CD40-targeted peptide proposed for type 1 diabetes therapy lacks relevant binding affinity to its cognate receptor. Reply to Pagni PP, Wolf A, Lo Conte M et al [letter]

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Abbreviation

RAG Recombination activating gene

To the Editor: We appreciate the letter by Pagni et al [1] that comments on our previous publication [2]. However, rather than definitively demonstrating that the KGY_Y₁₅ peptide does not work in pre-clinical studies, their report highlights the complexities of CD40 as a molecule and strengthens the need for a better understanding of CD40. It is easy to think that a molecule that has been studied for so long is fully understood when in reality the opposite is true.

The CD40 molecule has, over time, proven to be more complex than a simple cell-identifying molecule. Tone et al [3] demonstrated the existence of CD40 isoforms that were generated through alternative splicing of its RNA. We subsequently showed that there are differentially glycosylated forms of CD40 and those that are less glycosylated appear to represent a more active form of the protein [4]. In addition, we demonstrated that CD40 can form hybrid receptors with TNF-receptors 1 and 2. Further, we reported that CD40 interacts with galectin-9, controlling CD40 signalling [5], and with

recombination activating gene (RAG)1 and RAG2 proteins [6]. Clearly, these findings very rapidly compound problems in how to accurately assess any interactions of CD40 either in vitro or in vivo.

In their letter [1], Pagni et al used a recombinant mouse CD40 molecule, which is a disulfide-linked homodimer, along with a recombinant CD154. It is not clear whether the CD154 used was a monomer, dimer or trimer. At that juncture, it is uncertain how representative the interaction between these two recombinant proteins in vitro are of the situation in vivo in autoimmune mice. In addition, the assays were conducted in buffers that are wholly different from plasma. Some assays contained propylene glycol and polysorbate 80, while others contained EDTA and surfactant P20, and the KGY_Y₁₅ peptide was formulated in HEPES, propylene glycol and polysorbate 80. This is different from the KGY_Y₁₅ peptide formulation in PBS that was used for intravenous injections in our paper [2]. These parameters could affect how proteins and peptides retain their structure and, therefore, may affect certain types of binding while not affecting other types.

Pagni et al rightly point out that the interaction between recombinant CD40 and recombinant CD154 has several contact points in a crystallographic complex of the two proteins [1, 7]. They further point out that there are areas of interaction that would be more favourable in terms of strong interaction than the area in question for the binding of the KGY_Y₁₅ peptide. It is tempting to assume that a strong interaction is necessarily more favourable. Perhaps biological interactions are more plastic in nature than just no binding/strong binding. Always striving for the strongest interaction may not result in the most favourable outcome. It is probable that there are cases where biological interactions need modulation rather than complete prevention in order to have a measured and targeted effect. In the work by Pagni et al [1], it is therefore possible that the peptide binds with low affinity to one portion

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of the CD40 protein, while CD154 can still contact CD40 at other sites [7]. Therefore, the peptide may modulate CD40 signalling in a way that is favourable to prevent autoimmunity in vivo [2].

Pagni et al claim that the calculated half-life of the KGY_Y₁₅ peptide is ‘in the order of minutes’ [1]. This may be accurate according to their method of calculation. However, we have data that show that, in vivo, the peptide has a vastly longer half-life (D. H. Wagner Jr. and G. M. Vaitaitis, unpublished data).

Finally, Pagni et al appear to doubt our immunoprecipitation data that we obtained using the KGY_Y₁₅ peptide [1, 2]. We stand by our data and conclusions; we conducted experiments using appropriate controls and reported the findings in our paper [2].

It is our opinion that the limited set of in vitro (not in cellulo) experiments and calculations by Pagni et al are not sufficient to discount our in vivo success utilising the KGY_Y₁₅ peptide in treating autoimmunity. Regardless of the peptide mode of action, we clearly see an effect on autoimmune symptoms in both type 1 diabetes and murine experimental autoimmune encephalomyelitis, the latter being a model of multiple sclerosis [2, 8]. While the experiments in the work by Pagni et al [1] follow current standards in research, their conclusion that their data calls into question the rationale for progression toward clinical studies with the KGY_Y₁₅ peptide is an extreme overinterpretation. In our view, while more work may be necessary in order to understand the mode of action of KGY_Y₁₅, this peptide merits further consideration as a potential treatment in autoimmunity.

Duality of interest DHW is the founder and Chief Science Officer of Op-T LLC. DMW is the Chief Financial Officer of Op-T LLC. GMV performs consulting work for Op-T LLC.

Contribution statement GMV was responsible for drafting the article and DHW revised it critically for important intellectual content. All authors approved the version to be published.

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