Clarification on the source of anti-Zika virus antibody, ZAb-FLEP

In a recent opinion piece by Vasquez *et. al*[1] published in the journal mAbs, unsubstantiated allegations were made against Dr. Ram Sasisekharan that questioned, among other things, the source of the anti-Zika virus monoclonal antibody, ZAb-FLEP.

Dr. Sasisekharan is a co-founder of Tychan, a Company whose mission is to rapidly make available therapeutics and prophylactics against infectious diseases like Zika, which was declared a Public Health Emergency of International Concern in February 2016 by the World Health Organization.

The claims made by Vasquez et al. are factually incorrect and have not been subject to peer review. Additionally, Dr. Sasisekharan was never contacted by any of the authors or the mAbs journal for his input or perspective.

The foundations of our research at Tychan, however, are substantiated and peer-reviewed.

The three claims made by Vazquez *et al.* are incorrect and we have addressed each individually as follows:

Claim 1: Relevant antibody sequences were not published with the intent to "intentionally obfuscate the similarity of the sequence to previously published sequences".

The Sasisekharan lab's methodology explicitly calls for a focus on 3D "*structure*", not *"sequence"*. The "sequence" has no relevance in the innovativeness of our methodology.

Importantly, the target on Zika was chosen based on lessons learned studying the closely related dengue virus[2] – that is, to select regions on the virus surface that are:

1. Known to be effective targets for inactivation by antibodies[3]
2. Mutationally constrained and therefore minimizing drug resistance.

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[1] M. Vazquez, *et. al*. Connecting the sequence dots: shedding light on the genesis of antibodies reported to be designed in silico. mAbs (May 20, 2019).

[2] Tharakaraman et al., Cell Host Microbe, 2018.

[3] Chan et al, PNAS 2011, Robinson et al, Cell 2014

These criteria drove the identification through the analysis of the 3D structure using our network model of a region (aka 'epitope') involving two adjacent viral envelope proteins that are mutationally constrained and known to be effective targets for Dengue virus inactivation by antibodies.

Claim 2: The lab "arrived at the Zika mAb (ZAb_FLEP) by starting with C8 and making conservative modifications"

The discovery of the Zika mAb (ZAb_FLEP) did not start with C8. In fact it started with the distinct mutationally constrained epitope on Zika virus identified through our network model. A variety of human antibody sequences which are VH (variable heavy), VL (variable light), and CDR (complementarity-determining regions) from a variety of different antibodies from public databases was then used to derive scaffolds as a starting point for designing antibodies.

Using the iterative approach of computational analyses and experimental interrogation to optimize the structure, the final lead antibody, ZAb_FLEP, was created. This approach is fundamentally different from *ab initio* antibody design, where we believe the antibody mutational space is unconstrained.

More importantly, relying on an existing antibody such as C8 would be limiting as it will not allow quick response to any outbreaks caused by new viruses, which we want to develop our methodology to do. This is the fundamental objective of Tychan.

Claim 3: The Zika antibody is essentially the same as C8

The Zika antibody is not the same as C8. The epitope targeted by ZAb-FLEP has clear differences from those that fusion loop antibodies such as the C8 would bind to, including distinct amino acid contacts and different binding surface areas.

There are key biological activity differences between the antibodies, as evidenced by the stark contrast in how these antibodies react to the closely related dengue virus.

C8 has been shown to potently neutralize all four types of dengue virus whereas ZAb_FLEP poorly neutralized dengue virus types 3 and 4[4] due to differences in the epitope targeted.[5]

At the time that the Sasisekharan lab started their work, there were no reported structures for any antibody-Zika virus E protein complex. When we compared ZAb_FLEP to another antibody (C10) that bound to the "EDE1" epitope (also recognized by C8) and the only cryo-EM structure available at that time, we discovered key differences.[6] The EDE1 epitope is also recognized by C8, but there was no structural information available on the C8-EDE1 complex that we could compare to ZAb_FLEP.

Beyond these claims, we also note that all the authors of the mAbs opinion piece[7] are equity stakeholders in, and or employed by, Adimab LLC, which is involved in the discovery of therapeutic antibody-based products.

The Tychan board has been greatly satisfied with the progress of the anti-Zika virus monoclonal antibody, ZAb-FLEP. The rapid development using the methodology, from the start of its design to the first dose in human in 16 months, and the positive clinical trial results to date provide great hope not only for victims of the Zika virus, but as a platform with the potential to address other infectious diseases. These development will be published in due course.

At Tychan, we stand by our work and remain committed to our vision to bring life-saving treatments – such as the anti-Zika antibody therapy – to those in need. The integrity of our research work and clinical trials remain our topmost priority in our mission to save lives.

-- Tychan leadership team and board

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[4] *Tharakaraman et al., Cell Host Microbe, 2018*
[5] Cell Host and Microbe manuscript (Table 1, p. 620).

[6] Cell Host, Microbe. Figure 6 Pg 625. *The topological nature of ZAb_FLEP epitope combined with the differences in the arrangement of the E-protein near the different icosahedral vertices restricts the occupancy sites to 120. The spacing between the bound Fab fragments suggests the possibility of a bivalent binding pattern of ZAb FLEP, different from that of C10.*
[7] M. Vazquez, *et. al*. Connecting the sequence dots: shedding light on the genesis of antibodies reported to be designed in silico. mAbs (May 20, 2019).