PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (FLIMMA STUDY)</th>
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<tbody>
<tr>
<td>AUTHORS</td>
<td>Fink, Christine; Hofmann, Maja; Jagoda, Agata; Spaenkuch, Iris; Forschner, Andrea; Tampouri, Ioanna; Lomberg, Diana; Leupold, Dieter; Garbe, Claus; Haenssle, Holger</td>
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VERSION 1 - REVIEW

| REVIEWER | Toshiaki Saida, MD, PhD  
Shinshu University, Japan |
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<td>REVIEW RETURNED</td>
<td>11-Jun-2016</td>
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GENERAL COMMENTS

This is a well-written paper of a new diagnostic method of melanoma. The proposed project seems to be rational and worthy to be performed.

| REVIEWER | Mike Sathekge  
University of Pretoria  
South Africa |
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<td>REVIEW RETURNED</td>
<td>17-Jun-2016</td>
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GENERAL COMMENTS

I found the study protocol very well written and comprehensive. See below minor comments as follows:
1. An apparent contradiction with regards to funding. Whereas investigators wrote earlier under the strength and limitations of the study that they received funding only from the university and not from any private company, in their footnote, they said they received funding from a commercial sector. I guess the latter statement is an error. If however they did receive funding from a commercial enterprise, they must state the name of such company.
2. My other comments are on Declaration and ethics:
   - Will investigators inform the study participants of the results from the skin testing?
   - Investigator should provide study participants with a communication avenue such as a phone number that they can call if they ever have questions regarding the study later.
   - Investigators should include safe keep of collected data as well as duration of storage the same.
Reviewer 2:

1. „An apparent contradiction with regards to funding. Whereas investigators wrote earlier under the strength and limitations of the study that they received funding only from the university and not from any private company, in their footnote, they said they received funding from a commercial sector. I guess the latter statement is an error. If however they did receive funding from a commercial enterprise, they must state the name of such company.“

The latter statement regarding the funding was not correct and therefore revised: “5.1 Funding statement: The University of Tuebingen acts as the sponsor of the study. This research received no grant from a commercial sector.”

2. „Will investigators inform the study participants of the results from the skin testing?“

As this device is still under study and to avoid interference with following procedures (i.e. excision of the lesion) the participants should not be informed about the scoring results.

3. "Investigator should provide study participants with a communication avenue such as a phone number that they can call if they ever have questions regarding the study later.“

Ethics approval was provided by the ethic committee of the medical faculty of the University of Tuebingen, Heidelberg and Berlin, which demand exceptionally high standards. This includes detailed contact information for all participants provided on the information sheet and consent form.”

4. “Investigators should include safe keep of collected data as well as duration of storage.”

As requested, we added the following statement: “All records relating to this study are stored in an external archive and must be retained for at least ten years after completion of the research.” Changes in the text are shown using yellow highlight for additions, and strikethrough font/yellow highlights for deletions

**VERSION 2 – REVIEW**

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Mike Sathekge</th>
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<tr>
<td>University</td>
<td>University of Pretoria</td>
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| REVIEW RETURNED | 18-Aug-2016 |

| GENERAL COMMENTS | Under statistical analysis the authors state that “It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35%.” Perhaps justification of this statement could be made clearer under the introduction section. |

**VERSION 2 – AUTHOR RESPONSE**

1. Comments from Reviewer 2

1.1 “Please state any competing interests: None”
In the section 5 “Footnotes”, subsection 5.3 “Conflicts of interest” the authors declared that they have no competing interests.

2.2 “Under statistical analysis the authors state that “It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35%.” Perhaps justification of this statement could be made clearer under the introduction section.”

The sensitivity and specificity stated in the section “2.7 Statistical considerations” is based on the preclinical data available. As described in the introduction section the new diagnostic method showed a sensitivity of melanoma detection of 93.5% and a specificity of 80.0%. In a second study a sensitivity of melanoma detection of 82.5% and a specificity of 72.5% was detected. We completed the relevant sentence in the methods section as follows: “It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35% based on the available preclinical data described in more detail in the introduction section.”

2. Additional modification:

I have to apologize, as we had to make an additional modification in the reference section. By mistake, we included a wrong reference number 18. The correct reference was inserted instead (“Leupold D, Scholz M, Stankovic G et al. The stepwise two-photon excited melanin fluorescence is a unique diagnostic tool for the detection of malignant transformation in melanocytes. Pigment Cell Melanoma Res. 2011 Jun;24(3):438-45.”) Thank you very much for your effort.
Study protocol for a prospective, non-controlled, multicentre clinical study to evaluate the diagnostic accuracy of a stepwise two-photon excited melanin fluorescence in pigmented lesions suspicious for melanoma (FLIMMA study)

Christine Fink, Maja Hofmann, Agata Jagoda, Iris Spaenkuch, Andrea Forschner, Ioanna Tampouri, Diana Lomberg, Dieter Leupold, Claus Garbe and Holger A Haenssle

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