

Biomarkers in glioblastoma multiforme: status quo.

Nicola Montano, Quintino Giorgio D'Alessandris, Alessandro Izzo, Eduardo Fernandez, Roberto Pallini

Corresponding author:

Nicola Montano, Department Institute of Neurosurgery, Catholic University, Largo Agostino Gemelli, 800168 Rome, Italy

Handling editor:

Michal Heger

Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, the Netherlands

Review timeline:

Received: 26 February, 2016

Editorial decision: 15 March, 2016

Revision received: 26 March, 2016

Editorial decision: 26 March, 2016

Published online: 27 March, 2016

1st editorial decision:

Date: 15-Mar-2016

Ref.: Ms. No. JCTRes-D-16-00008

Biomarkers in Glioblastoma Multiforme. Evidences from a literature review

Journal of Clinical and Translational Research

Dear Dr. Montano,

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at <http://www.jctres.com/en/author-guidelines/> under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

I would kindly ask you to put some emphasis on the use of liquid biopsies (point 6 of reviewer 1) for the enumeration of biomarkers in the revision. These could be based on DNA or RNA sequencing, whereby the samples are derived from plasma or blood cells (e.g., platelets, see for example Cancer Cell. 2015 Nov 9;28(5):666-76).

Your revision is due by Mar 29, 2016.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

*****Reviewers' comments*****

Reviewer #1: General:

The authors describe in this literature review the potential role of the molecular markers EGFR, EGFRvIII, PTEN (and other aberrations in the RTK pathways), and VEGF expression as prognostic and/or predictive biomarkers in glioblastoma (GBM). The literature search was performed using pre-defined search terms in pubmed, and was summarized in several tables and the main text of the manuscript. The authors conclude that, so far, these biomarkers cannot be considered as prognostic or predictive biomarkers, as insufficient evidence is available yet. Before publication of this review, the authors should provide more insight into their reasoning and thoughts, provide more details to further broaden the scope of the review, and answer the following issues/questions:

Questions/issues:

- 1) Abstract: the abstract has to be supplemented by a short sentence stating the conclusion of the manuscript, as the abstract remains 'high-level' and more introduction-like.
- 2) What is key for a successful biomarker? Can the authors comment on that question in the introduction?
- 3) Final paragraph of the introduction: Here the authors propose several biomarkers that are in their view eligible for their literature search. However, for the reader the strength of these markers as potential targets for treatment remains unclear. In the past years, small- and larger-scale studies have assessed these markers as targets for treatment, but no definite answer about their efficacy is ready. Moreover, it seems that these drug targets will ultimately fail for the treatment of GBM. Can the authors comment on this and provide in the main text the strength of evidence that these drugs have potential for clinical use?
- 4) Methods: Indicate that date at which the search was performed. Also were, the search terms 'predictive', 'prognostic', or 'biomarkers' used in their search? Can the authors confirm that their search captured the complete published field (often for a literature search two databases are searched in parallel).
- 5) The Results-section starts somewhat confusing to me, as now also the predictive value of

these biomarkers is described. This biomarker value has to be more consistently mentioned in the abstract and introduction. Also, the authors have to specify for which specific, if applicable, RTK the biomarker is predictive. Finally, the results-section can be improved by providing percentages of total identified studies for each result in the main text.

6) The conclusion to which the authors reach is of course somewhat disappointing. The authors raise for each biomarker reasonable issues that can explain their conclusion. To further stimulate this field of research; can the authors provide here some examples of studies that have to be conducted, or suggestions to improve current clinical trials? How can continuous assessment of molecular markers be integrated in daily clinical practice? What could be the value of liquid biopsies? Which techniques / detection methods are the best to choose? And how can intratumor heterogeneity be overcome in this case?

Authors' rebuttal:

Date: 26-Mar-2016

Nicola Montano M.D., PhD.
Institute of Neurosurgery, Catholic University
Largo Agostino Gemelli, 8
00168 Rome, Italy
tel.: +39 0630154120,+39 0630154358;
fax: +39 063051343;
e-mail: nicolamontanomd@yahoo.it

Roma, 25 March 2016

Re: revision Ms. No. JCTRes-D-16-00008

Dear Dr. Heger

Thank you for giving us an opportunity to resubmit a revised version of our manuscript entitled "Biomarkers in Glioblastoma Multiforme. Evidences from a literature review"

We have addressed all comments of the reviewers using the track changes function in Word (attached as supplementary material not for publication). Moreover, every modification or rebuttal of the reviewer's comments is detailed per comment below in red italics.

We are grateful for the useful comments of the reviewers, as a result of which the paper has been considerably improved.

On behalf of the authors, kindest regards,

Nicola Montano

Reviewers' comments

Reviewer #1:

General:

The authors describe in this literature review the potential role of the molecular markers EGFR, EGFRvIII, PTEN (and other aberrations in the RTK pathways), and VEGF expression as prognostic and/or predictive biomarkers in glioblastoma (GBM). The literature search was performed using pre-defined search terms in pubmed, and was summarized in several tables and the main text of the manuscript. The authors conclude that, so far, these biomarkers cannot be considered as prognostic or predictive biomarkers, as insufficient evidence is available yet. Before publication of this review, the authors should provide more insight into their reasoning and thoughts, provide more details to further broaden the scope of the review, and answer the following issues/questions:

We are grateful for your commentary and suggestions, which we have addressed to the fullest extent as indicated below for every one of your comments. The abstract and the manuscript have been thoroughly reviewed in order to provide insights into the reasoning and broadening the scope of the review.

Questions/issues:

1) Abstract: the abstract has to be supplemented by a short sentence stating the conclusion of the manuscript, as the abstract remains 'high-level' and more introduction-like.

We have changed the “Relevance for patients” section of the abstract, adding the following sentence: “Unfortunately, current evidence is insufficient to draw a definite prognostic/predictive role for these biomarkers in GBM”

2) What is key for a successful biomarker? Can the authors comment on that question in the introduction?

We have changed the “Introduction” section. The following text has been added: “The ideal biomarker in GBM should be easy detectable by routine pathological techniques and highly reproducible among different laboratories and observers. Immunohistochemistry is a reliable, quick, no expensive and widely available technique, but sometimes the more accurate semiquantitative RT-PCR should be preferred. Moreover, an ideal biomarker should clearly identify patients with longer or shorter survival (prognostic role) and/or patients that can benefit

from a particular treatment (predictive role).”

3) Final paragraph of the introduction: Here the authors propose several biomarkers that are in their view eligible for their literature search. However, for the reader the strength of these markers as potential targets for treatment remains unclear. In the past years, small- and larger-scale studies have assessed these markers as targets for treatment, but no definite answer about their efficacy is ready. Moreover, it seems that these drug targets will ultimately fail for the treatment of GBM. Can the authors comment on this and provide in the main text the strength of evidence that these drugs have potential for clinical use?

In the Authors’ belief, these biomarkers deserve further analysis because they are the “target” of the most widely studied “targeted therapies”, namely bevacizumab, TKIs and rapalogs. This is stated in the Introduction section. Moreover, it is the Authors’ belief that the failure of clinical trials using these drugs should be a stimulus to the search of predictive biomarkers for response to targeted therapies. We have tried to clarify and reinforce these statements, also providing the state-of-art of clinical use of these targeted therapies, by adding the following paragraph to the Introduction: “There has been much enthusiasm in the past years regarding these “targeted therapies”, but results of clinical trials have been somewhat disappointing. Currently, bevacizumab remains a therapeutic option in recurrent GBM in the USA, whereas erlotinib and rapalogs are experimental drugs whose effectiveness has not been confirmed in unselected cohorts of patients. These evidences foster the search for biomarkers that can identify subgroups of patients who can benefit from targeted therapies.”

4) Methods: Indicate that date at which the search was performed. Also were, the search terms 'predictive', 'prognostic', or 'biomarkers' used in their search? Can the authors confirm that their search captured the complete published field (often for a literature search two databases are searched in parallel).

As suggested by the Reviewer, the date of the search was added in the “Search Strategy” section. The terms “predictive”, “prognostic” and “biomarkers” were not used. We decided to perform the search only in the PubMed database for quality reason; however, we acknowledge that this is a limitation of the work.

5) The Results-section starts somewhat confusing to me, as now also the predictive value of these biomarkers is described. This biomarker value has to be more consistently mentioned in the abstract and introduction. Also, the authors have to specify for which specific, if applicable, RTK the biomarker predictive is. Finally, the results-section can be improved by providing percentages of total identified studies for each results in the main text.

The inclusion in the paper of studies analyzing both the prognostic and the predictive role of biomarkers has been specified in the Abstract, sections “Aim” and “Relevance for patients”, and in the Introduction. As concerns details on RTK members and TKIs, they have been provided in Tables 1 and 2. According to Reviewer’s suggestion, percentages of studies have been included in the main text.

6) The conclusion to which the authors reach is of course somewhat disappointing. The authors raise for each biomarker reasonable issues that can explain their conclusion. To further stimulate this field of research; can the authors provide here some examples of studies that have to be conducted, or suggestions to improve current clinical trials? How can continuous assessment of molecular markers be integrated in daily clinical practice? What could be the value of liquid biopsies? Which techniques / detection methods are the best to choose? And how can intratumor heterogeneity be overcome in this case?

We have tried to stimulate this field of research and answer to the reviewer’s questions in the Discussion section. We have added the following paragraph: “In order to improve biomarkers detection and validation, current and future clinical trials need to prospectively assess their potential role. Several subgroups should be designed, with the aim of tailoring the treatment on patient’s molecular profile. This claims for large, collaborative, multicenter trials, able to reach adequate recruitment targets. In order to minimize biomarkers determination errors, a few pathology “reference” laboratories for each country should be identified that can validate the results from periphery. The problem of the intratumor heterogeneity can be solved by analyzing several tumor samples from different regions. When applicable, analysis of cancer stem cells profile can provide a reliable picture of the tumor’s landscape [60]. A new and intriguing opportunity for studying the genomic and/or proteomic profile of GBM for prognostic or predictive purposes is provided by the so-called “liquid biopsies”, i.e. the analysis of peripheral blood samples. The main target of these biopsies are the circulating tumor cells, which can be analyzed using standard immunocytochemical or molecular biology techniques. Liquid biopsies are currently used for prognostic/predictive purposes in several tumors. In GBM, the difficulty to identify affordable biomarkers to separate circulating tumor cells from normal blood cells [61] has hindered the development of this technique. Alternatives to circulating tumor cells, to be analyzed in a liquid biopsy, include RNA sequencing of “tumor-educated platelets” [62] and genomic profiling of microvesicles [63].”. Four references have been added.

2nd editorial decision:

Date: 26-Mar-2016

Ref.: Ms. No. JCTRes-D-16-00008R1

Biomarkers in Glioblastoma Multiforme. Evidences from a literature review

Journal of Clinical and Translational Research

Dear Dr. Montano,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Comments from the editor and reviewers can be found below.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger

Editor-in-Chief

Journal of Clinical and Translational Research