Exhibit 428

Report 05: Pfizer mRNA Construct- Why Spike Protein Causes Disease

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Report 05: Pfizer mRNA Construct – Why Spike Protein Causes Disease

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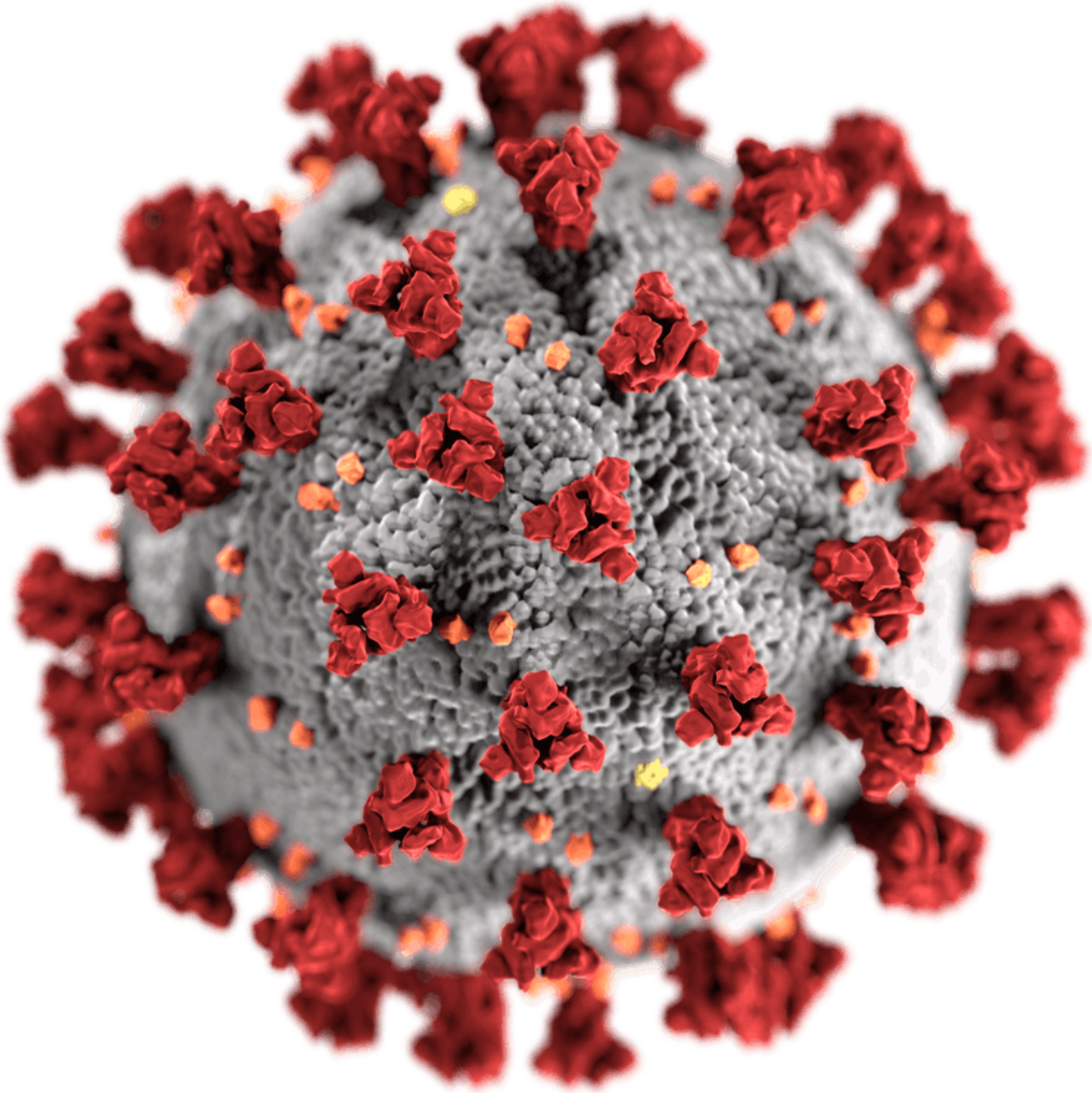

Introduction

In the first paragraph of Pfizer document 2.4 NONCLINICAL OVERVIEW, Pfizer states that “BNT162b2 is a nucleoside modified mRNA (modRNA) expressing full-length S [spike] with two proline mutations (P2) to lock the transmembrane protein in an antigenically optimal prefusion conformation” (p. 6, https://www.phmpt.org/wp-content/uploads2022/03/125742_S1_M2_24_nonclinical-overview.pdf (https://www.phmpt.org/wp-content/uploads2022/03/125742_S1_M2_24_nonclinical-overview.pdf)).

They list two references (Pallesen et al., 2017; Wrapp et al., 2020) as their justification for this design. That is the end of Pfizer's discussion on why that particular design was selected, and it appears that Pfizer conducted no further research before selecting this design (or construct) and proceeding with vaccine development. This, as it turns out, is quite important.

Concerns Regarding the Pfizer mRNA Construct

1. There are three primary concerns regarding the Pfizer approach used to design their mRNA vaccine.
2. The basic Pfizer construct utilizing two proline substitutions to stabilize the spike protein molecule is flawed, and the protein molecule as well as the mRNA itself, remain unstable.
3. The spike protein has been shown to cause disease; therefore, a vaccine based on the spike protein will promote pathogenesis, not prevent it.
   The S1 subunit of the spike protein has been shown to shed into the circulatory system, thereby furthering disease.

The following discussion expands on these three concerns.

Concern 1: Pfizer selected the Pallesen et al. (2017) construct as the basis for the Pfizer vaccine.

The work described by Pallesen et al. (2017) was performed on the MERS-CoV virus. Pallesen selected proline substitutions based on the work of others (Qiao et al., 1998; Sanders et al., 2002; Krarup, et al., 2015).

Pfizer also references a paper in the journal Science authored by Daniel Wrapp (Wrapp et al., 2020). Wrapp cites Pallesen et al. (2017) and the work of Robert Kirchdoerfer et al. (2018) who evaluated the Pallesen-style double proline substitutions (S2P) in the spike protein of SARS-CoV. Wrapp et al. (March 2020) assessed the 2P substitution in the spike protein of SARS-CoV-2, evaluating the construct for its affinity for the host cell receptor ACE2. Wrapp did not evaluate the SARS-CoV-2 S2P antigenicity nor the fate of the S1 subunit that is shed when the spike protein binds to the cell.

Wrapp et al. (March 2020) states that "Knowing the atomic level structure of the SARS-CoV-2 spike will allow for additional protein engineering efforts that could [emphasis added] improve antigenicity and protein expression for vaccine development." It appears that Pfizer took this article and used it as is to create a vaccine without "additional protein engineering efforts" as suggested by Wrapp et al. (2020).

Moreover, the purpose of introducing two proline substitutions into the spike protein as described by Pallesen and Wrapp (Pallesen et al., 2017; Wrapp et al., 2020; Pfizer, p. 6, https://www.phmpt.org/wp-content/uploads2022/03/125742_S1_M2_24_nonclinical-overview.pdf (https://www.phmpt.org/wp-content/uploads2022/03/125742_S1_M2_24_nonclinical-overview.pdf)) was to stabilize the spike protein to improve its thermal *stability, conformation and antigenicity*. But, as stated by Hsieh et al. (2020) with co-author Daniel Wrapp, "even with these (2P) substitutions the SARS-CoV-2 S-protein remains unstable and difficult to produce reliably in mammalian cells, hampering R&D of subunit vaccines."

Hsieh and Wrapp (Hsieh et al., July 2020) found that 26 of 100 variants that they created and tested had higher expression than the S-2P substitution that Pfizer selected. One of their variants, labeled Hexa-Pro, contained four proline substitutions in addition to the S-2P substitutions and had nearly 10X greater expression, had improved thermal stability and retained the desired conformation.

Numerous articles since then state that the 2P substitution used by Pallesen/Pfizer is unstable (McCallum et al., 2020, posted online Aug. 2020; Xiong et al., 2020; Brun et al., 2020; Juraszek et al., 2021). Brun et al. (posted November 2020) *even made suggestions for improving the Pfizer BNT162b2 vaccine after describing why it was a suboptimal design*.

Why did Pfizer select the Pallesen construct requiring storage in ultra-low-temperature freezers when the HexaPro construct is more stable, can be stored at room temperature and has much greater expression?

Concern 2: Pfizer did not address the well-documented pathogenesis caused by the coronavirus spike protein before release of their vaccine and before FDA approval.

In a 2005 article, Kuba demonstrated that SARS-CoV spike protein injected into mice worsened their lung disease (Kuba, 2005).

In 2008, Wang et al. demonstrated that the receptor binding domain (RBD) of the spike protein of SARS-CoV leads to internalization of ACE2, resulting in downregulation and subsequent lung injury (Wang et al., 2008). *The authors concluded that "because the RBD spike binding to ACE2 contributes to SARS pathogenesis, the use of subunit vaccines based on RBD spike should be considered carefully.*"

Wang et al. (2020) and Semimukai et al. (2020) noted that recombinant spike protein induced antibodies in mice and protected against SARS-CoV infection, but lung eosinophilic immunopathology was observed in the immunized mice after SARS infection.

Elizabeth M. Rhea and her co-authors reported on-line in December 2020 and published in March 2021 (Rhea et al., 2021) that S1 subunit labeled with radioiodine (I-S1) readily crosses the mouse blood-brain barrier (BBB) and could explain the adverse effects of S1 and/or SARS-CoV-2 such as encephalitis, respiratory difficulties and reduced ability to smell. I-S1 was also detected in kidney, liver and spleen.

In January 2021, Letarov et al. published an article in the journal *Biochemistry* (*Moscow*), titled *Free Sars-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection* (Letarov et al., 2021). They noted that the upregulation of cell surface expression of ACE1 and/or downregulation of ACE2 can lead to pulmonary damage. This occurs during SARS infection and by recombinant SARS-CoV spike protein. They hypothesize that S1 molecules carry intact RBDs, and their binding to ACE2 may induce ACE2 downregulation and deleterious downstream effects such as increased inflammation, thrombosis, and pulmonary damage.

Letarov et al. (2021) also reference the work of Zhang et al. (2020) who elucidated a spike protein mutation in SARS-CoV-2 (the D614G variant) that is associated with increased infectivity but reduced S1 shedding and mild symptoms. This is further evidence that the spike protein is responsible for pathogenesis.

Nuovo et al. (2021, posted online Dec. 2020) reported on the endothelial cell damage caused by the S1 subunit of the spike protein. They reported two main findings: 1) Human COVID-19 cases demonstrated microvessel endothelial damage in the brain and other organs, including the skin, due to circulating spike protein that induces cytokine production resulting in microencephalopathy; and 2) injection of the S1 full-length spike subunit into mice (but not the S2 subunit) induced an equivalent microvascular encephalopathy as seen in human COVID-19 cases. The authors further note that although their study "*focused on the brain, it should be stressed that there are other sites where there is a rich bed of microvessels with the ACE2 receptor, including skin/subcutaneous fat and the liver. As has been documented in human patients, microvessels at these sites can also display an endothelialitis that, in the skin/fat can induce complement activation/hypercoagulable state and the so called cytokine storm typical of fatal COVID-19*."

"*In sum, the data presented indicates that the full length S1 subunit of the spike protein of SARS-CoV-2 alone is capable, without the infectious virus, of inducing systemic microendothelial cell damage in mice with a cognate pattern of complement activation and increased cytokine expression and the concomitant thrombosis/hypercoagulable*

state. This disease pattern strongly parallels the extra-pulmonary manifestation of severe human COVID-19 and suggests that the latter may not represent systemic infectious virus. Thus, prevention of the CNS disease so common in severe COVID-19 may require neutralization/removal of the circulating pseudovirus."

Lei et al. (April 2021) created a pseudovirus exhibiting spike protein but containing no virus inside and concluded that the spike protein alone is sufficient to cause damage to the vascular endothelial cells.

With so much evidence demonstrating a direct link between the presence of the spike protein S1 subunit in the circulatory system and pathogenesis, why would Pfizer create a vaccine that not only injects spike protein into the patient, but converts the cells of the patient into "spike protein factories" that turn out the spike protein S1 subunit, the very molecule that causes illness?

Concern 3: Pfizer did not address the well-documented shedding of the coronavirus spike protein into the circulatory system, where it crosses over to multiple organ systems to cause pathogenesis, before release of their vaccine.

It was shown as early as 1994 (Bullough et al., 1994) that the surface spike protein of an enveloped virus (Influenza) would release a subunit after proteolytic cleavage of the structure upon binding to the host cell surface. Work by Alexandra Walls (2017) demonstrated that the proteolytic processing of coronavirus spike proteins allows
shedding of the S1 subunit.

Brun et al. (posted on-line November 2020) reported the process by which spike protein is processed within the host cell and soluble S1 subunit was secreted into the extracellular space via lysosomes. Their work indicated that the production of spike vaccine antigen protein without a virus to incorporate the protein into the viral envelope created an overexpression system and secretion of the protein by the cell (shedding). They suggest that the secreted spike proteins do not mimic the spike glycoproteins as they are presented on the actual virus and may effectively act as a decoy, eliciting more of the unwanted sub-optimal, non-neutralizing antibodies that are incapable of neutralizing the virus.

The authors state that the Pfizer BNT162b vaccines (and other similar type vaccines) rely on the supplied RNA sequence to use the host cell machinery to faithfully produce the spike protein in its fully folded, glycosylated and assembled state, resembling a natural infection, and they trigger a robust innate and humoral response; however, this does not happen. They go on to suggest a better vaccine design, one that abolishes the furin

cleavage site (which is intact in the Pfizer construct) and introduces mutations that lock the spike protein in the prefusion conformation to prevent shedding and elicit a more potent antibody response.

Rhea et al. (2021, posted on-line December 2020) noted that coronavirus spike proteins are often cleaved; therefore, S1 could be shed and shed S1 may cross the BBB. Shedding of the S1 subunit of the spike protein was also noted by Liu et al. (2020), Letarov et al. (2021), Rhea et al. (2020), Zhang et al. (2020) and Henderson et al.
(2020).

Given that the Pfizer mRNA construct design is sub-optimal; given that it has been well established (since 2005 to 2008) that spike proteins cause disease; and given that the spike protein S1 subunit is shed during binding of the virus or pseudovirus with the host cell, as well as secreted by host cells producing spike protein following injection with an mRNA-derived spike protein vaccine, why would Pfizer develop and release an mRNA vaccine that demonstrates all three of these deleterious qualities? Why would Pfizer develop and release an mRNA vaccine that demonstrates poor design with limited immunogenicity, requires storage at very low temperatures, and results in the production of a spike protein that readily sheds into the circulatory system to cause pathogenesis in multiple organ systems? And why would the FDA approve it?

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Dr. Demers has a PhD in pathology. He has spent more than 30 years working in DNA diagnostics and forensic genetics.

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1 reply added

Denise May 16, 2022 Reply

I guess according to this I'm doomed ,I got vaccinated to protect my very ill dad who is 81 that I live with 24/7 caring for him .
I got 3 Moderna shots