Author's response to reviews

Title: Selection, affinity maturation, and characterization of a human scFv antibody against CEA protein.

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Version: 2 Date: 23 January 2006

Author's response to reviews: see over
Dear Editors,

Please find the revised version of the manuscript “Selection, affinity maturation, and characterization of a human scFv antibody against CEA protein” by Pavoni et al., submitted on line.

I report below point by point how we have addressed the reviewers’ comments and suggestions, and which modifications have been introduced into the Revised Version.

Reviewer 1: Hua-Liang Huang
1. According to Reviewer’s suggestion, we provide additional information about primer design in Materials and Methods section, see p.4, lines 41-43 and p.5 lines 11-13, in the revised version. We also indicate a producer supplying the oligonucleotides with partially degenerated sequences, p.4, line 36. Moreover, the oligonucleotides for mutagenesis are described in detail on p.4, lines 31-41 and p.5, lines 8-13.

Reviewer 2: Udo Schumacher
1. We took into consideration the article cited by Reviewer and the various published reports dealing with murine CEACAM proteins. The only murine CEACAM protein having a counterpart in human CEACAM family, is CEACAM1. This protein is smaller than human CEA, and migrates with an apparent molecular weight between 115 and 150 kDa. We also performed several additional tests, analyzing normal and tumor murine tissue extracts by Western blot, with both our own and several commercially available anti-CEA antibodies. As a result, we find that a faint band of high molecular weight, recognized by all anti-CEA antibodies tested, migrates slightly higher than human CEA (180-200 kDa) and is present in both normal and tumor murine tissues, including those which do not express CEACAM1. This means that MA39 and E8 scFvs do not recognize murine CEA protein. According to these results, we have modified the manuscript and removed the parts concerning murine CEA. We thank the reviewer for pointing this out.

Reviewer 3: Rhona Stein
1. The Background and Discussion sections were re-written, strengthened by one-fourth and one-fifth, respectively.

2. Additional grammatical editing was done. The following grammatical and typing errors were corrected:
p.3, line 24: are → be
p.4, line 2: HB2151 bacterial host → The HB2151 bacterial host
p.4, line 14: Diversity → The diversity
p.4, line 30: from library ETH-2 → from the ETH-2 library
p.4, line 33: KM144 and KM145 → The KM144 and KM145
p.5, line 3: DH5αF’ competent cells → DH5αF’-competent cells
p.5, line 17: KM143 and KM148 external primers → The KM143 and KM148 external primers
p.5, line 38: 20 μg/ml was → 20 μg/ml were
p.5, line 42: hours → h
p.5, line 43: minutes → min
p.6, line 14: Tetramethylbenzidin → Tetramethylbenzidine
p.6, line 25: were applied; and ELISA → were applied, and ELISA
p.6, line 34: minutes → min
p.6, line 44: 7 min injection → 7-min injection
p.7, line 28: Erembodegen → Erembodegem
p.7, line 31: BD Bioscences, Erembodegen → BD Biosciences, Erembodegem
p.8, line 2: verified → verified
p.8, line 8: Rochford → Rockford
p.8, line 11: Becton-Dickinson → Becton, Dickinson and Company
p.8, line 15: Pittsburg → Pittsburgh
p.8, line 21: with counterstaining with → counterstaining with
p.8, line 38: ScFv antibody DNA characterization → DNA characterization of scFv antibodies
p.8, line 34: Perkin-Elmer → PerkinElmer
p.9, line 3: TRISTAR → TriStar
p.9, line 7: in Ethanol → in ethanol
p.9, line 9: 10μg/mL → 10 μg/mL
p.9, line 21: CEA as described → CEA, as described
p.9, line 37: Figure 1 → Figure 1
p.9, line 39: original the MA39 → the original MA39
p.10, line 10: nonsense → nonsense
p.10, line 18: Second → A second
p.10, line 24: subsequently panned → subsequently panned
p.10, line 29: original TGG → the original TGG
p.10, line 33: E8 scFv → The E8 scFv
p.11, line 1: significative → significant
p.11, line 23: we see no binding was with → we see no binding with
p.11, line 33: immunochemical → immunochemical
p.12, line 24: blocked → blocks
p.13, line 5: there is no reactivity was seen in → there is no reactivity in
in Table 4: Positivly → Positively

3. Comparison to other known anti-CEA antibodies is now included, p.12, lines 37-44.

Moreover, we checked the manuscript format as follows:
1. Title page is prepared as shown in “example title page”
2. All tables are now divided into cells with visible black line borders

All authors of the article agree to submit the manuscript in present form to BMC Cancer

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