Author's response to reviews

Title: Characterization of antibodies in single-chain format against the E7 oncoprotein of the Human papillomavirus type 16 and their improvement by mutagenesis

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Characterization of antibodies in single-chain format against the E7 oncoprotein of the Human papillomavirus type 16 and their improvement by mutagenesis

Gabriella M Dona, Colomba Giorgi and Luisa Accardi

Dear Professor Norton,

I wish to submit the following comments to the referees.

Comments to Lawrence Banks:

1. A panel showing the reactivity of scFv 43M1 and 43M2 against E7, in comparison with that of scFv43, has been included in Fig 4.

2. We fully agree with the reviewer's comments and suggestions. The aim of the present study was to perform the in vitro biophysical characterization of the anti-E7 scFvs, which is propaedeutic for their use in vivo. In fact, one main objective of the ongoing studies at our laboratory is determining whether or not the antibody with improved stability (scFv43M2) works better than the wt scFv43 in vivo. In a previous study we have shown the capability of scFv43 to inhibit the proliferation of the transfected SiHa cells. However, the SiHa cell transfection efficiency is usually very low (<15%) and does not permit the analysis of cell proliferation on the whole cell population. To bypass this problem we are presently setting up methodologies (retroviral system) that will permit analysing a higher percentage of scFv-expressing cells. These experiments are ongoing in our lab but in order to achieve the required results more time is needed.

3. We wish to point out that this matter is also the object of ongoing studies. We intend to use a combination of the most stable anti-E7 scFv fragments to inhibit the E7 action. This is of particular interest in view of preliminary results which indicate that scFv43M2 and 51 bind to different epitopes on the E7.

Comments to Massimo Tommasino:

A statement has been added to the text in accordance with the reviewer’s suggestions (page 14, line 2-3).

Thank you for your kind assistance and I hope that the revised paper meets your approval.

Kind regards

Luisa Accardi