:61-67, 2014). The use of steroid hormone will increase blood glucose thus potentially minimizing a beneficial effect of any KD therapy (Klement, Brain 140:e16, 2017). The confounding effects of these interactions should be discussed in the proposal. The authors must therefore address the reasons for not including a stand alone KD group considering the powerful anecdotal reports suggesting that therapeutic efficacy of the KD alone could be better than the efficacy of the current standard of care (http://www.dailymail.co.uk/health/article-3691808/Quitting-carbs-saved-life-Cancer-victim-given-months-live-refuses-chemo-claims-diet-meat-dairy-s-alive-two-years-later.html); (https://zerocarbzen.com/2015/05/31/healing-brain-cancer-with-a-zero-carb-ketogenic-diet-by-andrew-scarborough/). There are also indications that chemotherapy can interfere with the therapeutic action of ketogenic diets (Toth & Clemens. American J. of Med. Case Reports, 4:288-292, 2016). Considering the provocative effects of the standard of care on GBM energy metabolism (Winter et al., Critical Rev. Oncol/Hematol., 112:41-48, 2017), the authors could recruit several patients that might choose a stand alone KD if they understand that the SOC for GBM is only palliative and not curative, and that a stand alone KD could offer a better outcome based on anecdotal evidence. These issues should be clearly explained to all recruited patients regardless of the group that they are randomized to. In light of this information, the authors must address their reasoning for not including a stand-alone KD group.

4. There is growing evidence that the therapeutic benefit of ketogenic metabolic therapy could be enhanced if implemented prior to surgical resection. Surgical resection creates inflammation in the tumor microenvironment that facilitates tumor cell dissemination. The KD could reduce this effect if implemented at the time of diagnosis and prior to surgical resection in some select patients where a watchful waiting period is possible. It is important to recognize that most competent neurosurgeons can diagnose with a greater than 90% accuracy whether a patient would have high-grade glioma using non-invasive imaging procedures. The authors should also address in the protocol design their reasoning for not implementing the KD prior to surgical resection. There is also considerable flexibility as to when radiotherapy can be implemented following surgical resection. The authors should consider how patients might benefit most from the planned procedures. The authors should consider which of the proposed procedures would have the greatest therapeutic benefit while producing and the least toxicity to the patients.

5. The authors mention on line 165 that all patients will commence the diet at home, without a fasting start. It is well documented that calorie restriction or water-only fasting is a key reason for the therapeutic benefit of the KD against GBM (please read carefully the Klement review mentioned in comment 1. CR or fasting will target inflammation and angiogenesis, which drive GBM growth. To exclude fasting or calorie restriction is a major weakness in the proposal and will likely produce results that will be similar to those seen previously in the Rieger et al study (ERGO: a pilot study of ketogenic diet in recurrent glioblastoma,
In other words, the proposed study, as designed, will likely be a waste of time and resources. All recruited patients should know that the therapeutic action of KD therapy for brain cancer management is best under calorie restriction or water-only fasting. It should be recognized that ketogenic diets are naturally weight loss diets and thus some patients inadvertently restrict caloric intake. It is therefore essential that blood glucose and ketone levels be monitored on a regular basis. It is best to measure fasting blood glucose daily and blood ketones every day for the first two weeks then at least every few days with urine ketone measurements taken concurrently, as measures of urine ketones do not generally provide an accurate measure of blood ketones. This issue was discussed in the study from the Zucconi group (Nutrition & Metabolism 7:33, 2010). This is important, as there are suggestions that the success of KD for glioblastoma management is based on the ratio of blood glucose to ketones. It would therefore be important to collect data using the most accurate methods considering the enormous amount of time and resources that will be invested in the study. The authors mention these issues to some extent on lines 358-361. They suggest, however, that blood monitoring of ketone bodies is more invasive and expensive than urine monitoring. This is a weak argument, as glucose is also monitored from blood and similar procedures are used by millions of children and adults with diabetes. The expense excuse is also weak. How does the expense of blood ketone sticks compare to the expense of radiotherapy, TMZ, or with some of the other procedures that will be used? This should be justified in the protocol.

6. Exclusion criteria on line 123 is confusing. Exclusion criteria should also include those with a prior history of bariatric surgery. And what about valproic acid? This interferes with carnitine synthesis and may have an independent anti-tumor effect. What about thyroid disease requiring thyroid hormone?

7. The authors mention on line 210 that patients will be imaged using T1+ gadolinium. It will be important to recognize possible adverse effects from this procedure. Furthermore it will be important to document the influence of any procedure on blood glucose and ketone levels in the patients, as some procedures can elevate corticosteroid levels and blood glucose. Consequently, blood glucose and blood ketone levels should be monitored after the implementation of any invasive or uncomfortable procedure. No data should be used in analysis if a patient experiences any GI symptoms (nausea, vomiting, diarrhea) concurrent with testing. These conditions will ALL raise blood glucose levels.

8. The biochemical markers mentioned on line 287 should also include triglycerides, HDL, LDL, C-reactive protein, insulin, and thyroid hormone.

Level of interest
Please indicate how interesting you found the manuscript:

An article of importance in its field
Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organisation that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

'I declare that I have no competing interests'

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.