Author’s response to reviews

Title: Serial enumeration of circulating tumor cells predicts treatment response and prognosis in metastatic breast cancer: a prospective study in 393 patients

Authors:

Markus Wallwiener (markus.wallwiener@googlemail.com)
Sabine Riethdorf (s.riethdorf@uke.uni-hamburg.de)
Andreas D Hartkopf (andreas.hartkopf@med.uni-tuebingen.de)
Caroline Modugno (caroline.modugno@med.uni-heidelberg.de)
Juliane Nees (juliane.nees@t-online.de)
Dharaniya Madhavan (d.madhavan@dkfz-heidelberg.de)
Martin R Sprick (martin.sprick@hi-stem.de)
Sarah Schott (sarah.schott@med.uni-heidelberg.de)
Christoph Domschke (christoph.domschke@med.uni-heidelberg.de)
Irène Baccelli (i.baccelli@dkfz.de)
Birgitt Schönfisch (birgitt.schoenfisch@uni-tuebingen.de)
Barbara Burwinkel (barbara.burwinkel@med.uni-heidelberg.de)
Frederik Marmé (frederik.marme@med.uni-heidelberg.de)
Jörg Heil (joerg.heil@med.uni-heidelberg.de)
Christof Sohn (christof.sohn@med.uni-heidelberg.de)
Klaus Pantel (pantel@uke.de)
Andreas Trumpp (a.trumpp@dkfz.de)
Andreas Schneeweiss (andreas.schneeweiss@med.uni-heidelberg.de)

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Author's response to reviews: see over
To

Dafne Solera, PhD
Executive Editor *BMC Cancer*
c/o *BMC Cancer*

March 3rd, 2014

**Manuscript Submission to BMC Cancer:**
**Wallwiener et al. Serial enumeration of circulating tumor cells predicts treatment response and prognosis in metastatic breast cancer: a prospective study in 393 patients**

Dear Dr Solera

On behalf of my co-authors, I am submitting our manuscript for consideration for publication in *BMC Cancer*.

The manuscript reports the results from a large prospective study of 393 patients with metastatic breast cancer (MBC) starting a new line of systemic therapy. The study was jointly conducted by the National Center for Tumor Diseases, Heidelberg, Germany, and the Department of Obstetrics and Gynecology of the University of Heidelberg in close collaboration with the German Cancer Research Center (DKFZ) between March 2010 and December 2013.

We determined circulating tumor cell (CTC) status (considered positive if counts were \( \geq 5 \) CTCs/7.5 ml peripheral blood) at baseline (CTC\textsubscript{BL}) and after patients completed the first cycle of a new line of hormonal therapy or chemotherapy (CTC\textsubscript{1C}) as well as the changes in CTC status from CTC\textsubscript{BL} to CTC\textsubscript{1C} (CTC kinetics, CTC\textsubscript{KIN}) to assess the utility of serial CTC enumeration in predicting response, progression-free survival (PFS), and overall survival (OS).

In our study, investigators and technical staff performing or reviewing the CTC studies were blinded to patient history and treatment. Treatment regimens did not depend on CTC status, which was unknown to the treating physicians and patients. The study was not a clinical trial with prospective assignment of patients to interventions and therefore did not require registration in a public trial registry.

Our findings included significant reductions in median PFS and OS of CTC\textsubscript{BL}-positive vs. CTC\textsubscript{BL}-negative and CTC\textsubscript{1C}-positive vs. CTC\textsubscript{1C}-negative MBC patients. Unfavorable CTC\textsubscript{KIN} (persistent positive status or negative-to-positive transition) was significantly associated with progressive disease. Multivariate Cox regression analysis revealed that CTC\textsubscript{BL}-positive status,
persistent CTCs after one cycle, third- or higher-line therapy, and triple-negative receptor status were prognostic factors for shorter PFS. Similarly, CTC_{BL}-positive status, persistent CTCs after one cycle, bone and visceral/local metastases, and triple-negative receptor status were prognostic factors for shorter OS. Our results led us to conclude that CTC_{BL}, CTC_{1C}, and CTC_{KIN} are predictive of outcome in MBC and that serial CTC enumeration is useful in tailoring systemic treatment of MBC.

We expect our manuscript to be of great interest to breast cancer specialists worldwide, especially medical oncologists and gynecologists involved in the treatment of breast cancer patients with primary and metastatic disease as well as other clinicians and researchers with a special interest in the cellular and molecular aspects of the disease and its progression. We are confident that the results we report will also attract a wider international readership, including cellular and molecular biologists working in cancer research.

We confirm that the manuscript we are submitting has not been published elsewhere and is not currently under consideration for publication elsewhere.

Below please find a list of five experts we wish to suggest as potential reviewers.

Please do not hesitate to contact me with any queries you may have. My colleagues and I greatly look forward to your reply.

Yours sincerely,

Markus Wallwiener, MD
Suggested reviewers:

**Prof. Wolfgang Janni, MD**
Dept. of Obstetrics & Gynecology  
University of Ulm  
Prittwitzstr. 43  
D-89075 Ulm, Germany  
Tel: +49 731 500-58501  
Fax: +49 731 500-58502  
Email: wolfgang.janni@uniklinik-ulm.de

**Prof. Tanja Fehm, MD**
Dept. of Obstetrics & Gynecology  
University of Düsseldorf  
Moorenstr. 5  
40225 Düsseldorf  
direktion.frauenklinik(at)med.uni-duesseldorf.de  
Tel.: +49 211 81-17501  
Fax: +49 211 81-18483  
Email: tanja.fehm@med.uni-duesseldorf.de

**Prof. Jens Huober, MD**
Dept. of Obstetrics & Gynecology  
University of Ulm  
Prittwitzstr. 43  
D-89075 Ulm, Germany  
Tel: +49 731 500-58688  
Fax: +49 731 500-58502  
Email: jens.huober@uniklinik-ulm.de

**Peter A. Fasching, MD**
Dept. of Obstetrics & Gynecology  
University of Erlangen-Nuremberg  
Universitätsstr. 21–23  
91054 Erlangen, Germany  
Tel: +49 9131 85-33553  
Email: peter.fasching@uk-erlangen.de

**Prof. Volkmar Müller, MD**
Department of Gynecology  
University Medical Center Hamburg-Eppendorf  
Martinistraße 52  
D - 20246 Hamburg  
Tel.: +49 40 7410-23800  
Fax: +49 40 7410-54355  
E-Mail: vmueller@uke.de