Author's response to reviews

Title: The monoclonal antibody SM5-1 recognizes a fibronectin variant which is widely expressed in melanoma

Authors:

Uwe Trefzer (uwe.trefzer@charite.de)  
Yingwen Chen (Yingwen@lycos.com)  
Gunda Herberth (gunda.herberth@ufz.de)  
Felix Kiecker (felix.kiecker@charite.de)  
Maja A Hofmann (maja.hofmann@charite.de)  
Yajun Guo (yguo@unmc.edu)  
Wolfram Sterry (wolfram.sterry@charite.de)

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Author's response to reviews:

Response to reviewer 1:

1. The authors should describe how they used SOLR TM cells for Antibody Screening" on page 6 to 7.

Response: SOLR TM is of course not part of the antibody screening, but rather part of the in vivo excision. This has been clarified in the material and methods part.

2. The result of "FACS analyses of melanoma cell lines with mAb SM5-1 and fibronectin antibodies" on page 13 is unnecessary because it is already summarized on pp. 10-11.

Response: The result of "FACS analyses of melanoma cell lines with mAb SM5-1 and fibronectin antibodies" has been omitted and information on the reactivity of the fibronectin antibodiesTV-1 and FN15 was incorporated in the result of "mAb SM 5-1 is reactive with SMMUpos cells and other melanoma cell lines, but not with SMMUneg cells"

3. The figure number cited on page 17 (line 7 from bottom) should be specified.

Response: The figure is now numbered as figure 8 on page 13.

4. Methods" on pp. 4-10 should be shortened by citing their own papers.

Response: The methods part has been shortened by two pages (now pp. 4-8). Some parts were also fused: Instead of having the following sections:
Cell lines and antibodies, Flow cytometry and cyto spins, mRNA extraction and cDNA library construction, Antibody screening, In vivo excision using the exAssist/SOLR system, Restriction enzyme digestion, Plasmid DNA preparation, DNA Sequencing, RT-PCR, Western blotting

There are now the following sections:
Cell lines and antibodies, Flow cytometry and cyto spins, mRNA extraction and cDNA library construction, Immunoscreening of cDNA library, In vivo excision using the ExAssist/SOLR system, DNA Sequencing, RT-PCR, Western blotting

5. Discussion" on pp. 14-19 might also be condensed.

Response: The discussion has been condensed from about 6 pages to 4 pages. Accordingly, the number of references is now 56 instead of 63.

Response to reviewer 2:

1. Page 4: There is no information about the ethics.
Response: The information on the ethics has been added on page 4 (cell lines and antibodies) and reads as:
Informed consent was obtained from all patients prior to tissue sampling.

2. Page 5: Which chromogen was used with the LSAB Kit from DAKO?

Response: Diaminobenzidine (3,3'-diamino benzidine) was used as a chromogen. This is described on page 5 (section: Flow cytometry and cytospins). The kit used in this work is the LSAB and not the LSAB2 kit.

3. The authors describe several immunohistological methods, but there are no pictures given in the results. Some colour pictures of cytospins and skin specimens would improve the manuscript.

Response: Two more figures were added: Figure 4 depicts a cytospin staining with mAb SM5-1 and Figure 5 shows staining of mAb SM5-1 of a lymph node metastasis.