Comment on "Production of multivalent protein binders using a self-trimerization collagen-like peptide scaffold"

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The recently published manuscript by *Fan et al.* (1) describes the generation of multivalent antibody fragments using collagen sequence-based peptides to drive multimerization. In 2006, our group reported in *The International Journal of Cancer* that fusion of the N-terminal association subdomain (50 residues) of murine collagen XVIII NC1, responsible for noncovalent trimerization of collagen XVIII alpha chains, to the C-terminus of a single-chain variable fragment (scFv) confers their natural trimeric state to the fused antibody (2). The homo-trimeric molecules were isolated in functional active form from the cell culture supernatant of gene-modified human cells and showed high stability in the presence of relevant proteinases (2). The scFv used recognizes an angiogenesis-associated laminin epitope (3) and inhibits tumor angiogenesis and growth *in vivo* (4). We demonstrated that trimeric scFv-NC1 bound to laminin, and was more effective in blocking capillary morphogenesis in vitro, and in preventing tumor growth in vivo than its monomeric version (2).

Given the wide availability of Internet-based search engines, we regret the omission in the above mentioned paper of our original reference showing for the first time the ability of collagen-derived sequences to promote antibody trimerization. FJ

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Response to: "Comment on 'Production of multivalent protein binders using a self-trimerization collagen-like peptide scaffold'"

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I am writing in response to the concerns expressed by Dr. Cuestra and colleagues regarding not citing their work in our recently published manuscript. First, I would like to differentiate their work published in *The International Journal of Cancer* from ours. As found in the first paragraph under the "Discussion" section of our paper, we have pointed out that many researchers used different trimerizing domains, including the C-terminal non-collagenous (NC1) domain of collagens (in the case of Cuestra *et al.*, the NC1 domain of type XVIII collagen; others were derived from the C-propeptide of fibrillar collagens), to drive the trimerization of its fusion partners (antibody fragments, cytokines, growth factors, *et al.*). In contrast, we adopted a novel approach

by using a short self-trimerizing collagen-like peptide to fulfill the art. This was based on the finding of our previous work, in which the formation of the triple-helical structure of type XXI collagen is governed by the C-terminal collagenous (COL1) domain and the content of hydroxyproline in the COL1 is crucial for the collagen trimerization. We did an extensive work focusing on the characterization of the self-trimerizing $(GPP)_{10}$-scaffold fusion complexes. Due to manuscript size constraints and considering the above distinct mechanisms in terms of promoting antibody fragment trimerization with collagen-derived sequences, we did not emphasize their work in our paper. Nevertheless, I apologize that we did not cite their paper as a reference in the "Discussion" section of our manuscript. I thank Dr. Cuestraand colleagues for their comments and interest in our manuscript. FJ

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