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EHA DEFUSE project

There is some fascinating work on bat ecology and attempts to work out how bats are infected all of which are fascinating. That said, I'm not an expert in this field and would defer to knowledgeable researchers.

The virology is mixed. The search for more natural bat CoVs is a no brainer since SARS1. Post COVID we see the need for even more sampling on a wider geographical level. No problem.

There are two concerns:
1) Some of the genetic manipulations proposed are worrying.
2) The underlying reasons for performing them are unjustified.

Deliverables:
1) They will not advance our knowledge beyond generalities that cannot help a public health official, civil or military.
2) DEFUSE cannot deliver a robust app that will be of any use to warfighters.

The remaining comments are focused on the virology.

• **Predicting viral pandemics**
There is the pervading view that it is possible to predict the virus that will provoke the next pandemic. They are not alone; others feel this way.

Unfortunately, it is erroneous. See Wain-Hobson EMBO Molecular Medicine 2013 5:1-5 for detailed arguments. Others have said essentially the same thing, but so far, they have not been heeded.

Rather than repeat the detailed arguments, let's look at the track record in virology. Nobody predicted the COVID-19 pandemic, nor the SARS1, nor MERS outbreaks. Flu experts failed to predict the 2009 influenza A pandemic. They were hedging their bets on it emerging from an avian virus out of SE Asia. It emerged from swine in NW Mexico.

An old virus like Zika swept through the Pacific to Brazil and onwards to the Caribbean. We were all scared if it moved into SE USA. It didn't and nobody is talking up Zika today. We don't know how it went on its 2015-16 helter-skelter epidemic, nor how it imploded.

Nobody predicted the 2013-16 outbreak of Ebola in West Africa. Until then Ebola had only been seen in Central Africa. On top of which, it was the biggest outbreak by far of Ebola ever seen. Going back a further 30 years AIDS and its viruses flummoxed everybody. It took years to understand where they came from.

Let's focus on an example closer to home. Over the past 30 years the annual flu vaccine contained antigens from two flu A viruses and one B virus. More recently a second flu B virus started to co-circulate. Given the difficulty of predicting which, if at all, of the two B viruses would eventually dominate the other, it was decided to make a tetravalent flu vaccine which became available by 2012. This shows that for two viruses which WHO and the worldwide network of influenza reference labs were monitoring,

experts were unable predict which would overcome the other, if at all. Wisely they advised going with a tetravalent vaccine.

In short, virology's track record at predicting epidemics and pandemics is close to zero. To suggest otherwise is to go against, or ignore, a substantial corpus of data.

• Dual Use Research of Concern (DURC) or Gain of Function (GOF) research
The authors say their work is not DURC/GOF. No doubt this conforms to a very strict definition. I would disagree for the following reasons:

They are working with, indeed are making every effort to single out bat CoVs that can grow on human cells, or have Spike protein that allow infection of human cells as using viral pseudotypes. From the get go, they are singling out pleotropic viruses, those that replicate in cells from more than one animal. Maybe there is no subsequent human-to-human transmission of these viruses, but they do not know that.

To argue that making bat chimeras is not DURC/GOF because the origins of each segment is from a bat, is disingenuous, or at best simplistic. It is not the origin of the fragments that is important, it is the phenotype of the virus that matters. It's all about phenotype. Otherwise it wouldn't be called DURC. There would be no C.

I'm all for studying natural viruses, but making chimeras takes us into the realm of the unnatural. While the phenotypes could be attenuated, a few could be enhanced. The authors have no way of knowing before doing their experiments. They know that. But as they know that they are working with pleotropic viruses, or pleotropic Spike proteins, best be careful. On top of which, they are working with mutation machines in environments where selection is operative.

Given the basic premise of predicting a pandemic virus is mission possible, why perform this DURC/GOF work?

• Fatal attraction
Every attempt is made to identify high pathogenic bat CoVs. OK. But remember, a low path strain could be just one mutation away from a high path strain. Or mutate into a high path strain just after sampling was completed.

Why engineer the Spike protein to make it better adapted to infecting human cells? Protease cleavage sites, N-glycosylation sites and receptor binding sites will all be played with. They take care not to mention furin among the protease cleavage sites. It must be on their to do list.

Q: Will Nature take any of these courses? As we have seen that outbreak, epidemic or pandemic prediction is really hard, they cannot know what path Nature will take. But look at some data. Fouchier and Osterhaus inserted furin cleavage sites into influenza viruses around 2001-12. For a human H3N2 isolate insertion didn't change its pathogenesis in ferrets. For avian H6N1 and N5N1 it did increase pathogenesis in ferrets. Accordingly, we know that the outcome varies with the virus. The outcome is not a *fait accompl*i.

Q: And epistasis? Basically, mutations in one genetic background (one virus genome or gene) will not necessarily generate the same consequences in another. There is no mention of it in the proposal. To illustrate epistasis, two examples are given below:

One. The first is the splicing of furin cleavage sites into the hemagglutinin proteins of the flu viruses noted above – clear cases of GOF research when there is gain of function for the avian H5N1 and H6N1 flu viruses. For H3N2 there was no gain.
Two. A decade ago there were GOF transmission studies on avian H7N9 and H7N1 influenza A viruses. For one H7N9 experiment they found mutations in the hemagglutinin protein (H7) which surprised nobody. For the H7N1 experiment no changes were observed in the H7 hemagglutinin. The outcome depends on the virus or gene used.

The chimeric viruses to be made in the proposal will not be natural so any readout of pathogenesis in humanized mice must be taken as suggestive, but no more. Models are after all, models of human infection. The proposal notes that the N gene could distinguish low and high path, although this is not explored. As a consequence, readouts will be partial at best.

Terry Pratchett captured these shades of Prometheus or Icarus in contemporary language: "Some humans would do anything to see if it was possible to do it. If you put a large switch in some cave somewhere, with a sign on it saying 'End-of-the-World Switch. PLEASE DO NOT TOUCH', the paint wouldn't even have time to dry."

Having lived through the GOF debate and having had some time to reflect on it, my own take is that we're not here to make the world a more dangerous place. Making new viruses of unknown potential poses a small but finite risk of a leak, the consequences of which could be harrowing. As no benefits are forthcoming (no pandemic prediction, hence no preventive vaccines or drugs) the outcome of any risk/benefit analysis is a no brainer.

• **Sampling and representativity**
We have learnt recently (fall 2021) that a pretty good proximal origin to the SARS2 Spike gene can be found in Laotian caves. The consortium will be sampling just one cave of theirs for new viruses. They will splice in data from all over Asia.

Q: Is the quality of the data in Asia good enough compared to what they will generate? I guess data from outside the DEFUSE consortium is mainly sequence data with very little functional work. Will this dilute the effectiveness of the SE Asia wide ambitions of this project?

Q: What is the fraction of bat beta-CoVs they will be sampling as a function of the gamut of bat coronavirus in SE Asia? I don't expect a hard number as it's tough to answer, just a ball park one. I'd guess it would be <1% given the deep branch lengths in phylogenic trees of SARS CoVs. Even if it was 10% do the authors consider that is enough to make a robust app for DARPA?

• **No discussion of limitations**
There is no hypothesis breaking – what happens if they don't succeed here or then? For example, it they can't make a pan-bat interferon product then what? Where are the plan Bs? Unfortunately, they take a bulldozer approach and don't broach things not going according to plan. Murphy's law predicts they will not get everything right.