# Twitter Thread by Daoyu

**Daoyu**
@Daoyu15

## @WisdomRebel https://t.co/iHKwUoNLJ5

## https://t.co/sxm17Eu9hV

## https://t.co/FtjifSGItc

**PRRA is highly purified in the CaLu-3 cell line when FBS is added to inhibit trypsin. Stu lied about that in his defense on the FCS identity. https://t.co/GzM7uA6vup the Gallaher article contain glaring**

@AngloScot2 @WisdomRebel Mistakes and is completely impossible as HKU9 + RaTG13 only lead to a frameshift inactivated S and not a furin cleaved S.
https://t.co/gmDXK9OluN
The serological test the WIV claimed to be negative in Shi's addendum is the exact same test they claimed positive in 2012 in the phD

@AngloScot2 @WisdomRebel thesis, and Stu once again lied about it. https://t.co/Uiy8U9BTYp significant evidence in the form of contaminated SRA datasets suggest that the DEFUSE grant have been funded in China, as the full-length HKU4 is not a part of the 2016-2019 grant, but is found before the start

@AngloScot2 @WisdomRebel Date of the 2020-2024 grant of June 2020. The WIV also contained several HKU3-like Coronaviruses, particularly in mice in an 2017 GEO experiment, which is consistent with the published HKU3 and batified mice experiment in the DEFUSE document (evidence from an already conducted

@AngloScot2 @WisdomRebel Experiment). Either DEFUSE or fast-tracked form of the 2020-2024 grant in 2019 would have lead to full-length Sarbecovirus clones being used, leading to SARS-CoV-2 in the lab—they used HKU4 in stead of HKU5 for the MERS spike experiment indicating that backbone sequence distance

@AngloScot2 @WisdomRebel Can not be too high for CoV chimeras, requiring the 4991 backbone or full-length 4991 to be used. In addition, https://t.co/sxm17Eu9hV the 201801-202102 grant of the WIV fits perfectly to DEFUSE since this is consistent with neither the Understanding risk of bat coronavirus

@AngloScot2 @WisdomRebel Emergence grant nor the EID hotspots in southeast asia grant.

https://t.co/YwSTyD3N8B

https://t.co/ySjXCH0k2V
In addition, the claim that the first infection was connected to the wet market is incorrect since the WHO report and the Zenodo-cell article used a resolution

@AngloScot2 @WisdomRebel Of cases that were far too low (which resulted in assigning cases to the wrong side of the yantgtze river) and have intentionally omitted the headquarters of the WIV https://t.co/w7d6bwbAn9 in Wuchang. In addition, the first patient on the WHO report went only to a supermarket in

@AngloScot2 @WisdomRebel Jiangxia, not any wet markets, and it just happened that the RT-mart he visited https://t.co/khQSq8ULAp is in Jiangxia, right next to the BSL-4 building of the WIV.

@AngloScot2 @WisdomRebel In addition, in contradiction to the Stu's another claim that 4991 is "sequenced only after 2020", the full-length sequence of 4991 was already discussed in 2018, and the S protein show significant divergence from RaTG13 when compared against SARS-CoV Urbani. https://t.co/VI7I3PwJoK

> 5/6
>
> Finally, are you really the same as 4991 or are you a hair different in your spike protein? About 1.1% different so that the difference between your S gene and that of SARS1 would be 1.1% different than the diff reported for 4991 and SARS1 in 2019?https://t.co/K84RLuj55Y pic.twitter.com/IsSfMjenLI
>
> — Yuri Deigin (@ydeigin) June 1, 2021

@AngloScot2 @WisdomRebel https://t.co/ejV0sIlzj5

@AngloScot2 @WisdomRebel https://t.co/ELNPYGVn00
In addition, other anomalies are found in the RBD, especially that of T403 and D501.

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@AngloScot2 @WisdomRebel https://t.co/bJma8XExej
In addition, evidence in the form of out-of-whack substitution ratios was found in the RBD of RaTg13, indicating that accelerated evolution in the form of 5-Fluorouracil usage was present in the S protein—which they then go on to compensate in a completely

@AngloScot2 @WisdomRebel Unnatural way in the S2 and the ORF1b using loads and loads of dS changes, throwing the ratios of dS/dN out of whack in both locations. https://t.co/J2YFuLKOqY

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@AngloScot2 @WisdomRebel The CGG-CGG pair is at the end point of RNA editing and can not be mutated in the CoV system except for very rare circumstances of RdRp error—it mutates at a rate that is less than 1/10 the rate others can, and no immediately ZAP-beneficial mutation lead to a dS on the CGG pair.

@AngloScot2 @WisdomRebel If you count this low rate of mutation that is possible in the CGG-CGG pair, it is under neutral to slightly positive selection, and not negative or purifying selection. https://t.co/jDy6RoYdSM as for the multi-market hypothesis? Sichuan had loads and loads (1/4 of all sequences)

@AngloScot2 @WisdomRebel That had only T8782C but not C28144T, four of such genomes were found in early Wuhan as well. The 8782/28144 distinction in Wuhan appears in-host before other changes begin to show, so there is no need of two separate introductions for A and B lineage. It happened in humans.

@AngloScot2 @WisdomRebel The FCS PRRARS is highly purified in CaLu-3 cell cultures. Any change to the AA gives significantly reduced infectivity. Therefore, a canonical FCS of RRARR will simply mutate and revert into PRRAR within just 3 passages in the CaLu-3 cell line.

@AngloScot2 @WisdomRebel https://t.co/53dG1S7LnL

> Neil says: scientists would use established viral backbones to test new RBDs or a FCS
> Wrong
> P11 PREEMPT states that they wanted to generate full length backbones of 3-5 new bat-CoVs per year.
> The aim was to investigate novel viruses out there that could become pandemic.
>
> — valentin bruttel (@VBruttel) November 11, 2021

@AngloScot2 @WisdomRebel https://t.co/pNwWQ8a7jy

> Neil says: new backbones too much work
> Wrong.
> new viral backbones can be made in 1 week: https://t.co/FkQg7SVcXE
>
> — valentin bruttel (@VBruttel) November 11, 2021

@AngloScot2 @WisdomRebel And “when clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in VERO and HAE cultures”. The PRRA is most conserved upon conjugate gradient selection with CaLu-3 as main passage cells and VERO E6 for stock preparation https://t.co/c7UNQR1cnY

> Neil says: They didn't plan to introduce new FCS sites, would use a classical FCS to be sure it works
> Wrong
> P11 PREEMPT states they would \u201creview deep sequence data for SARSr-CoV that encode functional proteolytic cleavage sites, and introduce these into low risk parental strain\u201d
>
> — valentin bruttel (@VBruttel) November 11, 2021

@AngloScot2 @WisdomRebel between passages. https://t.co/nRTIU93WGP

@AngloScot2 @WisdomRebel https://t.co/Ff9XAc4ao3
In deed, CaLu-3 have 1/4 O-linked glycosylation that inhibits cleavage upon P681, saving the heparan sulfate binding peptide without compromising TMPRSS2 usage or immunogenicity. VERO E6 had even more O-linked glycosylation and HS binding as it have even

@AngloScot2 @WisdomRebel More uncleaved fractions with the PRRA compared to CaLu-3, and show a more pronounced advantage with PRRARS compared to RRRARS.

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@AngloScot2 @WisdomRebel In addition to the extremely high stability of PRRA in CaLu-3 and it's lack of stability in-vivo, some of the earliest patients in Wuhan harbored, by digital PCR and nanopore sequencing, large deletions across the QTQTN and SPRRARS https://t.co/YPHooePHko that are specific to

@AngloScot2 @WisdomRebel VERO E6 cell cultured SARS-CoV-2 strains--indicating that some of the quasispecies from the original cell culture were held over into the very first patients of SARS-CoV-2, strongly supporting the hypothesis that the PRRARS sequence being the result of composite selection

@AngloScot2 @WisdomRebel pressure generated from serial passage in CaLu-3 with stock preparation and storage in VERO E6 between passages, standard laboratory practices when it comes to the serial cell passage of viruses, which may also be employed if a transmissible vaccine (respiratory transmission with

@AngloScot2 @WisdomRebel no pathogenesis in aged WT BalB/C mice).