

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TAKEDA VACCINES, INC.
Petitioner,

v.

VALNEVA AUSTRIA GMBH,
Patent Owner.

Case No. IPR2023-00354
U.S. Patent No. 11,219,681

DECLARATION OF DAN H. BAROUCH, M.D., Ph.D.

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***Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)***

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TABLE OF ABBREVIATIONS

Abbreviation	Description
'664 PCT	PCT/EP2016/082664
'681 patent	U.S. Patent No. 11,219,681
'862 application	U.S. Application No. 16/813,862
DENV	Dengue virus
ELISA	enzyme-linked immunosorbent assay
EP '585	European Patent Application No. 15202585
GBS	Guillain-Barré syndrome
GMT	geometric mean titer
I-ODN	immunostimulatory oligodeoxynucleotide acid molecule
JE-PIV	Srivastava's JEV purified inactivated vaccine
JEV	Japanese Encephalitis virus
MN	microneutralization assay
NCBI	United States National Center for Biotechnology Information
ORF	open reading frame
PBS	phosphate buffered saline
POSA	person of ordinary skill in the art
ppb	parts per billion
PRNT	plaque reduction neutralization test
TBEV	Tick-Borne Encephalitis virus
WHO	The World Health Organization
WO '225	WO 2017/109225
WRAIR	Walter Reed Army Institute of Research
YFV	Yellow Fever virus
ZIKV	Zika virus

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I, Dan H. Barouch, hereby declare as follows.

I. INTRODUCTION

1. I am over the age of eighteen and competent to make this declaration.

2. I have been retained as an expert witness on behalf of Takeda Vaccines, Inc. (“Takeda”) for the above-captioned *inter partes* review (“IPR”).

I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$1000.00 per hour.

3. I understand that this Declaration accompanies a petition for IPR involving claims 1–15 and 21–25 of U.S. Patent No. 11,219,681 (“the ’681 patent”) (EX1001). I also understand that the ’681 patent is currently assigned to Valneva Austria GmbH (“Valneva”). Generally, the ’681 patent relates to Zika virus (“ZIKV”) vaccines. ZIKV is a member of the family of viruses known as *Flaviviridae*. Other viruses in this family include Yellow Fever virus (“YFV”), Tick-Borne Encephalitis virus (“TBEV”), Japanese Encephalitis virus (“JEV”), and Dengue virus (“DENV”). All of these viruses also belong to the genus of viruses known as flaviviruses.

4. I understand that the ’681 patent resulted from U.S. Patent Application No. 16/813,862 (“the ’862 application”), filed on March 10, 2020, as stated on the ’681 patent’s face page. EX1001, Items (21), (22). The ’681 patent also states on its face page that it is a continuation of U.S. Patent Application

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No. 16/063,007, which was filed as application No. PCT/EP2016/082664 (“the ’664 PCT”) on December 23, 2016 (EX1008).¹ *Id.*, Item (63). And, the ’681 patent states on its face page that it claims priority to a series of foreign patent applications, the earliest of which was filed on December 23, 2015. *Id.*, Item (30). I have reviewed the ’681 patent, the ’862 application, and each of the ’681 patent’s priority applications set forth above (EX1008; EX1004, 9–133; EX1014–EX1018).

5. As discussed below in Section X.B, claims 1–12, 15, and 21–25 cannot claim priority to the December 23, 2015 filing date to which the ’681 patent claims as its earliest priority date, because the December 23, 2015 application does not even mention ZIKV. Further, as discussed below in Section XI.B, claims 1–15 and 21–25 cannot claim priority to the December 23, 2016 filing date of the ’664 PCT, which the ’681 patent also alleges as a priority date, because the December 23, 2016 application does not adequately describe or enable the *full* scope of the invention claimed in any of claims 1–15 or 21–25 of the ’681 patent.

¹ I understand that the ’664 PCT was published on January 29, 2017, as International Patent Application Publication No. WO 2017/109225 (“WO ’225”) (EX1008).

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6. In preparing this Declaration, I have reviewed the '681 patent and each of the documents cited herein in light of general knowledge in the art as of the relevant dates for my analyses discussed in more detail below in Sections X and XI. I have also relied upon my experience, education, and knowledge in the relevant art. I have also considered the viewpoint of a person of ordinary skill in the art ("POSA") (i.e., a person of ordinary skill in the field of virology and vaccine research and development, as defined further below in Section VI) prior to the relevant dates for my analyses discussed in more detail below in Sections X and XI.

II. MY BACKGROUND AND QUALIFICATIONS

7. I am an expert in the field of virology and vaccine research and development, including the immunology and pathogenesis of viral infections and the development of novel vaccine and treatment strategies. My primary area of expertise includes the development of vaccine candidates for multiple pathogens of global significance, including HIV-1, ZIKV, and tuberculosis. Most recently, I participated in the development of a SARS-CoV-2 vaccine, which led to the Janssen/Johnson & Johnson COVID-19 vaccine (Ad26.COV2.S) that received Emergency Use Authorization from the United States Food & Drug Administration in February 2021 and multiple approvals throughout the world. I have actively been working in the vaccinology, virology, and immunology fields since 1995.

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8. In 1993, I received a Bachelor of Arts in Biochemistry, graduating *summa cum laude*, from Harvard University in Cambridge, Massachusetts. In 1995, I obtained a Doctor of Philosophy in Immunology from Oxford University in Oxford, England. I also received my Doctor of Medicine, *summa cum laude*, from Harvard Medical School in 1999 in Boston, Massachusetts.

9. From 1996 to 2002, I was a Research Fellow at the Beth Israel Deaconess Medical Center in Boston, Massachusetts. In the period from 1999 to 2001, I was also an Intern and Resident in Medicine at the Massachusetts General Hospital in Boston, Massachusetts. From 2001 to 2004, I was a Fellow in Infectious Diseases at Massachusetts General Hospital and Brigham and Women's Hospital.

10. Since 2001, I have also held Physician appointments at hospitals and affiliated institutions, including Massachusetts General Hospital, Brigham and Women's Hospital, Dana-Farber Cancer Institute, and Beth Israel Deaconess Medical Center. In 2012, I became the Founding Director of the Center of Virology and Vaccine Research at Beth Israel Deaconess Medical Center, a position that I have held for the past 10 years.

11. Since 2002, I have held Instructor, Assistant Professor, Associate Professor, and Professor positions at Harvard Medical School. I have also held Instructor and Lecturer Positions at Harvard College, Harvard School of Public

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Health, and Massachusetts Institute of Technology. Currently, I am the William Bosworth Castle Professor of Medicine at Harvard Medical School.

12. I have authored or co-authored over 350 published, peer-reviewed articles, as well as multiple abstracts and presentations, most of which are related to vaccinology, immunology, or virology.

13. In addition to my educational training and professional and research experiences described above, I have kept abreast of the fields of vaccinology, virology, and immunology by reading scientific literature; conferring with colleagues in the field; attending, presenting, and leading scientific conferences; and delivering regular and invited lectures.

14. Since 2004, I have served in an editorial capacity for several scientific journals including: Journal of Virology, Journal of Infectious Diseases, Clinical and Experimental Immunology, and Journal of Immunology. I have also served as a peer reviewer for several scientific journals including: Blood, Journal of Virology, Virology, Science, Nature Medicine, Nature Reviews Immunology, Journal of Infectious Diseases, Nature, Clinical Infectious Diseases, Journal of General Virology, Journal of Immunology, Nature Immunology, Clinical Vaccine and Immunology, Proceedings of the National Academy of Sciences, New England Journal of Medicine, Cell, Lancet Infectious Diseases, and Lancet.

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15. Starting in early 2016, my research group began work on developing a ZIKV vaccine. This led to a collaboration with researchers at the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, MD to develop and test vaccines against ZIKV. One of these vaccines was a purified inactivated ZIKV vaccine that stimulated neutralizing antibodies and conferred protection against ZIKV when administered to mice and rhesus monkey. EX1006, 476, Table 1; EX1062, 1129, FIG. 1. To my knowledge, we were the first group to publish data on a ZIKV vaccine in preclinical models.

16. Accordingly, I am an expert in the fields of virology and vaccine research, including vaccines for ZIKV. For that reason, I am qualified to provide an opinion as to what a POSA would have understood, known, or concluded as of the dates referenced herein. As an expert, I offer opinions throughout this declaration from the perspective of a POSA.

III. OVERVIEW OF THE '681 PATENT CLAIMS

17. I understand that claim 1 is the only independent claim that Takeda has challenged in the '681 patent. Claims 2–5, 10, 15, and 21–23 depend from claim 1. Claims 6, 7, and 9 depend from claim 5, and claim 8 depends from claim 7. Claims 11 and 13 depend from claim 10. Claim 12 depends from claim 11, and claim 14 depends from claim 13. Claims 24 and 25 depend from claim 2. It

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is my understanding that a dependent claim contains all the limitations of the claim from which it depends.

18. Claims 1, 5–11, and 15 of the '681 patent are generally directed to a ZIKV vaccine comprising a ZIKV having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72, wherein the ZIKV vaccine is capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects, wherein the neutralizing antibody titer is determined using a microneutralization assay (MN₅₀). EX1001, 445:34–41, 445:54–446:39, 446:53–448:10.

19. Claim 2 contains the additional requirement that the MN₅₀ is greater than 20. *Id.*, 445:42–43. Claim 3 contains the additional requirement that the MN₅₀ of greater than 15 is achieved in at least 75% of vaccinated subjects. *Id.*, 445:44–47. Claims 24 and 25 contain the additional requirement that the MN₅₀ is greater than 50 or greater than or equal to 90, respectively. *Id.*, 448:19–22.

20. Claim 4 contains the additional requirement that the ZIKV comprises an E protein having an amino acid sequence provided by any one of SEQ ID NOs: 14–69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14–69 and able to pack a virulent ZIKV. *Id.*, 445:48–53.

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21. Claim 12 contains the additional requirement that the ZIKV vaccine comprises aluminum hydroxide (i.e., $\text{Al}(\text{OH})_3$) as an adjuvant, wherein the aluminum hydroxide contains a concentration of less than 1.25 parts per billion (“ppb”) copper in the final pharmaceutical composition. *Id.*, 446:40–44.

22. Claims 13 and 14 contain the additional requirement that the ZIKV vaccine comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleotide acid molecule (I-ODN) (claim 13), wherein the peptide comprises the sequence KLKL₅KLK and the I-ODN comprises oligo-d(IC)₁₃ (claim 14). *Id.*, 446:45–52.

23. Claims 21–23 contain the additional requirement that the variant nucleic acid has at least 90%, 95%, or 99% identity to SEQ ID NO: 72, respectively. *Id.*, 448:11–18.

IV. OVERVIEW OF THE ART AND SUMMARY OF MY OPINIONS

24. For my analysis regarding the ability of claims 1–12, 15, and 21–25 to claim priority to the earliest foreign priority patent application listed on the face of the ’681 patent (European Patent Application No. 15202585 (“EP ’585”)), discussed in Section X.B, I considered the claimed ZIKV vaccines of the ’681 patent in relation to the general knowledge in the art as of December 23, 2015. It is my opinion that claims 1–12, 15, and 21–25 cannot claim priority to

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the December 23, 2015 filing date of EP '585 because EP '585 does not even mention ZIKV.

25. For my analysis of the obviousness of claims 1–12, 15, and 21–25 that is set forth in Section X.D, I considered the claimed ZIKV vaccines of the '681 patent in relation to the general knowledge in the art before March 18, 2016. The prior art references that I considered included (but were not limited to) the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), Thomas (EX1164), and Möhlen (EX1083).

26. **The WHO Zika Pipeline**² (EX1143) is a publication from the World Health Organization (“WHO”) that discloses “an emergency research and development plan [to] ... address[] research and development needs for novel means of [ZIKV] vector control, diagnostics, vaccines, and therapeutics” to address the “serious risk” of ZIKV. EX1143, 2.³ The WHO Zika Pipeline states that “[i]t [was] assumed that a ZIKV vaccine can be developed building on the same technologies that have been successfully used to develop human flavivirus

² Current Zika Product Pipeline, World Health Organization, *available at*: <https://web.archive.org/web/20160311113550/https://www.who.int/csr/research-and-development/zika-rd-pipeline.pdf> (Mar. 3, 2016).

³ Cites are to the original page numbers of the WHO Zika Pipeline.

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vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).” *Id.*, 5. The WHO Zika Pipeline also identifies eighteen active ZIKV vaccine programs, including “purified inactivated virus” vaccines, “[m]ost [of which were] building on existing flavivirus vaccine technology and know-how.” *Id.*

27. **Srivastava**⁴ (EX1163) discloses a successful technology that produced an immunogenic and protective purified inactivated vaccine for JEV, a flavivirus like ZIKV. Srivastava reported that their purified inactivated JEV vaccine stimulated neutralizing antibody titers in the range of 1,280–7,781 in 100% of vaccinated mice using a two-dose regimen. EX1163, Table 3. Srivastava’s JEV vaccine eventually became the FDA-approved product IXIARO[®] (Japanese Encephalitis Vaccine, Inactivated, Absorbed). EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48.

⁴ Srivastava, A.K., et al., “A Purified Inactivated Japanese Encephalitis Virus Vaccine Made in Vero Cells,” *Vaccine*, 19: 4557–4565 (2001).

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28. **Baronti**⁵ (EX1160) discloses the sequencing of a naturally occurring ZIKV strain, known as H/PF/2013, which was isolated from the serum of a subject infected by ZIKV during a ZIKV outbreak in 2013 in French Polynesia, and then grown on Vero cells. EX1160, 1. Baronti extracted the ZIKV RNA genome from ZIKV virions (i.e., viral particles)⁶ harvested from a cell culture supernatant and sequenced the virus's RNA genome. *Id.* Baronti states that "[t]he virus genome sequence ... [was] deposited in the GenBank database under the accession no. KJ776791" ("**the H/PF/2013 Sequence**"⁷ (EX1019)). EX1160, 1. Baronti discloses that the sequence obtained "includ[ed] the virus complete open reading frame (ORF) sequence." *Id.* As I discuss, the H/PF/2013 Sequence is 99% identical to the claimed SEQ ID NO: 72. *See* Section X.D.1.d, below.

⁵ Baronti, C., et al., "Complete Coding Sequence of Zika Virus from a French Polynesia Outbreak in 2013," *Genome Announcements*, 2(3): e00500-14 (2014).

⁶ Viruses exist outside of host cells as viral particles, i.e., virions. *See, e.g.,* EX1095, 168, FIG. 2.

⁷ GenBank: KJ776791.1, "Zika Virus Strain H/PF/2013 Polyprotein Gene, complete CDs," *available at*: <https://www.ncbi.nlm.nih.gov/nucleotide/KJ776791.1> (last modified June 13, 2014).

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29. **Thomas**⁸ (EX1164) discloses a microneutralization (“MN”) assay, which was an improved assay for detecting neutralizing antibodies stimulated by a vaccine. EX1164, 86. Thomas discloses that its MN assay improved upon the plaque reduction neutralization test (“PRNT”), which had traditionally been used in the art, by having higher throughput, being less labor-intensive, and less variable. *Id.* Thomas disclosed that its MN assay is sensitive for neutralizing antibody detection and has an estimated precision of 39% to 59%. *Id.*

A. Overview: Claims 1–12, 15, and 21–25 are not entitled to the December 23, 2015 filing date of EP ’585; and before the March 18, 2016 filing date of European Patent Application No. 16161068, the claims would have been obvious.

30. As discussed more fully below in Section X.D, claims 1–12, 15, and 21–23 are not entitled to the benefit of the December 23, 2015 filing date of EP ’585, because that application does not even mention ZIKV or ZIKV vaccines, let alone describe or enable the ZIKV vaccines claimed in the ’681 patent. Therefore, I have considered whether the ’681 patent claims would have been obvious before the filing date of the next patent application in the series, which is European Patent Application No. 16161068, filed March 18, 2016.

⁸ Thomas, S.J., et al., “A Phase II, Randomized, Safety and Immunogenicity Study of a Re-Derived, Live-Attenuated Dengue Virus Vaccine in Healthy Adults,” *Am. J. Trop. Med. Hyg.*, 88(1): 73–88 (2013).

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31. Before March 18, 2016, claims 1–11, 15, and 21–25 would have been obvious to a POSA in view of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas. Before March 18, 2016, a POSA would have recognized the need for a ZIKV vaccine from the WHO Zika Pipeline and general knowledge in the art. For example, the WHO Zika pipeline stated, “[g]iven the complexity of testing and using novel therapeutic drugs in pregnant women, it is unlikely at this stage that therapeutics [against ZIKV] will constitute a priority activity.” EX1143, 4. In contrast, the WHO Zika Pipeline acknowledged that vaccines could be targeted at pregnant woman, stating, “WHO has initiated a consultative process to develop a target product profile for a Zika vaccine for use in the emergency context and targeted at the protection of women in child-bearing age and pregnant women.” *Id.*, 5. The WHO Zika Pipeline suggested making a ZIKV vaccine using “the same technologies that have been successfully used to develop human flavivirus vaccines.” *Id.* A POSA, therefore, would have had a reason to use Srivastava’s flavivirus vaccine technology to develop a ZIKV vaccine, because it successfully resulted in a JEV vaccine, which eventually became the approved IXIARO product in 2009 and was safe, effective, and immunogenic due to it stimulating high neutralizing antibody titers, and economical to make. EX1163, 4563–4564, Table 3; EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48. A POSA who wanted to make a ZIKV vaccine, as

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suggested by the WHO Zika Pipeline, would have had a reason to use Srivastava's vaccine technology but substitute a known, infectious strain of ZIKV for the related flavivirus JEV. Baronti discloses such a known, infectious strain of ZIKV, and Baronti and the H/PF/2013 Sequence disclose its sequence.

32. A POSA would have also sought to make a ZIKV vaccine capable of stimulating high neutralizing antibody titers, e.g., > 1000 when following Srivastava's technology, because high titers were known in the art to be generally associated with better protection, and titers can wane over time, which could lead to a reduction in protection. EX1042, 708–709; EX1045, 1; *see* Section VII.E.4, below. A POSA would have also sought to obtain higher titers in as large a percentage of the vaccinated population as possible because it would have afforded broader coverage in the relevant population. Lastly, a POSA would have known the neutralizing antibody titers stimulated by a ZIKV vaccine could be assayed using an MN₅₀ assay, as described by Thomas, and a POSA would have had a reason to use such an MN₅₀ assay because it would have provided for higher throughput and less interassay variability compared to PRNT assays. EX1164, 86.

33. A POSA would have had a reasonable expectation of success in making a ZIKV vaccine comprising the H/PF/2013 ZIKV strain having an RNA genome comprising a sequence corresponding to the H/PF/2013 Sequence and capable of stimulating a MN₅₀ titer of greater than 90 in at least 70% of subjects.

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This is because the WHO Zika Pipeline stated that “technologies that have been successfully used to develop human flavivirus vaccine” can be used to develop a ZIKV vaccine. EX1143, 5. A POSA who was aware of general knowledge in the art would have also been aware of statements from prominent figures in the infectious disease and vaccinology fields, such as Dr. Anthony Fauci and Dr. Stanley Plotkin, which were consistent with the WHO Zika Pipeline’s statement that existing flavivirus technologies would be expected to be successfully applied to make a ZIKV vaccine. EX1162, 1–2; EX1052, 543.

34. Further, Srivastava discloses a flavivirus vaccine technology that stimulated neutralizing antibody titers $> 1,000$ in 100% of vaccinated subjects when administered twice. EX1163, Table 3. Given that ZIKV and other flaviviruses are closely related, a POSA would have reasonably expected that a ZIKV vaccine made following the technology Srivastava used for JEV and administered in a two-dose schedule would similarly stimulate high neutralizing antibody titers, i.e., > 1000 , in at least 70% subjects. A POSA would also have known from the general knowledge in the art that other flavivirus vaccines could stimulate high neutralizing antibody titers in at least 70% of vaccinated subjects when administered in a two-dose schedule. *See* Section VII.E, below.

35. As discussed more fully below in Section X.E, claim 12 would have been obvious in view the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013

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Sequence, Thomas, and **Möhlen**⁹ (EX1083). Srivastava discloses inactivating virions with formaldehyde and then neutralizing the formaldehyde with sodium bisulfite at the end of the virion inactivation process. EX1163, 4559. Srivastava also discloses formulating its vaccine with aluminum hydroxide (an aluminum salt) as an adjuvant. *Id.*, 4560. A POSA following Srivastava's vaccine technology to make a ZIKV vaccine, therefore, would have also (1) performed Srivastava's formaldehyde-inactivation process that neutralizes the formaldehyde with sodium bisulfite and (2) formulated the vaccine with aluminum hydroxide.

36. Möhlen discloses that heavy metals, such as copper, can react with residual sulfite used to neutralize the formaldehyde used to inactivate a virus, and cause oxidative degradation of the inactivated virions in the vaccine. EX1083, 11:14–22, 16:1–11. It was also general knowledge in the art that metal ions, such as copper, if present in biopharmaceutical drugs products, such as vaccine, can result in physicochemical degradation of the active molecule, e.g., antigen, over long-term storage. EX1077, 1173, 1177. Möhlen found that the stability of virions in a vaccine composition that includes an aluminum salt can be dependent on the

⁹ Möhlen, M., et al., International Patent Appl. Publ. No. WO 2013/083726 A1, "Aluminum Compounds for Use in Therapeutics and Vaccines" (filed Dec. 6, 2012; published June 13, 2013).

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content of heavy metals, such as copper, in the composition. EX1083, 3:23–28.

Möhlen discloses that an inactivated JEV virus in a JEV vaccine formulation comprising aluminum hydroxide and < 3 ppb copper was more stable than were formulations containing greater amounts of copper. *Id.*, 33:21–30, Tables 12–15, FIG. 8. Möhlen further discloses that

The amount of Cu [i.e., copper] in a composition or method of the inventions is in a particularly preferred embodiment less than 1,25 ppb [i.e., 1.25 ppb]¹⁰ based on the weight of the aqueous composition. In a particularly preferred embodiment said aqueous composition comprises Cu at a level that is below the detection limit of the method for the detection of copper.

Id., 27:27–28:2.

37. A POSA, therefore, using Srivastava’s vaccine technology that inactivates the virus using formaldehyde and then neutralizes the formaldehyde with sodium bisulfite would have had a reason to ensure that the level of copper in the aluminum hydroxide used in the ZIKV vaccine was below the limit of detection, including being below 1.25 ppb based on the weight of the aqueous

¹⁰ Möhlen is from a European group. A POSA would have understood that it is European style to use a comma where in the United States we would use a decimal point.

composition, as disclosed by Möhlen, as a matter of quality of assurance because it would reduce the chance that degradation of the ZIKV antigen would occur.

38. Möhlen discloses that “[m]ethods for measuring the level of one or more heavy metals in an aqueous solution are known in the art,” including “mass spectrometry, such as inductively-coupled-plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (F-AAS), and/or graphite furnace atomic absorption spectrometry (GF-AAS).” *Id.*, 28:18–25. A POSA, thus, would have had a reason to assess the level of copper in the vaccine composition using such methods to ensure that the level of copper was below the limit of detection, including being less than 1.25 ppb based on a final pharmaceutical composition comprising the ZIKV. And a POSA would have done so because it would have helped ensure production of a stable vaccine. Moreover, a POSA would have had a reasonable expectation of success in making a ZIKV vaccine having less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV because aluminum hydroxide was a commonly-used adjuvant in vaccines and Möhlen discloses techniques to measure copper levels, which would have allowed a POSA to determine if the aluminum hydroxide used results in copper levels above 1.25 ppb. *Id.* A POSA would simply have needed to change the lot of aluminum hydroxide used if copper levels were too high. Indeed, Möhlen discloses

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assaying lots of aluminum hydroxide for heavy metals, including copper, and finding lots with both high and low amounts of heavy metals. *Id.*, 26:7–13.

39. As explained in detail in Section X.F, I have also considered whether there is any objective evidence (i.e., objective indicia) supporting patentability of any of claims 1–12, 15, and 21–25 of the '681 patent.

B. Overview: Claims 1–15 and 21–25 are not entitled to the December 23, 2016 filing date of the '664 PCT; and before the March 10, 2020 filing date of the '862 application, the claims lacked novelty in view of the June 29, 2017 publication of WO '225.

40. In addition to forming the opinions summarized in Section IV.A above, I have also separately concluded that claims 1–15 and 21–25 lack novelty, as discussed in more detail in XI.C. Before assessing lack of novelty, I first considered whether any of claims 1–15 and 21–25 is entitled to the benefit of the December 23, 2016 filing date of the '664 PCT¹¹. As discussed in further detail below in paragraphs ¶¶251–327 in Section XI.B, I conclude that '681 patent claims are not entitled to the benefit of the filing date of the '664 PCT because the '664 PCT fails to both (i) *describe the full scope* of any of claims 1–15 or 21–25 of the

¹¹ I understand that the '664 PCT application was assigned U.S. Application No. 16/063,007, as shown on the face of the '681 patent. EX1001, Item (63).

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'681 patent and (ii) *enable* the *full scope* of any of claims 1–15 or 21–25 of the '681 patent¹².

41. In considering whether the '664 PCT, as of its December 23, 2016 filing date, adequately describes the invention claimed in the '681 patent, I have concluded that the '664 PCT would *not* have conveyed to a POSA that the inventors had possession of the vast genus of ZIKV vaccines encompassed by claims 1–15 and 21–25. Additionally, I have concluded that the '664 PCT, as of its December 23, 2016 filing date, would *not* have enabled a POSA to make and use the full scope of claims 1–15 and 21–25 of the '681 patent without undue experimentation.

42. As described further in Section XI.B.1.b.i, claims 1–15 and 21–25 encompass an unwieldy number of ZIKV vaccines due to the unwieldy number of nucleic acids claimed. However, as I explain in detail below, the '664 PCT does not provide a representative number of species within the scope of claims 1–15 and 21–25 of the '681 patent, nor does it disclose any correlation between the claimed function and the structure of the Zika virus vaccines. Therefore, the '664 PCT does

¹² I understand that the requirements to describe and enable the full scope of the claims are two separate requirements, which I address further in Sections XI.B.1 and XI.B.2.

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not convey to a POSA that the inventors possessed the enormous genus of the claimed ZIKV vaccines at the time they filed the '664 PCT. Further, regarding enablement, a POSA would have been forced to synthesize and screen an unwieldy number of ZIKV vaccine candidates to determine which embodiments of the '681 patent's claims 1–15 and 21–25 are operative. The amount of experimentation required to accomplish this task would have been undue as of the filing date of the '664 PCT. Therefore, for each of these independent reasons, claims 1–15 and 21–25 of the '681 patent are not entitled to the benefit of the December 23, 2016 filing date of the '664 PCT. Accordingly, in assessing novelty in view of the prior art, I have considered the knowledge in the art just before the next filing date in the series, i.e., just before the March 10, 2020 filing date of the '862 application.¹³

43. I understand that if, as I concluded, claims 1–15 and 21–25 are not entitled to the benefit of any filing date before March 10, 2020, International

¹³ I understand that if claims 1–15 and 21–25 of the '681 patent cannot claim priority to the '664 PCT, then they cannot claim priority to any of the earlier priority applications listed on the face of the '681 patent. I do not offer an opinion in this declaration as to whether the claims would have been adequately described or enabled *as of* the March 10, 2020 filing date of the '862 application.

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Publication WO 2017/109225 A1 (“WO ’225”; EX1008), published on June 29, 2017, according to its face page, becomes prior art to those claims. I also understand that WO ’225 is, as stated on its face page, an International Publication of the ’664 PCT (PCT/EP2016/082664). EX1008, Face Page, Item (21). As discussed in more detail in Section XI.C, WO ’225 discloses a single working example that is encompassed by claims 1–15 and 21–25 of the ’681 patent and that a POSA would have been able to make and use without undue experimentation before the March 10, 2020 filing date of the ’681 patent. Thus, WO ’225 discloses and enables an embodiment meeting each and every limitation of claims 1–15 and 21–25, arranged as claimed. WO ’225, therefore, anticipates claims 1–15 and 21–25.

V. DOCUMENTS CONSIDERED IN FORMULATING MY OPINIONS

44. In formulating my opinions, I have considered all the references and documents cited herein, including those below. I have accessed the websites that are identified in the table below and the corresponding exhibits are true and correct copies of what I observed on the websites when accessed. Exhibits EX1005–EX1009, EX1019–EX1025, EX1027–EX1078, EX1080–EX1085, EX1087–EX1090, EX1091–EX1096, EX1098–EX1101, EX1103–EX1121, EX1131–EX1138, EX1142, EX1160, EX1162–EX1164, EX1177–1180, and EX1190–EX1192 are materials expert witnesses would routinely rely upon to

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ascertain the state of the art at certain time periods, such as in the opinions set forth herein.

<i>Exhibit #</i>	<i>Description</i>
1001	Barbero Calzado, J., et al., U.S. Patent No. 11,219,681, “Zika Virus Vaccine” (filed March 10, 2020; issued Jan. 11, 2022)
1004	File History of U.S. Patent No. 11,219,681
1005	Yoshii, K., et al., “A Conserved Region in the prM Protein Is a Critical Determinant in the Assembly of Flavivirus Particles,” <i>J. General Virology</i> , 93: 27–38 (2012)
1006	Larocca, R.A., et al., “Vaccine Protection Against Zika Virus from Brazil,” <i>Nature</i> , 536: 474–478 (2016)
1007	Halloway, T., “WRAIR Technology Helps Create Japanese Encephalitis Vaccine,” <i>available at</i> : https://web.archive.org/web/20150920175227/http://www.army.mil/article/19643/WRAIR_Technology_helps_create_Japanese_Encephalitis_Vaccine/ (last accessed November 9, 2022)
1008	Barbero Calzado, J., et al., International Patent Appl. Publ. No. WO 2017/109225 A1, “Zika Virus Vaccine” (filed Dec. 23, 2016; published June 29, 2017)
1009	BLAST alignment of SEQ ID NO: 47 to SEQ ID NO: 73
1014	European Patent Appl. No. 15202585.4 (filed Dec. 23, 2015)
1015	European Patent Appl. No. 16161068.8 (filed Mar. 18, 2016)
1016	European Patent Appl. No. 16176025.1 (filed June 23, 2016)
1017	European Patent Appl. No. 16176049.1 (filed June 23, 2016)
1018	European Patent Appl. No. 16182845.4 (filed Aug. 4, 2016)
1019	GenBank: KJ776791.1, “Zika Virus Strain H/PF/2013 Polyprotein Gene, complete CDs,” <i>available at</i> : https://www.ncbi.nlm.nih.gov/nuccore/KJ776791.1 (last modified June 13, 2014) (last accessed November 9, 2022)
1020	BLAST alignment of KJ776791.1 to SEQ ID NO: 72

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Exhibit #	Description
1021	Watanaveeradej, V., et al., “Safety and Immunogenicity of a Rederived, Live-Attenuated Dengue Virus Vaccine in Healthy Adults Living in Thailand: A Randomized Trial,” <i>Am. J. Trop. Med. Hyg.</i> , 91(1): 119–128 (2014)
1022	Orenstein, W.A., et al., “Global Vaccination Recommendations and Thimerosal,” <i>Pediatrics</i> , 131(1): 149–151 (2012)
1023	Eckels, K.H., et al., “Japanese Encephalitis Virus Live-Attenuated Vaccine, Chinese Strain SA14-14-2; Adaptation to Primary Canine Kidney Cell Cultures and Preparation of a Vaccine for Human Use,” <i>Vaccine</i> , 6(6): 513–518 (1988)
1024	Martinez, L.J., et al., “Safety and Immunogenicity of a Dengue Virus Serotype-1 Purified-Inactivated Vaccine: Results of a Phase 1 Clinical Trial,” <i>Am. J. Trop. Med. Hyg.</i> , 93(3): 454–460 (2015)
1025	WHO Director-General Summarizes the Outcome of the Emergency Committee Regarding Clusters of Microcephaly and Guillain-Barré Syndrome, World Health Organization, (Feb. 1, 2016), <i>available at</i> : https://web.archive.org/web/20160204040311/http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/ (last accessed November 9, 2022)
1027	Li, X-F, et al., “Complete Genome Sequence of a Chikungunya Virus Isolated in Guangdong, China,” <i>J. Virology</i> , 86(16): 8904–8905.
1028	Putnak, R., et al. “Development of a Purified, Inactivated, Dengue-2 Virus Vaccine Prototype in Vero Cells: Immunogenicity and Protection in Mice and Rhesus Monkeys,” <i>J. Infectious Diseases</i> , 174: 1176–1184 (1996)
1029	Musso, D., et al., “Potential Sexual Transmission of Zika Virus,” <i>Emerging Infectious Diseases</i> , 21(2): 359–361
1030	Cox, B.D., et al., “Predicting Zika Virus Structural Biology: Challenged and Opportunities for Intervention,” <i>Antiviral Chemistry & Chemotherapy</i> , 24(3–4): 118–126 (2015)
1031	Duffy, M.R., “Zika Virus Outbreak on Yap Island, Federated States of Micronesia,” <i>New England J. of Med.</i> , 360(24): 2536–2543

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Exhibit #	Description
1032	Cao-Lormeau, V. and Musso, D., “Emerging Arboviruses in the Pacific,” <i>The Lancet</i> , 384: 1571–1572 (2014)
1033	Zika Virus Microcephaly and Guillain-Barré Syndrome, Situation Report, World Health Organization, <i>available at</i> : https://web.archive.org/web/20160320210243/http://apps.who.int/iris/bitstream/10665/204633/1/zikasitrep_17Mar2016_eng.pdf?ua=1 (Mar. 17, 2016) (last accessed November 9, 2022)
1034	Maurice, J., “WHO Reveals its Shopping List for Weapons Against Zika,” <i>The Lancet</i> 387: 733 (2016)
1035	Besnard, M., et al., “Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014,” 19(13): pii=20751 (2014)
1036	Monath, T.P., “Inactivated Yellow Fever 17D Vaccine: Development and Nonclinical Safety, Immunogenicity and Protective Activity,” <i>Vaccine</i> , 28: 3827–3840 (2010)
1037	Rasmussen, S.A., “Vaccines and Pregnancy: Past, Present, and Future,” <i>Seminars in Fetal & Neonatal Med.</i> , 19: 161–169 (2014)
1038	Plotkin, S.A. and Plotkin, S.L., “The Development of Vaccines: How the Past Led to the Future,” <i>Nature Reviews Microbiology</i> 9: 889–893 (2011)
1039	Heinz, F.X. and Stiasny, K., “Flaviviruses and Flavivirus Vaccines,” <i>Vaccine</i> 30: 4301–4306 (2012)
1040	Shan, C., “Zika Virus: Diagnosis, Therapeutics, and Vaccine,” <i>ACS Infectious Diseases</i> , 2: 170–172 (2016)
1041	Ishikawa, T., et al., “A Review of Successful Flavivirus Vaccines and the Problems with Those Flaviviruses for Which Vaccines are Not Yet Available,” <i>Vaccine</i> , 32: 1326–1337 (2014)
1042	Burton, D.R., “Antibodies, Viruses and Vaccines,” <i>Nature Review Immunology</i> 2: 706–713 (2002)
1043	Roehrig, J.T., et al., “Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses,” <i>Viral Immunology</i> , 21(2): 123–132 (2008)

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Exhibit #	Description
1044	Laurie, K.L., et al., “International Laboratory Comparison of Influenza Microneutralization Assays for A(H1N1)pdm09, A(H3N2), and A(H5N1) Influenza Viruses by CONSIZE,” <i>Clinical & Vaccine Immunology</i> , 22(8): 957–964 (2015)
1045	Klasse, P.J., “Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives,” <i>Advances in Biology</i> 2014(157895): 24 pages (2014)
1046	Bauer, K., “A Phase II, Randomized, Safety and Immunogenicity Trial of a Re-Derived, Live-Attenuated Dengue Virus Vaccine in Healthy Children and Adults Living in Puerto Rico,” <i>Am. J. Trop. Med. Hyg.</i> , 93(3): 441–453 (2015)
1047	Hombach, J., et al., “Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2–3 September, 2004,” <i>Vaccine</i> , 23: 5205–5211
1048	Lindenbach, B.D. and Rice, C.M., “Molecular Biology of Flaviviruses,” <i>Advances in Virus Research</i> 59: 23–61 (2003)
1049	Tauber, E., et al., “Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial,” <i>The Lancet</i> , 370: 1847–1853 (2007)
1050	Brinton, M.A. and Basu, M., “Functions of the 3’ and 5’ Genome RNA Regions of Members of the Genus Flavivirus,” <i>Virus Research</i> 206: 108–119 (2015)
1051	Okada, K., et al., “Safety and Immunogenicity of a Freeze-Dried, Cell Culture-Derived Japanese Encephalitis Vaccine (Inactivated) (JEBIK®V) in Children,” <i>Vaccine</i> ,
1052	Cohen, J., “The Race for a Zika Vaccine Is On,” <i>Science</i> 351(6273): 543–544 (2016)
1053	GenBank: AY632535.2, “Zika Virus Strain MR-766, Complete Genome,” <i>available at</i> : https://www.ncbi.nlm.nih.gov/nucleotide/AY632535 (last modified Nov. 23, 2010) (last accessed November 9, 2022)

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Exhibit #	Description
1054	GenBank: KU501215.1, “Zika Virus Strain PRVABC59, Complete Genome,” <i>available at</i> : https://www.ncbi.nlm.nih.gov/nuccore/KU501215.1 (last modified Feb. 1, 2016) (last accessed November 9, 2022)
1055	GenBank: KU497555.1, “Zika Virus Isolate Brazil-ZKV2015, Complete Genome,” <i>available at</i> : https://www.ncbi.nlm.nih.gov/nuccore/KU497555.1 (last modified Feb. 18, 2016) (last accessed November 9, 2022)
1056	Zhang, X., et al., “Genetic and Biochemical Characterizations of Zika Virus NS2A Protein,” <i>Emerging Microbes & Infections</i> , 8(1): 585–602 (2019)
1057	Sample GenBank Record <i>available at</i> : https://www.ncbi.nlm.nih.gov/genbank/samplerecord/#ModificationDateB (last accessed November 9, 2022)
1058	Musso, D., “Zika Virus Transmission from French Polynesia to Brazil,” <i>Emerging Infectious Diseases</i> 21(10): 1887 (2015)
1059	Schuller, E., et al., “Comparison of a Single, High-Dose Vaccination Regimen to the Standard Regimen for the Investigational Japanese Encephalitis Vaccine, IC51: A Randomized, Observer-Blind, Controlled Phase 3 Study,” <i>Vaccine</i> , 27: 2188–2193 (2009)
1060	Schuller, E., et al., “Long-term Immunogenicity of the New Vero Cell-Derived, Inactivated Japanese Encephalitis Virus Vaccine IC51 Six and 12 Month Results of a Multicenter Follow-Up Phase 3 Study,” <i>Vaccine</i> , 26: 4382–4386 (2008)
1061	Assessment Report for IXIARO, European Medicines Agency, (2009), <i>available at</i> : https://www.ema.europa.eu/en/documents/assessment-report/ixiaro-epar-public-assessment-report_en.pdf (last accessed November 9, 2022)
1062	Abbink, P., “Protective Efficacy of Multiple Vaccine Platforms Against Zika Virus Challenge in Rhesus Monkeys,” <i>Science</i> , 353(6304) 1129–1132 (2016)

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Exhibit #	Description
1063	Schlegl, R., et al., “Influence of Elemental Impurities in Aluminum Hydroxide Adjuvant on the Stability of Inactivated Japanese Encephalitis Vaccine, IXIARO [®] ,” <i>Vaccine</i> , 33: 5989–5996 (2015)
1064	Vaccines, Blood & Biologics: IXIARO [®] - Summary Basis for Regulatory Action, (March 30, 2009), <i>available at</i> : https://web.archive.org/web/20090619154725/https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm142580.htm (last accessed November 9, 2022)
1065	Duggan, S.T. and Plosker, G.L., “Japanese Encephalitis Vaccine (Inactivated, Adsorbed) [IXIARO [®]],” <i>Drugs</i> 69(1): 115–122 (2009)
1066	Schellack, C., et al., “IC31, A Novel Adjuvant Signaling via TLR9, Induces Potent Cellular and Humoral Immune Responses,” <i>Vaccine</i> , 24: 5461–5472 (2006)
1067	Annunziato, F., et al., “The 3 Major Types of Innate and Adaptive Cell-Mediated Effector Immunity,” <i>J. Allergy & Clinical Immunology</i> , 135(3): 626–635 (2014)
1068	Smith, D.W. and Mackenzie, J., “Zika Virus and Guillain-Barré Syndrome: Another Viral Cause to Add to the List,” <i>The Lancet</i> , 387: 1486–1488 (2016)
1069	Benson, D.A., et al., “GenBank,” <i>Nucleic Acids Research</i> , 41: D36–D42 (2012)
1070	Samarasekera, U. and Triunfol, M., “Concern Over Zika Virus Grips the World,” <i>The Lancet</i> 387:521-524 (2016)
1071	BLAST alignment of translation of KJ776791.1 ORF to SEQ ID NO: 73
1072	Fox, M., “Could We Have A Zika Vaccine Soon?” NBCNews.com (Jan. 30, 2016), <i>available at</i> : https://web.archive.org/web/20160130130441/https://www.nbcnews.com/storyline/zika-virus-outbreak/could-we-have-zika-vaccine-soon-n507186 (last accessed November 9, 2022)
1073	Fauci, A.S. and D.M. Morens, “Zika Virus in the Americas – Yet Another Arbovirus Threat,” <i>The New England Journal of Medicine</i> , 374(7): 601–604 (2016)

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Exhibit #	Description
1074	Dyer, O., “Zika Vaccine Could Be In Production By Year’s End, Says Maker,” <i>BMJ</i> 352:i630 (2016)
1075	BLAST alignment of PRVABC59 to SEQ ID NO: 72
1076	BLAST alignment of translation of PRVABC59 ORF to SEQ ID NO: 40
1077	Kumar, S., et al., “Metal Ion Leachates and the Physio-Chemical Stability of Biotherapeutic Drug Products,” <i>Current Pharmaceutical Design</i> , 20: 1173–1181 (2014)
1078	Declaration of Scott Bailey, Ph.D.
1079	<i>Curriculum Vitae</i> of Scott Bailey, Ph.D.
1080	Luca, V.C., et al., “Crystal Structure of the Japanese Encephalitis Virus Envelope Protein,” <i>J. Virology</i> , 86(4): 2337–2346 (2012)
1081	Klema, V.J., et al., “Dengue Virus Nonstructural Protein 5 (NS5) Assembles into a Dimer with a Unique Methyltransferase and Polymerase Interface,” <i>PLOS Pathogens</i> , 12(2): e1005451 (2016)
1082	Mukhopadhyay, S., et al., “A Structural Perspective of the <i>Flavivirus</i> Life Cycle,” <i>Nature Review Microbiology</i> , 3: 13–22 (2005)
1083	Möhlen, M., et al., International Patent Appl. Publ. No. WO 2013/083726 A1, “Aluminum Compounds for Use in Therapeutics and Vaccines” (filed Dec. 6, 2012; published June 13, 2013)
1084	GenBank entry for KJ776791.2 <i>available at</i> : https://www.ncbi.nlm.nih.gov/nuccore/KJ776791.2 (last modified Aug. 31, 2016) (last accessed November 9, 2022)
1085	Ledgerwood, J.E., “A West Nile Virus DNA Vaccine Utilizing a Modified Promoter Induces Neutralizing Antibody in Younger and Older Healthy Adults in a Phase I Clinical Trial,” <i>J. Infectious Diseases</i> , 203: 1396–1404 (2011)
1087	Delrue, I., et al., “Inactivated Virus Vaccines from Chemistry to Prophylaxis: Merits, Risks and Challenges,” <i>Expert Review of Vaccines</i> , 11(6): 695–719 (2012)

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Exhibit #	Description
1088	Stephenson, K.E., et al., “Safety and Immunogenicity of a Zika Purified Inactivated Virus Vaccine Given Via Standard, Accelerated, or Shortened Schedules: A Single-Centre, Double-Blind, Sequential-Group, Randomised, Placebo-Controlled, Phase 1 Trial,” <i>Lancet Infectious Disease</i> , 20: 1061–1070 (2020)
1089	Collette, N.M., et al., “Single Amino Acid Mutations Affect Zika Virus Replication In Vitro and Virulence In Vivo,” <i>Viruses</i> , 12(11): 1295 (2020)
1091	DiNunno, N.M., et al., “Identification of a Pocket Factor That Is Critical to Zika Virus Assembly,” <i>Nature Communications</i> , 11: 4953 (2020)
1092	Barnard, T.R., et al., “Molecular Determinants of Flavivirus Virion Assembly,” <i>Trends in Biochemical Sciences</i> , 46(5): 378–390 (2021)
1093	Tan, T.Y., et al., “Capsid Protein Structure in Zika Virus Reveals the Flavivirus Assembly Process,” <i>Nature Communications</i> , 11: 895 (2020)
1094	Ma, X., et al., “Identification and Characterization of Key Residues in Zika Virus Envelope Protein for Virus Assembly and Entry,” <i>Emerging Microbes & Infections</i> , 11(1): 1604–1620 (2022)
1095	Pierson, T.C. and Diamond, M.S., “Degrees of Maturity: The Complex Structure of Biology of Flaviviruses,” <i>Current Opinion in Virology</i> , 2: 168–175 (2012)
1096	Barzon, L., et al., “Zika Virus: From Pathogenesis to Disease Control,” <i>FEMS Microbiology Letters</i> , 363(18): 1–17 (2016)
1098	BLAST alignment of KJ776791.2 to SEQ ID NO: 72
1099	Poland, G.A., et al., “Zika Vaccine Development: Current Status,” <i>Mayo Clinic Proceedings</i> , 94(12): 2572–2586 (2019)
1100	Aubry, F., et al., “Flavivirus Reverse Genetic Systems, Construction Techniques and Applications: A Historical Perspective,” <i>Antiviral Research</i> , 114: 67–85 (2015)
1101	Shan, C., et al., “An Infectious cDNA Clone of Zika Virus to Study Viral Virulence, Mosquito Transmission, and Antiviral Inhibitors,” <i>Cell Host & Microbe</i> , 19: 891–900 (2016)

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Exhibit #	Description
1103	Bennett, J., “How Many Particles Are in the Observable Universe?”, <i>Popular Mechanics</i> , July 11, 2017, <i>available at</i> : https://www.popularmechanics.com/space/a27259/how-many-particles-are-in-the-entire-universe/ (last visited December 14, 2022)
1104	File History of U.S. Patent Application No. 16/063,007
1105	BLAST alignment of SEQ ID NO: 2 and SEQ ID NO: 72
1106	BLAST alignment of SEQ ID NO: 3 and SEQ ID NO: 72
1107	BLAST alignment of SEQ ID NO: 4 and SEQ ID NO: 72
1108	BLAST alignment of SEQ ID NO: 5 and SEQ ID NO: 72
1109	BLAST alignment of SEQ ID NO: 6 and SEQ ID NO: 72
1110	BLAST alignment of SEQ ID NO: 7 and SEQ ID NO: 72
1111	BLAST alignment of SEQ ID NO: 8 and SEQ ID NO: 72
1112	BLAST alignment of SEQ ID NO: 9 and SEQ ID NO: 72
1113	BLAST alignment of SEQ ID NO: 10 and SEQ ID NO: 72
1114	BLAST alignment of SEQ ID NO: 11 and SEQ ID NO: 72
1115	BLAST alignment of SEQ ID NO: 12 and SEQ ID NO: 72
1116	BLAST alignment of SEQ ID NO: 13 and SEQ ID NO: 72
1117	BLAST alignment of SEQ ID NO: 11 and SEQ ID NO: 12
1118	Nema, S. and Brendel, R.J., “Excipients and Their Role in Approved Injectable Products: Current Usage and Future Directions,” <i>PDA J. Pharmaceutical Science & Technology</i> , 65(3): 287–332 (2011)
1119	Chen, R. and Vasilakis, N., “Dengue – Quo tu et quo voids?,” <i>Viruses</i> , 3: 1562–1608 (2011)
1120	Manikandan, S., “Measures of Central Tendency: The Mean,” <i>J. Pharmacology & Pharmacotherapeutics</i> , 2(2): 140–142 (2011)
1121	COLLEGE PHYSICS 790 (Franklin Miller, Jr. ed., 4th ed. 1977)
1131	INTRODUCTORY STATISTICS (Prem S. Mann ed., 7th ed. 2010)
1132	Summation Notation, <i>available at</i> : http://www.columbia.edu/itc/sipa/math/summation.html)

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Exhibit #	Description
1133	Harvey, A. and Howell, E., “How Many Stars Are in the Universe?,” <i>available at</i> : https://www.space.com/26078-how-many-stars-are-there.html
1134	Lopez, M.J. and Mohiuddin, S.S., “Biochemistry, Essential Amino Acids,” <i>NCBI Bookshelf</i> (2022), <i>available at</i> : https://www.ncbi.nlm.nih.gov/books/NBK557845/?report=printable
1135	Shchelochkov, O.A., “Open Reading Frame,” <i>National Human Genome Research Institute</i> (2002), <i>available at</i> : https://www.genome.gov/genetics-glossary/Open-Reading-Frame
1136	Genomics Education Programme, “Read,” NHS Health Education England, <i>available at</i> : https://www.genomicseducation.hee.nhs.uk/glossary/read/
1137	Kurnaz, M.L., et al., “A Statistical Analysis of the Robustness of Alternate Genetic Coding Tables,” <i>Int. J. Mol. Sci.</i> , 9: 679–697 (2008)
1138	Enfissi, A., et al., “Zika Virus Genome from the Americas,” <i>The Lancet</i> , 387: 227–228 (2016)
1143	Copy of <i>Zika Pipeline</i> archived on March 11, 2016 by the Internet Archive, <i>available at</i> : https://web.archive.org/web/201603111113550/http://www.who.int/csr/research-and-development/zika-rd-pipeline.pdf , accessed and obtained on November 10, 2022
1160	Baronti, C., et al., “Complete Coding Sequence of Zika Virus from a French Polynesia Outbreak in 2013,” <i>Genome Announcements</i> , 2(3): e00500-14 (2014)
1162	Sifferlin, A., “U.S. Launches ‘Full-Court Press’ for a Zika Vaccine,” <i>TIME</i> (Jan. 21, 2016, 3:24 PM), <i>available at</i> : https://time.com/4188973/zika-virus-vaccine-nih/
1163	Srivastava, A.K., et al., “A Purified Inactivated Japanese Encephalitis Virus Vaccine Made in Vero cells,” <i>Vaccine</i> , 19(3): 4557–4565 (2001)

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Exhibit #	Description
1164	Thomas, S. J., et al., “A Phase II, Randomized, Safety and Immunogenicity Study of a Re-derived, Live-attenuated Dengue Virus Vaccine in Healthy Adults,” <i>American Journal of Tropical Medicine and Hygiene</i> , 88(1): 73–88 (2013)
1177	Plevka, P., et al., “Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres,” <i>EMBO Reports</i> , 12(6): 602-606 (2011)
1178	Rodrigues, A.F., et al., “Viral vaccines and their manufacturing cell substrates: New trends and designs in modern vaccinology,” <i>Biotechnol. J.</i> , 10: 1329-1344 (2015)
1179	Souza, M.C.O., et al., “Production of yellow fever virus in microcarrier-based Vero cell cultures,” <i>Vaccine</i> , 27: 6420-6423 (2009)
1180	Pereira, R.C., et al., “An Inactivated Yellow Fever 17DD Vaccine Cultivated in Vero Cell Cultures,” <i>Vaccine</i> , 33: 4261-4268 (2015)
1190	BLAST alignment of KU955593.1 to SEQ ID NO: 72
1191	BLAST alignment of FSS13025 NS2A protein and SEQ ID NO: 73 (annotated)
1192	BLAST alignment of MR-766 E protein and SEQ ID NO: 73 (annotated)

VI. PERSON OF ORDINARY SKILL IN THE ART

45. As an expert, I have offered opinions throughout this declaration from the perspective of a POSA. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art, and is a person of ordinary creativity.

46. It is my opinion that a POSA relevant to the '681 patent would have had knowledge of the scientific literature related to virology, including

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flaviviruses, and vaccines and have had skills relating to methods for developing and testing viral vaccines, including flavivirus vaccines. A POSA also would have been knowledgeable of laboratory techniques and strategies for passaging viruses, designing vaccines, conducting *in vitro* studies testing and analyzing immune responses to vaccines, and performing and interpreting preclinical and clinical vaccine studies.

47. Typically, a POSA would have had an M.D. and/or a Ph.D. degree, specializing in infectious diseases, virology, immunology, vaccinology, or a related discipline (e.g., biology, molecular biology, biochemistry, microbiology, structural biology, pathology), as well as, typically, at least 3 years of research and development experience specializing in developing and testing antiviral vaccines.

48. A POSA would also have worked as part of a multi-disciplinary team and drawn upon not only his or her own skills, but also taken advantage of certain specialized skills of others in the team, to solve a given problem. For example, such a team may be comprised of a bioinformatician, molecular biologist, epidemiologist, manufacturing specialist, and/or pharmaceutical formulator.

VII. STATE OF THE ART BEFORE MARCH 18, 2016

49. I present below a summary of what the art would have conveyed to a POSA before March 18, 2016, with regard to flaviviruses such as ZIKV and the development of vaccines targeting such viruses.

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A. The art recognized that ZIKV was a global threat because ZIKV infections were associated with fetal abnormalities and neurological disorders.

50. ZIKV belongs to the *Flaviviridae* family of positive strand RNA viruses that include human pathogens such as West Nile virus (“WNV”), Yellow Fever virus (“YFV”), Japanese Encephalitis virus (“JEV”), and Dengue virus (“DENV”). EX1029, 359; EX1030, 118; EX1040, 170.

51. Before a 2007 outbreak of ZIKV on Yap Island in the Federated States of Micronesia, “[n]o outbreaks and only 14 cases of human Zika virus disease [had] been [] documented.” EX1031, 2538. In 2013–2014, ZIKV caused a large epidemic in French Polynesia. EX1032. In 2015, ZIKV spread to Brazil, causing an estimated 0.4–1.3 million cases of infection. EX1033, 2. “Phylogenetic studies showed that the closest strain to the one that emerged in Brazil was isolated from samples from case-patients in French Polynesia and spread among the Pacific Islands ... both strains belong to the Asian lineage.” EX1058, 1887. Before March 18, 2016, the virus had spread to at least 33 countries and territories in the Americas. EX1033, 1. Until that time, ZIKV infection had been only “characterized by [incidences of] rash, conjunctivitis, and arthralgia.” EX1031, 2536. But as a result of the 2013–2015 outbreaks, the WHO stated that “[t]he mounting evidence from observational, cohort and case-control studies indicates

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that Zika virus is highly likely to be a cause of microcephaly, GBS¹⁴, and other neurologic disorders.” EX1033, 1.

52. As more infections became detected, links arose between ZIKV infection and serious complications during pregnancy. *Id.*, 6. Indeed, the WHO reported that “[t]he prevalence of microcephaly among newborn children in 15 [Brazilian] states with laboratory-confirmed Zika virus transmission (2.8 cases per 10 000 live births) significantly exceeded that in four states without confirmed Zika virus transmission (0.6 cases per 10 000 live births).” *Id.* The WHO additionally reported that a “causal role for Zika virus” and birth defects was “highly likely,” stating:

Among the 6480 cases of microcephaly and/or CNS malformation reported in Brazil, 182 child deaths occurred after birth or during pregnancy (including miscarriage or stillbirth); 40 of these were confirmed as having microcephaly and/or CNS malformation potentially linked to congenital Zika virus infection....

An outbreak of Zika virus in French Polynesia was followed by an increase in the number of CNS

¹⁴ GBS is Guillain-Barré syndrome, which “is a serious immune-mediated illness manifesting as progressive paralysis over 1–3 weeks.” EX1068, 1486.

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malformations in children born between March 2014 and May 2015....

Given the temporal and geographical associations between Zika virus infections and microcephaly, the association between Zika virus and microcephaly observed in prospective and retrospective studies of women during pregnancy, and the repeated discovery of virus in fetal brain tissue, a causal role for Zika virus is highly likely.

Id., 7–8. The WHO, therefore, concluded that the “most significant health risks [were] for pregnant women.” *Id.*, 10.

B. Before March 18, 2016, the art recognized an urgent need for developing ZIKV vaccines and multiple vaccine programs were already in progress and planned.

53. In response to the escalated concern over ZIKV infection and its association with clusters of microcephaly and Guillain-Barré syndrome, the WHO issued a press release declaring ZIKV a “Public Health Emergency of International Concern.” EX1025, 2. In that press release, the WHO Director-General noted that he convened an Emergency Committee that advised that “the recent cluster of microcephaly cases and other neurological disorders reported in Brazil, following a similar cluster in French Polynesia in 2014, constitute[d] an ‘extraordinary event’ and a public health threat to other parts of the world.” *Id.*, 1. In the Committee’s view, “a coordinated international response [was] needed to

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minimize the threat in affected countries and reduce the risk of further international spread.” *Id.* The WHO Director-General also stated in this press release that “[the] coordinated international response [was] needed ... to expedite the development of diagnostic tests and vaccines to protect people at risk, especially during pregnancy.” *Id.*, 2.

54. Realizing the growing global threat of ZIKV, the field recognized that there was an “urgent need” for developing a ZIKV vaccine. EX1034, 733.

Similarly, Dr. Anthony Fauci, the head of the United States National Institute of Allergy and Infectious Diseases, described the need to develop ZIKV vaccines and other interventions as requiring “all hands on deck.” EX1162, 1. Dr. Fauci also said that he would also be “advising scientists about money available to fund more research into the virus.” *Id.* He planned to “look at grants already awarded to scientists studying similar viruses, and then supplement those grants with money for more Zika-specific work.” *Id.*

55. The WHO also identified ZIKV “as a serious risk, necessitating further action as soon as possible.” EX1143, 2. The WHO further stated that ZIKV vaccines can be developed “building on the same technologies that have been successfully used to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).” *Id.*, 5. Marie-Paule Kieny, the WHO Assistant Director-General, Health Systems and Innovation, likewise stated

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that “[a]n encouraging factor in developing a vaccine against Zika virus, ... is that ‘Zika is a flavivirus and vaccines have been successfully produced against other flaviviruses.’” EX1034, 733.

56. Dr. Fauci similarly stated that when developing vaccines for viruses that are similar, such as ZIKV and other flaviviruses, “you can translate the technologies that you’ve developed to hasten the end game goal of what you want for a virus like Zika.” EX1162, 1. Dr. Fauci additionally stated that “[i]t is to our advantage we already have existing platforms to use as a sort of jumping off point.” EX1072, 1. Therefore, the field believed that there was likely “a good shot at success” in developing a vaccine against ZIKV. EX1052, 543. “Vaccine pioneer Stanley Plotkin,” moreover, emphasized “that Zika belongs to the flavivirus family, and vaccines exist for several of its relatives, including dengue, yellow fever, and Japanese Encephalitis.” *Id.* He further stated that he did not “see any technical issues [in making a ZIKV vaccine,] such as the ones that obviously exist for vaccines against HIV, tuberculosis, and may other agents.” *Id.*

57. In a March 3, 2016 document, the WHO announced “an emergency research and development plan ... [to] address[] research and development needs for novel means of [ZIKV] vector control, diagnostics, vaccines, and therapeutics” to address the “serious risk” of ZIKV. EX1143, 2. In that report, the WHO disclosed that multiple Zika virus vaccines had started undergoing development.

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Id., 14. The WHO stated that the different types of ZIKV vaccines already undergoing development included purified inactivated virus vaccines, live recombinant vaccines, virus-like particles, plasmid DNA vaccines, as well as vectored vaccines. *Id.* According to the WHO report, eighteen different programs were actively developing or considering ZIKV virus vaccines and “[m]ost [were] building on existing flavivirus vaccine technology and know-how.” *Id.*, 5.

58. The field also recognized by March 18, 2016, that an inactivated ZIKV vaccine had “the best chance of winning regulatory approval as a product that pregnant women might use,” which was of note given the connection between ZIKV infection in pregnant women and birth defects. EX1052, 543; *see also* ¶52 above.

59. Starting in January 2016, my laboratory at Beth Israel Deaconess Medical Center and others at WRAIR were engaged in developing ZIKV vaccines. Shortly after March 18, 2016, we were the first to publish pre-clinical studies using a purified inactivated ZIKV vaccine. EX1006. Our ZIKV vaccine stimulated neutralizing antibodies in mice and protected against ZIKV challenge. *Id.*, 476, Table 1. Follow-up studies revealed that the vaccine could stimulate log₁₀ MN₅₀

titers of 3.86, i.e., MN_{50} of 7,244 ($10^{3.86} = 7,244$),¹⁵ when administered in two doses to rhesus monkeys and a mean titer of 1154 when administered in two doses to humans. EX1062, 1129, FIG. 1B; EX1088, FIG. 2, Table 3.

C. Aspects of ZIKV structure and maturation were known in the art.

60. Before March 18, 2016, the art generally disclosed that for flaviviruses, “[t]he viral ORF is translated into a single polyprotein that is co- and post-translationally cleaved by host and viral proteases to produce ten proteins, including three structural proteins (capsid, pre-membrane, and envelope) and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).” EX1081, 2. It was also known that the flavivirus genome was replicated via “a negative-strand intermediate, which serves as a template for additional positive-strand RNAs.” EX1048, 24. Before March 18, 2016, the field determined genomic and polypeptide sequences of multiple strains of ZIKV. *See*, e.g., EX1053; EX1054; EX1055. This allowed Baronti to confirm, before March 18, 2016, that ZIKV, like other flaviviruses, is also composed of capsid, prM, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 proteins. EX1160, 1.

¹⁵ To convert the $\log_{10}$ values into an integer value, the $\log_{10}$ values are treated as the n th power of 10. EX1121, 790.

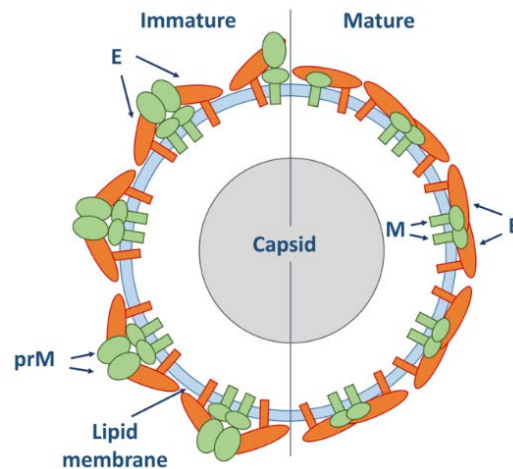
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61. The three flaviviral structural proteins play important roles in the life cycle of flaviviruses, such as ZIKV, including replication, assembly, maturation, and fusion. EX1082, 14. Studies showed that non-infectious immature virions (i.e., virus particles) become infectious mature virions after “dramatic structural changes” during their release from cells. EX1095, 168–170. Immature virions containing the capsid, E, and premembrane/membrane (“prM”) proteins form “trimeric spikes” in the host cell’s endoplasmic reticulum. EX1005, 27, 31. Prior to release from the cell, “the immature virion particles are cleaved by the host protease furin, resulting in mature, infectious particles” after dissociation of the ‘pr’ portion from the virion. EX1082, 14. Mature virions enter host cells by receptor-mediated endocytosis. *Id.* “The acidic environment of the endosome triggers an irreversible trimerization of the [envelope] E protein that results in fusion of the viral and cell membranes.” *Id.* “After fusion has occurred, the NC [nucleocapsid] is released into the cytoplasm, the capsid protein and RNA dissociate, and replication of the RNA genome and particle assembly is initiated.” *Id.*

62. All seven NS proteins of ZIKV are involved in flavivirus replication. EX1081, 2. They form a viral replication complex in the host cell’s endoplasmic reticulum to carry out the functions during viral replication. *Id.* The art also disclosed that the flavivirus RNA genome contains 5’- and 3’-noncoding

regions that play key roles in regulating translation of the genome. EX1048, 28–29. The art also reported that interactions between the 5'- and 3'-noncoding regions were important for negative-strand and further positive-strand RNA synthesis. EX1050, 109–110.

63. A simplified depiction of a Zika virion is shown below:



EX1096, FIG. 2B. The left side of the figure shows the immature version of the virus before cleavage of the pr domain from the virus's prM protein, which yields the mature version of the virus, shown on the right. *Id.* Generally, a ZIKV virion has to be mature for it to be infectious¹⁶ (and thus to be able to replicate in host cells). EX1177, 602.

¹⁶ The inventors of the '681 patent agree: “[m]osaic and immature forms are generally not infectious-*only mature virions are infective.*” EX1001, 3:52–54 (emphasis added).

D. Skilled artisans used neutralizing antibody titers to assess the likelihood of protectiveness of vaccines.

64. The goal of vaccination is to stimulate the vaccinated subject's immune system, including generating antibodies that neutralize the virus to prevent or treat viral infection or limit the consequences of infection. EX1042, 709. The art disclosed that "[n]eutralization has been defined as 'the loss of infectivity which ensues when antibody molecule(s) bind to a virus particle, and usually occurs without the involvement of any other agency.'" *Id.*, 706.

65. A POSA would have also known that "[t]he level of neutralizing antibody that is induced [by a vaccine] correlates with the degree of protection against disease for several viral vaccines." *Id.*, 709. A vaccine's ability to stimulate a neutralizing antibody titer is, therefore, a customary parameter for measuring an immune response in vaccine development. As such, those in the art would have used neutralization assays to determine the presence and titer of neutralizing antibodies in a subject's serum after administration of a vaccine. EX1045, 10–11.

66. The presence of neutralizing antibodies after vaccination can be determined by mixing infectious virus with the serum of vaccinated subjects and observing the ability of antibodies present in the serum to prevent infection of mammalian cells *in vitro*. EX1043, 126. Before March 18, 2016, methods of quantifying vaccine-elicited neutralizing antibodies included plaque reduction neutralization test (PRNT) assays and microneutralization (MN) assays. *See, e.g.,*

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EX1163, 4562–4563; EX1024, 458; EX1164, 86. PRNT assays were commonly used by skilled artisans to measure a serum’s neutralizing antibodies ability to inactivate virus. EX1047, 5209. But the art noted advantages of MN assays compared to PRNT assays, such as higher throughput and less interassay variability. EX1164, 86; *see also* Section VII.D.1, below.

67. A skilled artisan would have known that “[g]enerally speaking, protection is achieved when the neutralizing antibody titre in the serum of the animal at the time of virus challenge is relatively high, usually ... [when] the serum of the animal can be diluted 100-fold and 90% neutralization still be achieved *in vitro*.” EX1042, 707–708; EX1045, 1.

68. Before March 18, 2016, the art established that neutralizing antibody titers of 10 were “the minimum protective level” against JEV and YFV. *See, e.g.,* EX1047, 5209–5210; EX1041, 1327, 1330, 1334. In addition, although the neutralizing antibody titer for seroprotection has not been defined for the flaviviruses TBEV and WNV, a neutralizing antibody titer of over 10 was generally used, in line with the minimum protective titer established for JEV and YFV. *See, e.g.,* EX1041, 1330, 1334.

1. Plaque reduction neutralization test (PRNT) assays

69. As discussed above, PRNT assays were used in the art to determine the neutralizing antibody titer stimulated by a given vaccine. A POSA would have

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understood that “[t]he basic design of the PRNT allows for virus-antibody interaction to occur in a test tube or microtiter plate.” EX1043, 126. To perform the assay, virus-susceptible cells are mixed with a known quantity of virions and serial dilutions of a vaccinated subject’s sera. *Id.* The mixture is then plated and overlaid with a semi-solid medium that restricts spread of progeny virus. *Id.* Viral plaques are then counted and compared with the starting concentration of virus to determine the percentage reduction in total virus infectivity. *Id.* Neutralizing antibodies in serum samples decrease the amount of infectious virus, and thus fewer plaques are observed in cell culture after incubation. Thus, the field used PRNT assays to determine whether neutralizing antibodies (1) are present in a vaccinated subject’s serum; and (2) the degree to which the neutralizing antibodies can reduce virus infectivity.

70. The art typically assayed for PRNT₅₀, which measured the serum dilution capable of preventing 50% of plaque formation by virus in cell cultures. *See, e.g.,* EX1163, 4563. The PRNT₅₀ titer is the reciprocal of the serum dilution that neutralizes 50% of the targeted virus, e.g., a PRNT₅₀ of 15 means that a 1/15 dilution of the vaccinated subject’s serum neutralized 50% of the targeted virus. The WHO recommended that a PRNT₅₀ titer ≥ 10 should be used as the primary end point for demonstrating immunogenicity in trials for JEV vaccines. EX1047, 5210.

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71. PRNT assays were noted in the art, however, as having certain disadvantages in certain contexts: “A disadvantage of the PRNT is that it is labor intensive and therefore not readily amenable to high throughput, making it difficult to use for large-scale surveillance and vaccine trials.” EX1043, 126; *see also* EX1164, 86 (stating that PRNT has “limitations, such as its low throughput, unacceptable labor-intensive nature, and high degree of interassay variability”).

2. Microneutralization (MN) assays

72. Various groups developed different MN assay protocols, including those that used a 2-day enzyme-linked immunosorbent assay (“ELISA”) technique and a 3-day hemagglutination protocol, each used for detecting viral infection, as well as a 7-day hemagglutination protocol used for detecting neutralizing antibodies stimulated by an influenza vaccine and that of Thomas et al. (discussed below) that was a 4-day ELISA assay used for detecting neutralizing antibodies stimulated by a flavivirus vaccine. EX1044, 957; *see* ¶73, below. None of these assays were validated, such that assays done at different times in different laboratories cannot be directly compared to demonstrate superiority of one vaccine compared to another. This was due to the difficulties of standardizing cell culture conditions, which are “laboratory specific.” *Id.*, 963.

73. In 2013, Thomas developed an MN₅₀ assay to determine the titer of neutralizing antibodies stimulated by a live-attenuated DENV vaccine. EX1164,

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86. Thomas noted that his MN assay was sensitive for detecting neutralizing antibodies and had advantages over PRNT assays because it was more high-throughput, less labor intensive, and had less interassay variability. *See, e.g., id.*, 86. In the MN₅₀ assay of Thomas, like for PRNT assays, a constant amount of virus was added to different serum dilutions obtained from vaccinated subjects and incubated with monolayers of Vero cell. *Id.*, 75. Thomas measured for a reduction in virus infectivity as measured by ELISA after a 4-day incubation period. *Id.* Also, as with PRNT₅₀ assays, the neutralizing antibody titer is the reciprocal number of the last dilution of serum that neutralizes $\geq 50\%$ of the targeting virus, e.g., if a 1/15 dilution of serum neutralizes 50% of the target virus the MN₅₀ titer is 15. *Id.* “The precision of the assay was estimated to range from 39% to 59%.” *Id.*, 86. Also, as with PRNT₅₀ assays, Thomas defined seropositivity, i.e., the development of neutralizing antibodies to an antigen (also known referred to in Thomas as seroconversion), as a MN₅₀ titer ≥ 10 . *Id.*

E. Various flavivirus vaccines that stimulated neutralizing antibodies were developed before March 18, 2016.

74. Before March 18, 2016, there were at least twelve “licensed [i.e., approved] vaccines for four flaviviruses: [Yellow Fever virus] YFV (live-attenuated), [Tick-borne encephalitis virus] TBEV (inactivated), JEV (both inactivated and live-attenuated), and DENV virus (recombinant chimeric live-attenuated).” EX1040, 171; *see also* EX1041, 1327, Tables 1 and 2. For most

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flavivirus vaccines that had been developed, at least two doses of the vaccines were required for seroconversion. *See* Section VII.E, below.

75. Scientists had developed live-attenuated YFV vaccines as early as the 1930s. EX1041, 1327. The field considered the YFV vaccine known as YFV-17D to be the “most successful live-attenuated human vaccine” and had used YFV-17D to develop other vaccines for JEV, DENV, and WNV by chimerization.” *Id.*, 1327, 1330, 1332, 1334. In 2010, Monath developed an inactivated YFV vaccine. EX1036, 3827. The first TBEV vaccine was approved in Austria in 1976, which was then again approved under the TicoVac[®] trade-name in 2000. *Id.*, 1330. Various DENV vaccines were developed and tested by those in the field, including live-attenuated vaccines, purified inactivated vaccines, and chimeric vaccines before March 18, 2016. *Id.*, 1331–1333. The first JEV vaccine developed was a mouse brain-derived inactivated vaccine developed in Japan in 1954. *Id.*, 1328. By 2001, Srivastava developed a purified, inactivated JEV vaccine made in Vero cells using methods “originally developed for making [a] dengue-2” purified inactivated vaccine. EX1163, 4558. Srivastava’s purified inactivated JEV vaccine eventually became the approved vaccine known as IXIARO in 2009. EX1007, 1; EX1061, 10; EX1064, 3.

76. In general, purified, inactivated virus vaccines were preferred in the art over live-attenuated vaccines because they can be given to “elderly persons . . . ,

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immunosuppressed individuals, persons with a history of thymectomy, infants <9 months of age, and even pregnant and nursing women.” EX1036, 3828. Indeed, “[i]n developing recommendations for pregnant women,” the United States Advisory Committee on Immunization Practices reported that it “considers whether the vaccine is inactivated or live: inactivated viral or bacterial vaccines or toxoids have been considered to be safe during pregnancy, whereas live vaccines are considered potentially to be of concern because of the theoretical risk of transmission of the live vaccine component to the fetus.” EX1037, 162.

77. Thus, before March 18, 2016, the art was replete with guidance for developing flavivirus vaccines, including multiple inactivated flavivirus vaccines. And a POSA reviewing the art, therefore, would have had an expectation that an inactivated ZIKV vaccine would be effective. See ¶¶55–58, above.

78. Outlined below are the neutralizing antibody titers that were reported in the art for various DENV and JEV vaccines developed before March 18, 2016.

1. DENV vaccines

79. In 1996, Putnak published data from two purified, inactivated DENV-2 vaccines using the S16803 strain of DENV-2. EX1028, 1177. A POSA would have understood the following from Putnak: Putnak made vaccines using Vero cells that were passaged in media containing serum (WR-3) or serum-free medium (WR-4). *Id.*, 1178. Putnak administered both the WR-3 and WR-4 vaccines to mice

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as a single dose or two doses and assayed the mice's sera for neutralizing antibodies using a PRNT₅₀ assay. *Id.*, 1178, Table 4. Putnak observed neutralizing antibody titers ranging from 160–340 after one dose and 350–2,500 after two doses for the WR-3 and WR-4 vaccines. *Id.*, Table 4.

80. Putnak then administered the WR-4 vaccine to rhesus monkeys using a three-dose schedule and, again, assayed for neutralizing antibody titers stimulated by the vaccine using a PRNT₅₀ assay. *Id.*, 1179, Table 5. Of the seventeen monkeys that had not previously been exposed to DENV, ten (58%) developed neutralizing antibodies titers in the range of 10–160 after the first dose when the sera was assayed at day 28 post-injection. *Id.*, Table 5. After the second dose, all seventeen monkeys (100%) developed neutralizing antibodies titers in the range of 30–1,230 when the sera were assayed at day 28 post-second injection (day 56 post-first injection). *Id.*

81. Putnak found that “[p]rior to the third dose at 182 days [post-first injection], [neutralizing antibodies] had decayed to undetectable levels in 5 of 17 monkeys,” and titers in the remaining monkeys ranged from 10–250. *Id.*, 1179. Administering the third dose stimulated an increase in titers that were observed in the range of 20–1,680 when sera were assayed day 28 post-third injection (corresponding to Study Day 210). *Id.*, Table 5. However, similarly to the observations after two doses, neutralizing antibody titers dropped again to levels

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within the range of 40–800 at Study Day 238, i.e., 28-days post administration of the third dose. *Id.*

82. Putnak’s data are reproduced below. Monkey DA86 is the monkey previously exposed to DENV before vaccination. *Id.*, 1179. The column labeled “Inoc 2, day 28” shows the neutralizing antibody titers 28 days after administration of the first dose and before administration of the second dose. The column labeled “Day 56” shows the neutralizing antibody titers 56 days after administration of the first dose and 28 days after administration of the second dose. The column labeled “Inoc 3, day 182” shows the neutralizing antibody titers before administration of the third dose and 154 days after the administration of the second dose. The column labeled “Day 210” shows the neutralizing antibody titers 28 days after administration of the third dose.

Table 5. Neutralizing antibody responses and protection from viremia in rhesus monkeys following vaccination with DEN-2 purified, inactivated vaccine lot WR-4 and challenge with DEN-2 virus strain S16803.

Vaccine dose (ug)	Monkey	Inoc 1, day 0	Inoc 2, day 28	Day 56	Inoc 3, day 182	Day 210	Challenge, day 238	Day 308	Viremia on days 1–10 after challenge									
									1	2	3	4	5	6	7	8	9	10
75	4B6	<10	20	610	70	720	120	ND	–	ND	ND	ND	ND	ND	ND	ND	ND	ND
	CH137	<10	160	1040	140	900	210	150	–	–	–	–	–	–	–	–	–	–
	882C	<10	10	800	80	1000	150	4800	–	–	–	–	+/<1.2	–	–	–	–	–
25	033A	<10	80	1100	110	1000	170	7200	–	–	+/<1.2	–	–	–	–	–	–	–
	891558	<10	80	1170	210	680	150	3600	–	–	–	+/<1.2	+/<1.2	–	–	–	–	–
	68A	<10	<10	100	20	850	100	2300	–	–	–	–	–	–	–	–	–	–
7.5	3MI	<10	10	320	40	900	150	5300	–	–	–	–	–	–	–	–	–	–
	547A	<10	20	980	250	950	330	3900	–	–	–	–	+/1.2	–	–	–	–	–
	6PR	<10	160	320	50	1680	800	1860	–	–	–	–	–	–	–	–	–	–
2.5	84456	<20	30	1220	<10	1200	120	6400	–	–	–	+/4.1	–	–	–	–	–	–
	931C	<20	20	960	70	140	90	10,600	–	–	–	–	–	–	–	–	–	–
	B652	<20	<20	1230	<10	200	90	7600	–	–	–	+/4.1	+/4.1	–	–	–	–	–
0.75	3JT	<20	<20	330	<10	80	40	6700	–	–	–	+/4.4	+/5.5	+/5.2	–	–	–	–
	P991	<20	<20	170	20	150	70	5200	–	–	–	–	+/4.0	+/4.7	–	–	–	–
	907T	<20	<20	60	10	100	120	4400	–	–	–	–	+/<1.2	–	–	–	–	–
0.25	DA86	20	700	1950	300	8800	1320	1350	–	–	–	–	–	–	–	–	–	–
	159D	<20	<20	30	<10	20	60	4800	–	–	–	+/3.4	+/4.7	+/4.8	–	–	–	–
	N839	<20	<20	40	<10	400	60	4600	–	–	–	+/1/2	+/2.7	–	–	–	–	–
Untreated	CH139	<10	ND	ND	ND	ND	<10	2300	–	–	+/1.5	+/3.5	+/4.8	+/5.0	+/4.2	–	–	+/<1.2
	E42	<10	ND	ND	ND	ND	<10	520	+/3.9	+/<1.2	+/1.2	+/1.3	+/3.5	+/4.3	+/3.4	+/2.0	–	–
	E363	<10	ND	ND	ND	ND	<10	410	–	+/1.5	+/<1.2	–	–	+/2.3	+/<1.2	+/3.5	+/2.4	–

NOTE. Data are plaque reduction neutralization 50% end points or isolation of virus by amplification in mosquito cells (+ or –)/direct plaque assay of virus from serum (log₁₀ pfu/mL). Samples not found viremic by amplification in mosquito cells (–) were not titrated by direct plaque assay. No virus was recoverable on days 11–14 after challenge. Monkey 4B6 died 2 days after challenge. Untreated (control) monkeys were given PBS–aluminum hydroxide on inoculation (Inoc) days. ND, not done.

EX1028, Table 5.

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83. A POSA would have understood from Putnak's study that high neutralizing antibody titers could be obtained in 100% of vaccinated mice and 88% of vaccinated monkeys after two doses of Putnak's DENV vaccine and neutralizing antibodies can wane over time.

84. A POSA also would have understood that Thomas reported in 2013 on two different formulations of a live-attenuated tetravalent DENV vaccine (TDEN F17 and TDEN F19) based on strains of DENV-1 (West Pac 74), DENV-2 (S16803), DENV-3 (CH53489), and DENV-4 (341750). EX1164, 74.¹⁷ Thomas formulated TDEN F19 to have approximately 10-fold less DENV-4 than TDEN F17. *Id.*

85. Thomas published data from a phase II study that assessed the neutralizing antibody titer stimulated by the vaccines in humans after receiving two doses of the vaccine using a MN₅₀ assay. *See* ¶73, above. In subjects that were not already seropositive for a DENV serotype (non-primed), Thomas disclosed MN₅₀ titers ranging from 13 to 2,202 for DENV-1, 24 to >2,430 for DENV-2, 11 to 504 for DENV-3, and 13 to >2,430 for DENV-4 one month post-dose two in

¹⁷ DENV-1, DENV-2, DENV-3, and DENV-4 are different serotypes of DENV. EX1164, 73. The DENV serotypes are genetically related but distinguishable from each other based on the antigens they present. EX1119, 1563.

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individuals considered to have become seropositive (i.e., individuals with titers ≥ 10). EX1164, 86, Table 8. Thomas also disclosed administering a third dose and observing that neutralizing antibody titers stimulated by the second dose had waned over time, as measured prior to administration of the third dose. *Id.*, Table 8.

86. Thomas's data is reproduced below. *Id.* The yellow highlighted subjects in the table were the non-primed subjects, i.e., they had not been previously exposed to a DENV serotype. Subjects with neutralizing antibodies < 10 for a given DENV serotype after receiving the second or third dose were considered to not have become seropositive for that DENV serotype.

Individual DENV antibody titers from subjects with data available for all three doses (ATP cohort)													
Groups and subject ID	Primed	Post-dose 2				Pre-dose 3				Post-dose 3			
		DENV-1	DENV-2	DENV-3	DENV-4	DENV-1	DENV-2	DENV-3	DENV-4	DENV-1	DENV-2	DENV-3	DENV-4
F17													
14	Yes	2,202	> 2,430	504	> 2,430	147	236	53	1,522	166	210	77	1,550
29		< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	460	< 10	828	14
35		549	>2,430	1,551	228	426	930	1,327	310	552	1,416	1,893	228
49	Yes	1,208	672	494	213	638	129	137	49	819	583	418	202
65		13	184	13	14	10	31	< 10	< 10	33	73	25	14
67		20	776	17	13	11	234	< 10	< 10	15	219	12	13
88		53	189	26	1,825	26	32	< 10	651	20	58	16	485
95		< 10	64	< 10	93	< 10	< 10	< 10	< 10	< 10	1,281	< 10	< 10
F19													
20		233	1,588	277	1,255	20	449	63	204	105	466	89	350
34		512	26	17	< 10	202	< 10	< 10	< 10	253	19	< 10	< 10
46		< 10	326	< 10	< 10	< 10	66	< 10	< 10	11	250	< 10	< 10
54		618	337	98	22	399	67	60	< 10	571	157	110	24
61	Yes	> 2,430	1,372	1,417	1,134	> 2,430	939	1,435	1,343	> 2,430	800	1,405	1,172
64		25	24	11	1,887	11	< 10	< 10	944	38	171	21	1,080
71		< 10	41	< 10	< 10	< 10	< 10	< 10	< 10	15	1,540	< 10	< 10
75	Yes	> 2,430	1,780	1,536	628	> 2,430	482	1,392	437	> 2,430	790	1,605	326
82		188	261	53	51	133	87	19	27	119	115	40	22

Post-dose 2 = blood sample taken 1 month after dose 2 administration; post-dose 3 = blood sample taken 1 month after dose 3 administration; pre-dose 3 = blood sample taken before dose 3 administration (at the dose 3 visit).

EX1028, Table 8 (annotated).

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87. A POSA would have understood from the publication of Thomas's study that neutralizing antibody titers > 100 could be obtained in humans after two doses of Thomas's DENV vaccine and neutralizing antibodies can wane over time.

88. In 2014, Watanaveeradej published data from a phase II study with Thomas's TDEN vaccines. Watanaveeradej disclosed measuring neutralizing antibody titers in subjects who received a first and second dose of the TDEN vaccines. EX1021, 121. Watanaveeradej disclosed that only after the second dose were the titers measured using Thomas's MN_{50} assay significantly > 10 for each serotype and ranging from 14–2,430 across all four DENV serotypes in 100% of non-primed individuals that were vaccinated. *Id.*, 121 (citing reference 10, which is Tomas (EX1164)), Table 6. A POSA would have understood from Watanaveeradej's data that high neutralizing antibody titers could be obtained in humans after two DENV vaccine administrations.

89. In 2015, Bauer also published data from a phase II study with Thomas's TDEN F17 and F19 vaccines. Bauer disclosed measuring geometric mean titers ("GMTs")¹⁸ after subjects received the first and second dose of the

¹⁸ A geometric mean is the arithmetic mean of values taken on a log scale and is appropriately used when values change exponentially, such as when using serological dilutions. EX1120, 141.

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TDEN vaccines, finding that only one month after the second dose were GMTs measured using Thomas's MN₅₀ assay significantly > 10 and ranging from 60.3–377.7 across all four DENV serotypes. EX1046, 443 (citing reference 12, which is Tomas (EX1164)), Table 6. Bauer's data further demonstrate that two doses of a flavivirus vaccine are typically needed to obtain DENV neutralizing antibody titers > 10 in vaccinated subjects.

90. In 2015, Martinez published a study on a purified inactivated DENV-1 vaccine based on DENV-1 strain (West Pac 74). EX1024, 455. Martinez disclosed assessing the neutralizing antibody titer stimulated by the vaccines in a phase I study of humans receiving one or two doses of the vaccine using the same MN₅₀ assay Thomas published. *Id.*, 456 (citing reference 22, which is Thomas (EX1164)).

91. Martinez disclosed observing that on day 28 after the first dose of the DENV-1 vaccine, only two subjects had seroconverted (i.e., had MN₅₀ titers >10). *Id.*, 456, Table 3. Martinez disclosed then administering the second dose and observed MN₅₀ titers in the range of 11–1,868 in 100% of subjects two weeks after receiving the second dose. Martinez also reported that MN₅₀ titers waned over time, dropping below 10 in almost 40% of the individuals at Day 90 (7 out of 18 subjects for which neutralizing antibodies were determined). *Id.*

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92. Martinez's MN₅₀ data is reproduced below. *Id.* The column labeled "Day 0" shows the neutralizing antibody titers Martinez observed on the day the first dose was administered. The column labeled "Day 28" shows the titers observed 28 days after administration of the first dose and on the day the second dose was administered. The column labeled "Day 42" shows the titers observed 14 days after administration of the second dose, the column labeled "Day 56" shows the titers observed 28 days after administration of the second dose, and the column labeled "Day 90" shows the titers observed 62 days after administration of the second dose.

TABLE 3
Neutralizing antibody responses in subjects vaccinated with 2.5 and 5.0 µg doses of DENV-1 PIV on days 0 and 28

Time points	Day 0	Day 28	Day 42	Day 56	Day 90
Subject no.			DENV-1 PIV 2.5 µg dose		
01	< 10	< 10	34	20	30
02	< 10	< 10	557	227	177
03	< 10	< 10	24	< 10	< 10
04*	< 10	< 10	ND	ND	ND
05	< 10	< 10	16	11	< 10
06	< 10	< 10	69	53	< 10
07	< 10	< 10	12	< 10	< 10
08	< 10	109	460	80	27
09	< 10	< 10	32	29	26
10	< 10	< 10	20	35	18
GMT	< 10 (3)	< 10 (5)	49	24	13
95% CI	3-3	2-12	17-146	8-70	4-38
% SC	0 (0/10)	11 (1/10)	100 (9/9)	78 (7/9)	56 (5/9)
			DENV-1 PIV 5.0 µg dose		
11	< 10	< 10	1,868	167	101
12	< 10	< 10	228	21	14
13	< 10	< 10	113	27	27
14	< 10	< 10	61	33	< 10
15	< 10	< 10	25	< 10	ND
16	< 10	< 10	107	< 10	< 10
17	< 10	12	511	159	44
18	< 10	< 10	11	< 10	< 10
19	< 10	< 10	633	137	109
20	< 10	< 10	146	21	13
GMT	< 10 (3)	< 10 (4)	145	24	< 10 (9)
95% CI	3-3	3-5	48-434	8-73	6-48
% SC	0 (0/10)	10 (1/10)	100 (10/10)	70 (7/10)	67 (6/9)

MN₅₀ titers are expressed as reciprocal dilutions of serum tested in the assay. CI = confidence interval; DENV-1-PIV = dengue virus serotype-1 purified-inactivated vaccine; GMT = geometric mean titer; MN = microneutralization; % SC = % seroconversion.
 *Subject did not receive a second vaccine dose

EX1024, Table 3.

93. A POSA would have understood from Martinez's data that neutralizing antibody titers > 100 could be obtained in 70% of human subjects administered two 5 μg doses of the DENV-1 vaccine.

2. JEV vaccines

94. In 2001, Srivastava published on the development of a purified, inactivated JEV vaccine (JE-PIV) made using Vero cells and the JEV SA₁₄₋₁₄₋₂ strain. Srivastava discloses assaying JE-PIV for its ability to stimulate neutralizing antibodies using a PRNT₅₀ assay in mice. EX1163, 4560, Table 3. Srivastava reported administering two doses given four weeks apart. *Id.*, 4561–4563. Srivastava disclosed comparing JE-PIV to another inactivated JEV vaccine, JE-Vax, by assaying for neutralizing antibody titers two weeks after the administration of the second dose of each vaccine. *Id.*, 4561–4563, Table 3. Srivastava disclosed that (1) JE-PIV stimulated neutralizing antibody titers in the range of 1,280–7,781 in 100% of vaccinated mice when administered at 800 ng and 34–563 in 100% of mice when administered at 32 ng; and (2) JE-Vax stimulated neutralizing antibody titers in the range of 283–3,754 when administered at 1200 ng and 55–150 in 70% of mice when administered at 48 ng. Srivastava's PRNT₅₀ data are reproduced below. *Id.*

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Table 3
Virus neutralizing antibody titers (PRNT₅₀) in mice vaccinated with JE PIV lot 0574 or commercial JE Vax

Vaccine	Dose (ng)	PRNT ₅₀ GMT	PRNT ₅₀ Range	Seroconversion rate	Immunizing dose 50% (ID ₅₀)
JE PIV	800	3842	1280–7781	10/10	4 ng (2–7 ng, 95% CI)
	160	1373	168–3026	10/10	
	32	113	34–563	10/10	
	6	ND ^b	ND	7/10	
	1	ND	ND	1/10	
JE Vax ^a	1200	733	283–3754	10/10	15 ng (14–17, 95% CI)
	240	117	18–863	10/10	
	48	90	55–150	7/10	
	10	ND	ND	6/10	
	2	ND	ND	1/10	

^a For JE-Vax, the virus protein concentration as reconstituted from the vial was 6 micrograms (6000 ng) per ml from information supplied by the manufacturer.

^b Not determined.

EX1163, Table 3.

95. A POSA would have understood from Srivastava’s data that JE-PIV stimulated neutralizing antibody titers > 1000 in 100% of vaccinated mice after two doses.

96. In 2007, Tauber published a phase III study with Srivastava’s JE-PIV before it eventually became the approved IXIARO product. EX1049, 1848; EX1041, 1329 (discussing IXIARO and citing Tauber’s study as reference 38); EX1007, 1; EX1061, 10; EX1064, 3.¹⁹ Tauber disclosed that, when dosed twice, the vaccine stimulated a GMT of 244 and titers in a range of 5–19,783 using a PRNT₅₀ assay, with titers above 10 being observed in 98% of vaccinated subjects.

¹⁹ The ’681 patent also acknowledges that Srivastava’s JE-PIV vaccine developed into the approved IXIARO product. EX1001, 8:41–48.

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EX1049, 1850. Figure 2 from Tauber, reproduced below, also shows that neutralizing antibody titers $\geq 81-160$ were stimulated in $\sim 90\%$ of subjects.²⁰

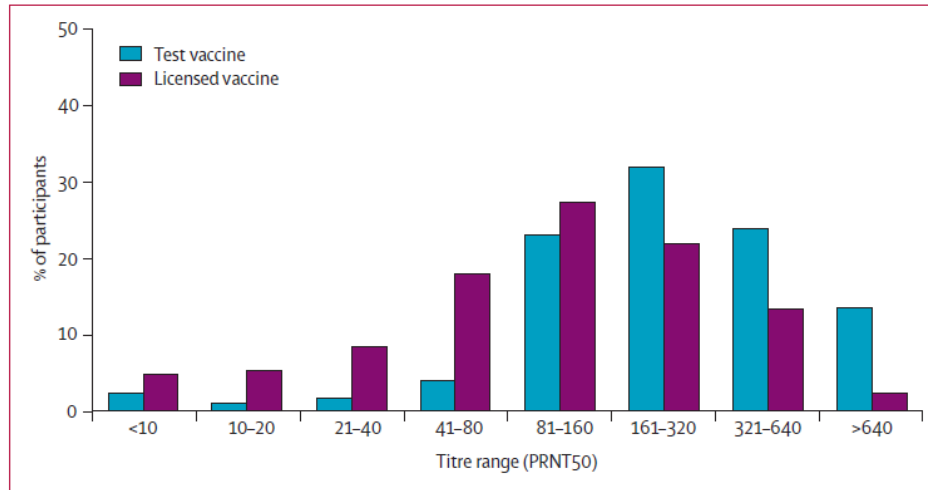


Figure 2: Frequency distribution of JEV-neutralising antibody levels at day 56 in immunised participants
Number of participants based on per-protocol analysis (test vaccine, n=361; licensed vaccine, n=364).
PRNT50=50% plaque reduction neutralisation test.

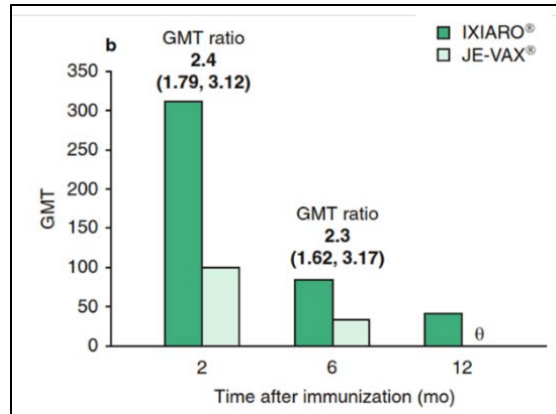
Id., FIG. 2 (IXIARO is the “Test vaccine” and JE-Vax is the “Licensed vaccine”).

97. Similarly, as with DENV vaccines above, GMTs induced by two doses of IXIARO and three doses of inactivated JEV vaccine JE-Vax in humans were reported as waning over time in a publication by Duggan and Plosker.

EX1065, 119. Figure 2b from Duggan and Plosker illustrating this is reproduced below:

²⁰ I determined the values of 98% and $\sim 90\%$ discussed above by estimating the Y-axis values of the bars in Tauber’s Figure 2 and adding them together.

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Id., FIG. 2b. The values of 2, 6, and 12 on the X-axis indicate the months after subjects received their first dose of IXIARO, which was also 1, 5, and 11 months after subjects received their second dose. *Id.* The GMT ratios of 2.4 and 2.3 indicate that GMTs were 2.4- and 2.3-fold higher for IXIARO compared to JE-Vax at the given time points. *Id.* The GMT for JE-Vax was not measured at month 12. *Id.* The values in parenthesis represent the 95% confidence interval range, i.e., the range of values that one can be 95% confident contain a true mean of the population.

98. A POSA would have understood from the above studies with IXIARO that high neutralizing antibody titers could be obtained in ~90% of subjects receiving two doses of the vaccine and that neutralizing antibody titers can diminish over time.

99. In 2012, Okada disclosed data from assaying an inactivated JEV vaccine (JEBIK) based on the JEV Beijing-1 strain. EX1051, 5968. Okada disclosed determining that after a second dose, JEBIK stimulated neutralizing

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antibody titers “ ≥ 20 in all 123 children (100%) who received 5 μg of viral protein and in 121/122 (99.2%) and 113/119 (95.0%) of the children who received doses of 2.5 and 1.25 μg , respectively,” as measured by using a PRNT₅₀ assay. *Id.*, 5970. Okada reported observing GMTs of about 391, 263, and 129 for the 5 μg , 2.5 μg , and 1.25 μg dosages, respectively, in subjects after they received their second dose. *Id.*, Table 3.²¹ Okada then disclosed observing neutralizing antibodies waning 6 to 12 months after vaccination. *Id.* Okada disclosed administering a third dose and observing that JEBIK stimulated GMTs of about 9,057, 5,834, and 3,148 for the 5 μg , 2.5 μg , and 1.25 μg dosages, respectively. *Id.*

100. A POSA would have understood from Okada that high neutralizing antibody titers could be obtained in subjects receiving two doses of JEBIK and that neutralizing antibody titers can diminish over time.

²¹ Okada reported the GMTs as determined by PRNT₅₀ in values of $\log_{10}$. EX1051, Table 3. Okada reports $\log_{10}$ GMT values of 2.592, 2.420, and 2.109 for the 5 μg , 2.5 μg , and 1.25 μg dosages, respectively, after two doses, which are $10^{2.592} = 391$, $10^{2.420} = 263$, and $10^{2.109} = 129$. *Id.* Okada reports $\log_{10}$ GMT values of 3.957, 3.766, and 3.498 for the 5 μg , 2.5 μg , and 1.25 μg dosages, respectively, after three doses, which are $10^{3.957} = 9,057$, $10^{3.766} = 5,834$, and $10^{3.498} = 3,148$. *Id.*

3. Summary of neutralizing antibody titers of flavivirus vaccines

101. I have provided the table below, compiling the neutralizing antibody titers stimulated by some of the flavivirus vaccines discussed above that were reported in the art before March 18, 2016:

Reference / Virus	Neutralizing Antibody Titer Range	Assay Used
Putnak et al. (EX1028) DENV-2	30 – 1,230 (28 days post-dose two, monkeys)	PRNT ₅₀
Thomas (EX1164) DENV-1 – DENV-4	11 – >2,430 (1 month post-dose two, humans)	MN ₅₀
Martinez (EX1024) DENV-1	11 – 1,868 (2 weeks post-dose two, humans)	MN ₅₀
Watanaveeradej (EX1021) DENV-1 – DENV-4	18 – 2,430 (1 month post-dose two, humans)	MN ₅₀
Bauer (EX1046) DENV-1 – DENV-4	84.4 – 377.7 (1 month post-dose two, humans)	MN ₅₀
Srivastava (EX1163) JEV	34 – 7,781 (2 weeks post-dose two, mice)	PRNT ₅₀
Tauber (EX1049) JEV	5 – 19,783 (4 weeks post-dose two, humans)	PRNT ₅₀

4. Skilled artisans understood that neutralizing antibody titers could wane over time after vaccination.

102. Neutralizing antibody titers were known in the art to typically wane over time after vaccination. For instance, as discussed above in Section VII.E, the flavivirus vaccines assayed for neutralizing antibody titers were observed to have decreases in neutralizing antibody titers between doses. See ¶¶81–83, 85–87, 91–93, above. In line with this, researchers studying IXIARO also noted that

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GMTs for neutralizing antibodies were 311 two months after subjects received their second dose. EX1060, Table 1. The GMTs then dropped to 84 and 41 by six and twelve months, respectively, after subjects received their second dose, evidencing that titers waned over time. *Id.* The researchers noted that this was expected, stating:

The decline in GMT over time following IC51 vaccination seen in this follow-up study (a decrease to one-third of the initial GMT from 2 to 6 months and a decrease to one-seventh from 2 to 12 months) is expected after immunization with an inactivated vaccine and compares well with data from other inactivated JE vaccines.

Id., 4386.

103. Consequently, before March 18, 2016, a POSA would have had a reason to develop a vaccine that would stimulate high neutralizing antibody titers to account for the fact that titers typically wane over time and can lead to a reduction in the vaccine's protection.

VIII. THE '681 PATENT AND CLAIMS

104. The '681 patent is generally directed to "Zika virus vaccines and compositions and methods of producing and administering said vaccines to subjects in need thereof." EX1001, Abstract.

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105. I understand that in claims 1–15 and 21–25 of the '681 there is one independent claim. Independent claim 1 recites:

A Zika virus vaccine comprising a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72, wherein said Zika virus vaccine is capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects, wherein the neutralizing antibody titer is determined using a microneutralization assay (MN50).

Id., 445:34–41.

106. I understand that dependent claims contain all the limitations of the claims from which they depend, and add further requirements to the claims from which they depend. Claims 2–15 and 21–25 of the '681 patent depend directly or indirectly from claim 1 and specify additional limitations.

IX. THE MEANING OF CLAIM TERMS

107. I understand from counsel that the claim terms of a United States patent are to be given their ordinary and customary meaning in light of the specification and prosecution history—that is, the meaning that the terms would

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have to a POSA as of the filing date.²² After reviewing the '681 patent's specification, it is my opinion that a POSA reading the '681 patent would have understood that the claim terms of claims 1–15 and 21–25 should be given their ordinary and customary meaning, except as discussed below. It is my understanding that a dependent claim contains all the limitations of the claim from which it depends.

108. **“variant nucleic acid having at least 80% [90%, 95%, or 99%] identity”**: Claim 1 recites that the ZIKV virus has “a variant nucleic acid having at least 80% identity to SEQ ID NO: 72.” EX1001, 445:34–37. Claims 21–23 recite that the percent identity to SEQ ID NO: 72 is 90%, 95%, or 99%, respectively. *Id.*, 448:11–18.

109. The '681 patent states:

The terms “identical” or percent “identity” in the context of two or more nucleic acids or amino acid sequences refer to two or more sequences or subsequences that are

²² I have interpreted the claims as of March 18, 2016 for my analysis regarding the obviousness of the claims (discussed in Section X) and as of March 10, 2020 for analysis regarding anticipation of the claims (discussed in Section XI.C). There were no pertinent differences in my interpretation of the claims for my analyses herein.

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the same. Two sequences are “substantially identical” if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (e.g., at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) *over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window*, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection....

Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms....

Id., 127:33–128:31.

110. A POSA, therefore, would understand the term “variant nucleic acid having at least 80% identity” (claim 1), and 90%, 95%, and 99% identity (claims 21–23), to encompass variants having the specified percent identity to SEQ ID NO: 72, for example, using an algorithm such as BLAST, with the percent identity resulting from the maximum correspondence over the two sequences, and with the nucleic acid sequence of interest not being required to be identical to SEQ ID NO: 72 over any particular region and able to overlap with SEQ ID NO: 72, provided that the specified percent identity is maintained.

X. BASIS OF MY ANALYSIS REGARDING OBVIOUSNESS

111. As discussed below in Section X.B, it is my opinion that claims 1–12, 15, and 21–25 of the '681 patent are not entitled to a priority date earlier than March 18, 2016. As also discussed below, it is my opinion that (1) claims 1–11, 15, and 21–25 would have been obvious over the combination of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164); and (2) claim 12 would have been obvious over the combination of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, Thomas, and Möhlen (EX1083). Claim charts showing where the limitations of claims 1–12, 15, and 21–25 are disclosed in the prior art are in Appendix C, attached hereto.

A. My understanding of the law of obviousness

112. I understand that an obviousness analysis involves determining the scope and content of the prior art; comparing the differences between the claimed invention and the prior art; and resolving the level of ordinary skill in the pertinent art.

113. I understand that combining or modifying the teachings of the prior art to achieve the claimed invention is a way to establish obviousness. I understand that the reason to combine prior art references can come from a variety of sources, not just the prior art itself or the specific problem the patentee was trying to solve.

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And I understand that the references themselves need not provide a specific hint or suggestion of the alteration needed to arrive at the claimed invention; the analysis may include recourse to logic, judgment, and common sense available to a person of ordinary skill that does not need to be explicit in any reference.

114. I also understand that where there is a reason to modify or combine the prior art to achieve the claimed invention, there must also be a reasonable expectation of success in so doing. I understand that this does not require absolute predictability of success.

115. I understand that when considering the obviousness of an invention, one should also consider whether there is any objective evidence that supports the non-obviousness of the invention. I understand that objective evidence of nonobviousness (also known as secondary considerations of non-obviousness) includes factors such as failure of others; unexpectedly superior results; skepticism followed by praise in the industry; long-felt, but unmet, need; copying; and commercial success. I also understand that near-simultaneous invention by another is evidence of the level of skill in the art and constitutes objective evidence that POSAs understood the problem and its solution.

B. Claims 1–12, 15, and 21–25 are not entitled to a priority date earlier than March 18, 2016.

116. As stated above in ¶13, I understand that the '681 patent claims priority to a series of foreign patent applications, the earliest being EP15202585 ("EP

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'585"; EX1014), filed on December 23, 2015.

117. It is my understanding from counsel that for a patent to gain the benefit of the filing date of an earlier application (also known as claiming priority to an earlier application), the earlier application—as of its filing date (here, December 23, 2015)—must both (i) enable and (ii) provide adequate written description of the full scope of the subject matter that is later claimed in the patent. I further understand from counsel that priority is assessed on a claim-by-claim basis. In other words, the subject matter of each patent claim must be compared to the disclosure of the earlier application to determine whether the claimed subject matter is adequately described and enabled by the earlier application, such that the patent claim is entitled to rely on the filing date of the earlier application. I set forth my understanding of the written description and enablement requirements in Sections XI.B, below.

118. I have reviewed EP '585 and have found no disclosures in it regarding ZIKV, ZIKV vaccines, or any flaviviruses. In fact, the disclosures of EP '585 are completely directed to “processes for the purification of infectious *Chikungunya virus particles*, i.e., mature, functional virus particles” and “use of any of the processes ... for manufacturing a composition for immunization against a *Chikungunya virus infection*.” EX1014, 8, 3, 6. *Chikungunya virus* (although

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having certain similarity to ZIKV) is a member of the family of viruses known as *Togaviridae*, and is not a member of the *Flaviviridae* family of viruses. *Id.*, 1.

119. Consequently, EP '585 does not describe a ZIKV or a ZIKV vaccine, and a POSA would not have concluded that the inventors deemed ZIKV vaccines to be part of the alleged invention in EP '585. Thus, a POSA would not have concluded that the inventors possessed any ZIKV vaccine as of the December 23, 2015 filing date of EP '585. EP '585, therefore, does not provide written description support for claims 1–12, 15, and 21–25 of the '681 patent.

120. EP '585, moreover, does not enable claims 1–12, 15, and 21–25 of the '681 patent claims. As discussed below in Section XI.B.1.b.i, the breadth of claims 1–12, 15, and 21–25 is vast, encompassing myriad candidate ZIKV vaccines. The EP '585 specification, however, provides *zero* guidance on how to make a ZIKV vaccine and has *zero* working examples of a ZIKV vaccine. And as discussed below in Section XI.B.2, the quantity of experimentation needed to make and use the myriad ZIKV vaccines encompassed by claims 1–12, 15, and 21–25 would be highly undue even as of December 23, 2016, let alone as of December 23, 2015, particularly given the complete lack of guidance in the EP '585 specification on how to make *any* ZIKV vaccine, let alone the millions of variant candidates embraced by the claims.

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121. Because EP '585 does not contain any disclosures regarding ZIKV or any flaviviruses, it is my opinion that a POSA would not understand EP '585 to provide written description or enablement for any of claims 1–12, 15, and 21–25 of the '681 patent. Therefore, claims 1–12, 15, and 21–25 of the '681 patent cannot claim priority to the December 23, 2015 filing date of EP '585. Thus, the *earliest* possible priority date for claims 1–12, 15, and 21–25 of the '681 patent is March 18, 2016, the next filing date in the '681 patent's alleged priority chain, which is the filing date of European Patent Application No. 16161068 ("EP '068").²³

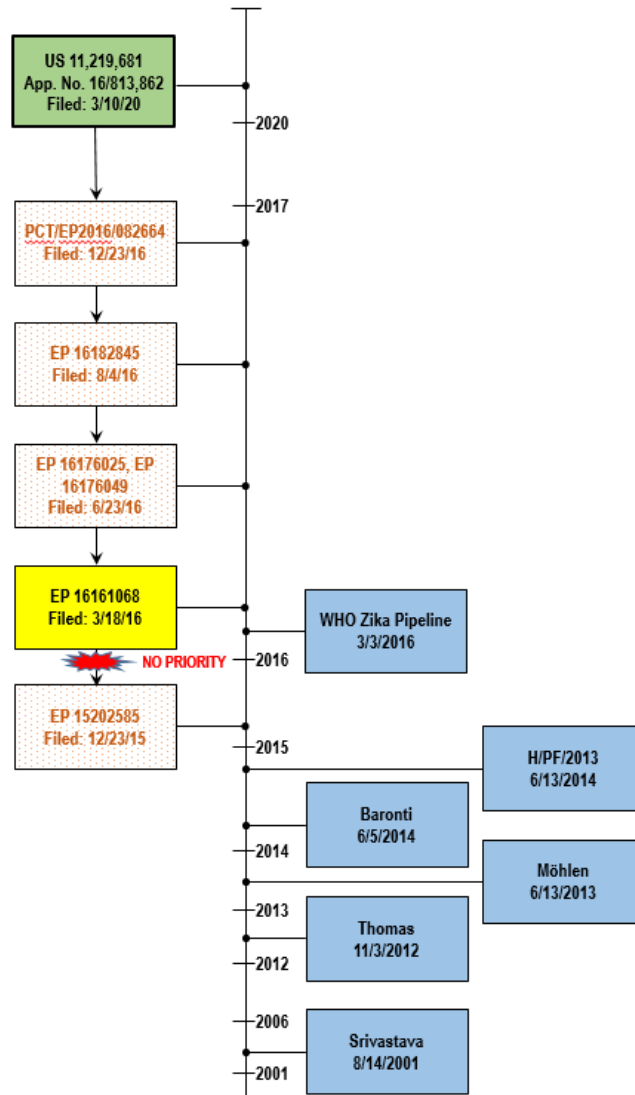
C. I understand that the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, Thomas, and Möhlen are all prior art that was publicly available to a POSA.

122. Srivastava (EX1163) indicates that it published in 2001, Baronti (EX1160) indicates that it published in 2014, Thomas (EX1164) indicates that it published in 2013, and Möhlen (EX1083) indicates that it published in 2013. The GenBank entry for the H/PF/2013 Sequence indicates that the H/PF/2013

²³ As I discuss below in Section XI.B, claims 1–12, 15, and 21–25 of the '681 patent are not entitled to the December 23, 2016 filing date of the '664 PCT, and therefore also cannot be entitled to any earlier filing date in the broken priority chain.

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Sequence would have been readily accessible to a POSA on June 13, 2014, as discussed below in ¶123. The figure below illustrates the dates associated with each reference in relation to the March 18, 2016 filing date of EP '068:



123. Baronti states that it deposited the sequence it obtained of the H/PF/2013 ZIKV strain's RNA in GenBank as accession number KJ776791. EX1160, 1. I am familiar with GenBank, and I have used it to search for deposits of nucleotide and protein sequences on multiple occasions, including before

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March 18, 2016. GenBank is a well-known and well-utilized public, government database of genetic sequence information built and distributed by the United States National Center for Biotechnology Information (“NCBI”), a division of the National Library of Medicine, which is a part of the National Institutes of Health. EX1069, D36. “NCBI makes the GenBank data available at no cost over the Internet, through FTP and a wide range of web-based retrieval and analysis services.” *Id.* A POSA would have been familiar with searching, accessing, and interpreting GenBank records before March 18, 2016, due to GenBank being a central source of genomic and protein sequence information. I accessed the H/PF/2013 Sequence by navigating to the GenBank website located at <https://www.ncbi.nlm.nih.gov/genbank/> and entering the KJ776791 accession number Baronti discloses. That returned a webpage (EX1084) with the current version of the sequence, KJ776791.2. I then navigated to the earlier version Baronti submitted, located at <https://www.ncbi.nlm.nih.gov/nuccore/KJ776791.1>, using a link located on the webpage returned when I searched KJ776791 (EX1019). The GenBank record for KJ776791.1 (“the H/PF/2013 Sequence”) indicates it was last modified on June 13, 2014. EX1019 (*see* LOCUS field for accession number KJ776791.1); EX1057, 4–7 (“The date in the LOCUS field is the date of last modification.”). The information in GenBank, including the June 13, 2014 date in the LOCUS field, is information that expert witnesses, such as

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myself, would reasonably rely upon given GenBank's curation by a reputable government agency. Thus, the H/PF/2013 Sequence would have been disseminated or otherwise readily accessible to a POSA as of June 13, 2014 and before March 18, 2016.

124. In view of the above, I have assumed that the WHO Zika Pipeline, Srivastava, Baronti, H/PF/2013 Sequence, Thomas, and Möhlen qualify as prior art to claims 1–12, 15, and 21–25, as of those claims' earliest possible March 18, 2016 priority date for the purposes of my analysis below.

D. Claims 1–11, 15, and 21–25 would have been obvious over the combination of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas.

125. Claims 1–11, 15, and 21–25 of the '681 patent would have been obvious to a POSA over the combination of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164) before March 18, 2016. I provide an overview of my opinions immediately below, and I provide a claim-by-claim analysis further below, beginning in Sections X.D.1–X.D.5.

126. A POSA reading the WHO Zika Pipeline would have recognized the need for a ZIKV vaccine to address the international public health crisis arising from ZIKV's spread. EX1143, 2, 5. The WHO Zika Pipeline (and the general knowledge in the art) would have directed a POSA to employ known "technologies

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that have been successfully used to develop” vaccines for other flaviviruses, such as JEV. *Id.*, 5. A POSA would have also recognized that, in particular, pregnant women would have been the focus of ZIKV vaccine development, which would have provided a reason for a POSA to use an inactivated virus vaccine platform. *See, e.g., id.*; EX1036, 3828; EX1037, 162. Indeed, safely administering a ZIKV vaccine to pregnant individuals, moreover, would have been of high importance due to ZIKV infection being linked to birth defects. *See* ¶¶51–52, above. A POSA, moreover, would have further been motivated to make a inactivated ZIKV vaccine because inactivated virus vaccines had been preferred in other populations, such as “elderly persons ..., immunosuppressed individuals, persons with a history of thymectomy, infants <9 months of age, ... and nursing women.” EX1036, 3828.

127. A POSA would have also understood that ZIKV generally has an African and Asian lineage, but also that the sequence homology amongst naturally occurring ZIKV strains is high, suggesting that any ZIKV belonging to one of these lineages would be useful for such an inactivated virus vaccine. EX1138, 227. For instance, Baronti reported that the ZIKV strain H/PF/2013 belonging to the Asian lineage “shar[es] common ancestorship and ca. 99.9% nt [i.e., nucleotide] and aa [i.e., amino acid] identities with isolates circulating in southern Asia and the Pacific islands in the late 2000s.” EX 1010, 1.

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128. Srivastava discloses such a vaccine technology that was used to successfully make a purified inactivated JEV vaccine from a known JEV strain and that eventually became approved as IXIARO. EX1163, 4564; EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48. A POSA, therefore, would have followed Srivastava’s vaccine technology to make a purified inactivated ZIKV vaccine by swapping in a known ZIKV strain in the place of JEV.

129. Baronti discloses a known, naturally occurring ZIKV strain, the H/PF/2013 strain, which a POSA would have used with Srivastava’s vaccine technology to make a ZIKV vaccine because Srivastava used a known strain of JEV. EX1160, 1. Baronti discloses that the H/PF/2013 strain’s RNA genome comprises a sequence corresponding to the DNA sequence disclosed in the H/PF/2013 Sequence, which provides the full open reading frame (“ORF”) of the H/PF/2013 strain’s polyprotein. *Id.*, 1; EX1019. The H/PF/2013 strain belongs to the Asian lineage showing high identity with previous known strains and being closely related to the one that emerged in Brazil, with both being related to fetal defects. EX1058, 1887; EX1033, 7–8.

130. A POSA would have also sought to make a ZIKV vaccine that could stimulate neutralizing antibody titers greater than 15, e.g., > 100, because, generally speaking, “[n]eutralizing antibodies ... are the best correlate of protection from viral infection after vaccination” and “[g]enerally speaking,

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protection is achieved when the neutralizing antibody titre in the serum of the animal at the time of virus challenge is relatively high.” EX1045, 1; EX1042, 707–708.

131. A POSA, moreover, would have known that neutralizing antibody titers could be assessed using a MN assay, which Thomas discloses. *See* Section VII.D.2, above; EX1164, 86. Thomas discloses that its MN₅₀ assay is more convenient and less variable compared to PRNT₅₀ assays, and Thomas used that assay to assess neutralizing antibodies stimulated by a flavivirus vaccine. EX1164, 86. Lastly, a POSA would have known from the general knowledge in the art that obtaining neutralizing antibody titers in a high percentage of the vaccinated population would have been desirable to afford protection in as many vaccinated individuals as possible. *See, e.g.,* EX1047, 5210 (recommending that new JEV vaccines should achieve at least 75% seroconversion); EX1059, 2192 (finding a standard two-dose regimen of IXIARO that resulted in 100% seroconversion preferable over a single-dose, high-dosage regimen that resulted in 60% seroconversion and single-dose, regular-dosage regimen that resulted in 20% seroconversion).

132. My analysis below shows where the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas teach each and every limitation of claims 1–11, 15, and 21–25.

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133. A POSA would have had a reason to combine the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas to arrive at the claimed ZIKV vaccines because, before March 18, 2016:

- (1) The WHO Zika Pipeline (and general knowledge the art) identified a need for vaccines to address the “serious risk” of ZIKV, especially for pregnant women (*see*, e.g., EX1143, 5);
- (2) The WHO Zika Pipeline suggested “building on the same technologies that have been successfully used to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis [JEV], Dengue)” (*id.*, 2, 5);
- (3) Srivastava discloses such a “technolog[y] ... successfully used to develop” a purified inactivated JEV vaccine from a natural JEV strain, which Srivastava discloses as having several advantages (EX1163, 4557, 4564);
- (4) Baronti discloses a naturally occurring ZIKV strain isolated from a subject infected during the 2013 ZIKV outbreak in French Polynesia, the H/PF/2013 strain (EX1160, 1); Baronti passaged the H/PF/2013 strain in cell culture, harvested the virus, and sequenced the strain’s RNA genome (*id.*); Baronti deposited the corresponding DNA

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sequence in GenBank as the H/PF/2013 Sequence (*id.*); the RNA genome of the H/PF/2013 strain thus comprises a sequence corresponding to the H/PF/2013 sequence; and a POSA would have known from the general knowledge in the art that the H/PF/2013 strain was “the closest strain to the one that emerged in Brazil,” both of which were associated with fetal defects (EX1033, 7–8; EX1058, 1887);

- (5) Before March 18, 2016, a POSA would have sought to make a ZIKV vaccine that could stimulate high neutralizing antibody titers, e.g., > 1000 when following Srivastava’s technology. This is because a POSA would have known from the general knowledge in the art that high neutralizing antibody titers are generally considered to provide better protection and counteract the waning of titers below effective levels, which was generally known in the art as able to take place over time (EX1045, 1; EX1042, 707–708; *see* Section VII.E.4, above). A POSA, therefore, would have sought a vaccine that stimulated high titers because it would have been considered to result in better protection, which also would have been expected to be longer in duration.

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- (6) A POSA would have known that Thomas provided a 4-day MN assay that (1) detected neutralizing antibodies against a flavivirus vaccine; (2) could be used to assess a ZIKV vaccine for its ability to stimulate neutralizing antibody titers; and (3) would “overcome many of PRNT’s limitations, such as [PRNT’s] low throughput, unacceptable labor-intensive nature, and high degree of interassay variability” (EX1164, 86); and
- (7) A POSA also would have sought to make a ZIKV vaccine that could stimulate high neutralizing antibody titers in a high percentage of the vaccinated population in order to enable affording protection to as many vaccinated individuals as possible. See ¶131, above.

134. Additionally, a POSA would have had a reasonable expectation of success in making a purified inactivated ZIKV vaccine using the known H/PF/2013 strain of ZIKV from Baronti, which would be capable of stimulating a MN_{50} of greater than 15 in at least 70% of subjects.

135. This is because, first, Srivastava disclosed that a purified inactivated flavivirus vaccine that could stimulate neutralizing antibodies had been successfully made using a technology that had been successful for other flaviviruses. EX1163, 4558 (“The JE-PIV described in the present report was

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produced using methods originally developed for making the dengue-2 PIV.”). A POSA, therefore, would have reasonably expected that vaccine platforms used for other flaviviruses, such as JEV, could be applied to ZIKV.

136. Indeed, the WHO Zika Pipeline stated that “[i]t [was] assumed that a ZIKV vaccine can be developed building on the same technologies that have been successfully used to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).” EX1143, 5. The WHO Zika Pipeline also identified eighteen active ZIKV vaccine programs, “includ[ing] purified inactive virus” vaccines, “[m]ost [of which were] building on existing flavivirus vaccine technology and know-how.” *Id.* A POSA would have also found the WHO Zika Pipeline’s assessment that existing flavivirus technologies could be applied to make a ZIKV vaccine supported by the general knowledge in the art. For example, Sifferlin reported that Dr. Fauci stated that “you can translate the technologies that you’ve developed to hasten the endgame goal of what you want for a virus like Zika.” EX1162, 1. Cohen likewise reported that “[v]accine pioneer Stanley Plotkin ... predict[ed] ‘a straight forward developmental pathway’ for a vaccine,” as “Zika belongs to the flavivirus family, and vaccines exist for several of its relatives,” in particular JEV vaccines have been successfully developed inactivated virus vaccine platforms. EX1052, 543.

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137. Second, Srivastava disclosed that neutralizing antibody titers > 1000 could be obtained in at least 70% of subjects for a purified inactivated flavivirus vaccine administered using a two-dose schedule. EX1163, Table 3. A POSA would also have known from the general knowledge in the art that other flavivirus vaccines given in at least two doses were capable of stimulating high neutralizing antibody titers in at least 70% of subjects.²⁴ See Section VII.E, above. And given the close relationship between ZIKV and other flaviviruses, in particular JEV, a POSA would have reasonably expected that a purified inactivated ZIKV vaccine made following the technology of Srivastava and administered in a two-dose schedule would similarly stimulate high neutralizing antibody titers in at least 70% of subjects.

138. In reaching my opinions regarding the obviousness of claims 1–11, 15, and 21–25, I considered the possibility of objective evidence (as discussed in

²⁴ A POSA would have conservatively reasonably expected that the neutralizing antibody titers for a ZIKV vaccine dosed twice would not be any lower than ≥ 100 in at least 70% of the vaccinated subjects in view of the general knowledge in the art of the data from flavivirus vaccines other than Srivastava's. See Section VII.E, above.

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Section X.E) but am not aware of any that would support the patentability of claims 1–11, 15, and 21–25.

1. Claim 1

139. Claim recites:

A Zika virus vaccine comprising a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72, wherein said Zika virus vaccine is capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects, wherein the neutralizing antibody titer is determined using a microneutralization assay (MN₅₀).

EX1001, 445:34–41.

a. Before March 18, 2016, a POSA would have had a reason to make a purified inactivated ZIKV vaccine using a known ZIKV strain.

140. Before March 18, 2016, a series of ZIKV outbreaks occurred in 2007, 2013–2014, and 2015–2016. After the 2015–2016 outbreaks, ZIKV infections became known to be associated with neurological disorders, such as Guillain-Barré syndrome and congenital disorders that included fetal neurological malformations and fetal death. EX1033, 1–2, 6, 10.

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141. Therefore, in a March 3, 2016 document, after identifying ZIKV “as a serious risk, necessitating further action as soon as possible,” the WHO stated in the WHO Zika Pipeline that the “WHO ha[d] initiated an emergency research and development plan” to respond to ZIKV. EX1143, 2. The WHO Zika Pipeline identified at least eighteen ZIKV vaccines programs that were under development or actively being considered, “[m]ost [of which were] building on existing flavivirus vaccine technology and know-how.” *Id.*, 5. The WHO Zika Pipeline identified various types of vaccines being developed, including purified inactivated, live-attenuated, chimeric, and vectored vaccines. *Id.*

142. A POSA would have understood that the WHO Zika Pipeline’s assessment of the need for a ZIKV vaccine was consistent with the general knowledge in the art, which likewise disclosed an urgent need for ZIKV vaccine. For example, as reflected in the *Time* magazine article Sifferlin published, it was generally known in the art that Dr. Fauci “‘made it clear that’” the United States “‘want[ed] to put a full-court press’” on addressing ZIKV through vaccine development and it was “‘all hands on deck for Zika.’” EX1162, 1. The WHO had also declared that ZIKV “constitute[d] a Public Health Emergency of International Concern” after assessing “the cluster of microcephaly cases and other neurological disorders reported in Brazil, following a similar cluster in French Polynesia.” EX1025, 1–2.

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143. Accordingly, before March 18, 2016, a POSA would have had a reason to make a ZIKV vaccine to aid in addressing the international health threat ZIKV posed.

144. The WHO Zika Pipeline suggested using known vaccines platforms that had been successfully used for other flaviviruses to make a ZIKV vaccine, stating:

It is assumed that a ZIKV vaccine *can be developed building on the same technologies* that have been *successfully used* to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).

EX1143, 5.

145. A POSA, moreover, would have known from the general knowledge in the art that multiple purified inactivated flavivirus vaccines using known virus strains and capable of stimulating high neutralizing antibody titers had been successfully developed before March 18, 2016.

146. In fact, Srivastava discloses a successful vaccine technology for making an immunogenic and protective purified inactivated virus vaccine for JEV, a flavivirus like ZIKV, which stimulated neutralizing antibody titers in the range of 1,280–7,781 in 100% of vaccinated mice after a second dose of the vaccine.

EX1163, Table 3. Srivastava's JE-PIV eventually became IXIARO, which was

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assayed by Tauber. EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48; EX1041, 1329 (discussing IXIARO and citing Tauber (EX1049) as reference 38).

147. A POSA would have also known from general knowledge in the art that Tauber observed that IXIARO stimulated high titers, e.g., a GMT of 244 and as high as 19,783 and titers ≥ 81 –160 in ~90% of humans. EX1049, 1850. A POSA would have further known from that general knowledge that other flavivirus vaccines were capable of stimulating high neutralizing antibody titers in greater than 70% of subjects when administered at least twice. For instance, a purified inactivated DENV-2 vaccine stimulated antibody titers in the range of 350–2,500 in 100% of vaccinated mice and 100–1,230 in 82% of vaccinated rhesus monkeys. EX1028, Tables 4 and 5. A live-attenuated DENV vaccine stimulated MN₅₀ titers in the range of 139–2,430 in 83% of vaccinated subjects when assessed for neutralizing antibodies to each serotype. EX1021, Table 6. And a purified inactivated DENV-1 vaccine stimulated MN₅₀ titers in the range of 107–1,868 in 70% of subjects. EX1024, 456, Table 3.

148. A POSA, further, would have had a reason to make a purified inactivated ZIKV vaccine because it could be more safely given to “elderly persons ..., immunosuppressed individuals, persons with a history of thymectomy, infants <9 months of age, and even pregnant and nursing women.” EX1036, 3828; *see also* EX1037, 162. Additionally, safely vaccinating pregnant individuals would

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have been of high importance due to the link between ZIKV infection and fetal disorders and death. See ¶¶51–52, above.

149. A POSA, therefore, would have had a reason to follow Srivastava’s vaccine technology to make a ZIKV vaccine. This is because Srivastava discloses a technology that made a purified inactivated JEV vaccine (“JE-PIV”) that was “more immunogenic and as effective as preventing encephalitis in mice” when compared to the existing inactivated FDA-approved JEV vaccine (“JE-Vax”) at the time Srivastava’s vaccine was made. EX1163, 4557.

150. A POSA would have understood a vaccine with good immunogenicity to be important. This is because good immunogenicity would be indicative of the ability of the vaccine to stimulate high neutralizing antibody titers, which would have the potential of better and longer lasting protection because high neutralizing antibody titers were generally associated with better protection and neutralizing antibodies could wane over time. EX1045, 1; EX1042, 707–708; *see also* Section VII.E, above (discussing observation of neutralizing antibody titers diminishing over time for flavivirus vaccines).

151. Srivastava also made its JE-PIV using a technology previously used to produce a vaccine for another member of the flavivirus family, DENV-2. EX1163, 4558. Thus, Srivastava demonstrates the ability to apply a common vaccine platform to various members of the flavivirus family. A POSA would also have

been aware from the general knowledge in the art that various other vaccines using flaviviruses were capable of stimulating high neutralizing antibody titers. See Section VII.E, above.

152. The foregoing also supports the WHO Zika Pipeline’s recommendation to use “the same technologies that have been successfully used to develop human flavivirus vaccines.” EX1143, 5. A POSA, therefore, would have reasonably expected that Srivastava’s flavivirus vaccine technology could be successfully applied to make a ZIKV vaccine. In fact, Srivastava’s purified inactivated JEV vaccine eventually became the approved IXIARO product in 2009. EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48.

b. Srivastava discloses a technology for the production of a purified inactivated JEV vaccine that is safe, effective, and economical.

153. Srivastava obtained JEV virions from the live-attenuated JEV strain known as SA₁₄-14-2,²⁵ which was obtained from an already approved vaccine. EX1163, 4559. Srivastava then passaged the SA₁₄-14-2 JEV virions in Vero cells to obtain a viral production seed that was used to further infect Vero cells for vaccine production. *Id.* Srivastava next took infected Vero cells, harvested the JEV

²⁵ SA₁₄-14-2 was originally derived from serial passage of the wild-type JEV SA14 strain in primary dog kidney cells. EX1023, 513, Table 1.

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virions from the cell medium supernatant on various days after infection, and obtained a viral concentrate. *Id.* Srivastava then purified the viral concentrate using protamine sulfate purification, followed by sucrose gradient centrifugation of the resulting supernatant and pooling of fractions containing active virus. *Id.*

154. To completely inactivate the purified JEV virions, Srivastava incubated the JEV virions in formalin (aqueous formaldehyde) for 10 days at 22 °C. *Id.*, 4559–4561. Srivastava then dialyzed the purified inactivated JEV virions into phosphate buffered saline (“PBS”) and filtered them to generate a bulk lot for vaccine formulation. *Id.* Srivastava’s JE-PIV contained the purified inactivated JEV virions, 0.1% aluminum hydroxide as an adjuvant, and 0.1% thimerosal in PBS. *Id.*, 4560. Srivastava states that its “manufacturing process was efficient in generating a high yield of virus, essentially free of contaminating host cell proteins and nucleic acids.” *Id.*, 4557.

155. Srivastava compared the immunogenicity of its JE-PIV to the then-existing FDA-approved JE-Vax, which also contained inactivated JEV virions. *Id.*, 4561. To do this, Srivastava injected mice with “graded doses of JE-PIV ... or with commercially obtained licensed vaccine, Biken JE-Vax.” *Id.* Srivastava administered each vaccine to ten mice each, in two separate doses given 28 days apart for each dosage tested. *Id.*, 4561–4563.

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156. Srivastava assayed the post-injection sera after the second administration of each vaccine “for JE virus-neutralizing antibodies” using PRNT₅₀ assays. Srivastava discloses that the lowest dose of its JE-PIV that elicited neutralizing antibodies was 32 ng, compared to JE-Vax that did so when dosed at 48 ng. *Id.*, 4562–4563, Table 3. Srivastava’s PRNT₅₀ data from Table 3 of Srivastava is reproduced below:

Table 3
Virus neutralizing antibody titers (PRNT₅₀) in mice vaccinated with JE PIV lot 0574 or commercial JE Vax

Vaccine	Dose (ng)	PRNT ₅₀ GMT	PRNT ₅₀ Range	Seroconversion rate	Immunizing dose 50% (ID ₅₀)
JE PIV	800	3842	1280–7781	10/10	4 ng (2–7 ng, 95% CI)
	160	1373	168–3026	10/10	
	32	113	34–563	10/10	
	6	ND ^b	ND	7/10	
	1	ND	ND	1/10	
JE Vax ^a	1200	733	283–3754	10/10	15 ng (14–17, 95% CI)
	240	117	18–863	10/10	
	48	90	55–150	7/10	
	10	ND	ND	6/10	
	2	ND	ND	1/10	

^a For JE-Vax, the virus protein concentration as reconstituted from the vial was 6 micrograms (6000 ng) per ml from information supplied by the manufacturer.

^b Not determined.

EX1163, Table 3.

157. Srivastava’s Table 3 show that JE-PIV dosed at 800 ng stimulated neutralizing antibodies titers in the range of 1,280–7,781 in 100% of mice, with a GMT of 3,842. *Id.* JE-Vax stimulated titers in the range of 283–3,754 in 100% of subjects, with a GMT of 733, at a higher dose of 1200 ng. *Id.* As such, Srivastava concluded that its “results showed that at equivalent doses ... JE-PIV was more immunogenic.” EX1163, 4564. Srivastava also found that JE-PIV had protective efficacy in mice. *Id.*, 4557, 4563.

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158. Based on the results obtained, Srivastava further concluded “that the processes and materials used for production of the new JE-PIV will result in a product that is safe, effective and economical” and that its “manufacturing methods are sufficiently robust, demonstrating good scalability, efficiency, and product yield.” *Id.*, 4564. Additionally, Srivastava stated that the JE-PIV manufacturing process had several advantages over the existing live-attenuated vaccine: the ability to (1) maintain the Vero cells in serum-free media to support growing the virions to a high titer, (2) harvest a large production lot, (3) increase safety due to lack of serum products, and (4) purify the virions using efficient and inexpensive methods. *Id.* A POSA would have understood the advantages Srivastava identified to be applicable to ZIKV because they arise from Srivastava’s process of using Vero cell culture to propagate the virus and are independent of the specific flavivirus species Srivastava used. *Id.* Indeed, Srivastava discloses that its process was also used with DENV-2. EX1163, 4558. And JEV, DENV, and ZIKV are all closely related. EX1030, 118; EX1031, 2537.

- c. **A POSA seeking to make a ZIKV vaccine would have had a reason to follow Srivastava’s vaccine technology and substitute JEV with the H/PF/2013 ZIKV strain from Baronti because Baronti discloses a known ZIKV strain and Srivastava discloses a successful flavivirus vaccine technology.**

159. In view of the disclosures of Srivastava, discussed in Section X.D.1.b, a POSA would have understood Srivastava to disclose a successful technology for

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the production of a purified inactivated flavivirus vaccine that is safe, effective, and economical, and which could be applied when developing a purified inactivated ZIKV vaccine.

160. And as discussed above in Section X.D.1.a, a POSA would have had a reason to follow Srivastava's technology for JEV because the WHO Zika Pipeline suggested using "*the same technologies* that have been *successfully used* to develop human flavivirus vaccines," which Srivastava exemplifies. EX1143, 5. A POSA would have understood that the WHO Zika Pipeline's assessment of the need for a ZIKV vaccine was consistent with the general knowledge in the art. Indeed, before March 18, 2016, Sifferlin reported in *Time* magazine Dr. Fauci's statement reflecting the view that "[w]hen you are developing ... vaccines ... for viruses that are similar, you can *translate the technologies that you've developed to hasten the end game goal* of what you want for a virus like Zika." EX1162, 1. Srivastava also discloses that its technology was based on a process successfully used for producing immunogenic DENV-2 vaccine compositions. EX1163, 4558. And Srivastava's purified inactivated JEV vaccine eventually became the approved IXIARO product, as the '681 patent acknowledges. EX1007, 1; EX1061, 10; EX1064, 3; EX1001, 8:41–48.

161. The WHO Zika Pipeline, in a March 3, 2016 document, also identified eighteen ZIKV vaccines undergoing development or actively being considered,

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including four that were based on using inactivated ZIKV. As discussed in Section X.D.1.a, a POSA would have been motivated to make an inactivated ZIKV vaccine because pregnant individuals, whose fetuses would be at risk for ZIKV-induced microcephaly and fetal abnormalities, and individuals with immunodeficiency conditions, would have been more safely administered an inactivated ZIKV vaccine.

162. A POSA would have had a reason to choose Srivastava's vaccine technology because Srivastava disclosed that it was a safe, effective, and economical technology that had been applied to both JEV and DENV, and it stimulated high titers of neutralizing antibodies, i.e., > 1000, after two doses using a known strain of JEV. *See* Section X.D.1.b, above. Thus, a POSA would have had a reason to follow Srivastava's vaccine technology and make a purified inactivated ZIKV vaccine using a known strain of ZIKV.

163. Baronti discloses a known naturally occurring strain of ZIKV (H/PF/2013) isolated from a subject infected during the French Polynesia outbreak of ZIKV in 2013, which was a ZIKV strain associated with incidences of "CNS malformations in children." EX1160, 1; EX1033, 7–8. Baronti also reported that the H/PF/2013 strain "shar[es] common ancestorship and ca. 99.9% nt [i.e., nucleotide] and aa [i.e., amino acid] identities with isolates circulating in southern Asia and the Pacific islands in the late 2000s." EX 1010, 1. A POSA, therefore,

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would have understood the H/PF/2013 strain to be one of various ZIKV strains that each would have been useful in making a ZIKV vaccine.

164. Baronti obtained the H/PF/2013 strain, harvested ZIKV virions from a cell culture supernatant from Vero cells infected with the virus, and extracted the strain's RNA. EX1001, 1. Baronti then sequenced the virus's genome and deposited the corresponding DNA sequence in GenBank in 2014 as accession no. KJ776791 ("the H/PF/2013 Sequence"). EX1160, 1; EX1019. Baronti discloses that the sequence obtained "includ[ed] the virus complete open reading frame (ORF) sequence." EX1160, 1. As discussed below in Section X.D.1.d, the H/PF/2013 Sequence is 99% identical to SEQ ID NO: 72. The H/PF/2013 Sequence ORF encodes the same amino acid sequence as SEQ ID NO: 73, which is the translation of the ORF of SEQ ID NO: 72. EX1071, 3–6 (showing a BLAST alignment of the translation of the H/PF/2013 Sequence's ORF and SEQ ID NO: 73); EX1001, 129:46–51.

165. Like the JEV strain used by Srivastava, the ZIKV strain Baronti studied had been passaged in Vero cells. *Id.* Baronti's strain would also have been of interest because it was a ZIKV strain that had a connection to fetal birth defects, had a role in a large ZIKV outbreak, and was known to be "the closest strain to the one that emerged in Brazil" in 2015. EX1033, 7–8; EX1058, 1887. Thus, a POSA reading the WHO Zika Pipeline and Srivastava in combination with Baronti and

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the H/PF/2013 Sequence would have had a reason to make a vaccine using the H/PF/2013 strain disclosed in Baronti, which would comprise a sequence corresponding to the H/PF/2013 Sequence in its RNA genome. A POSA would have just substituted the H/PF/2013 ZIKV strain for JEV in the technology of Srivastava.

d. Baronti and the H/PF/2013 Sequence disclose a ZIKV strain with an RNA genome comprising a sequence corresponding to a DNA sequence having at least 80% identity to SEQ ID NO: 72.

166. As discussed above, Baronti states that the H/PF/2013 Sequence “includ[es] the virus complete open reading frame (ORF) sequence,” and the H/PF/2013 Sequence was deposited into GenBank under accession no. KJ776791. EX1160, 1. The H/PF/2013 Sequence discloses a 10,617 nucleotide-long sequence. EX1019. The H/PF/2013 Sequence has 99% identity to the claimed SEQ ID NO: 72 when aligned using BLAST, and the H/PF/2013 Sequence is actually 100% identical when compared using just the regions where the two sequences completely overlap.²⁶ EX1020, 3–11. In August 2016, Baronti updated its

²⁶ In this declaration, to determine the percent identity of sequences when aligned, I used the Global Align function of BLAST, which the '681 patent discloses as a suitable alignment program to determine percent identity. See Section IX.

Sequences being compared were entered into the Global Align program and aligned using Global Align's default parameters (*available at:* https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=blastn&BLAST_SPEC=GlobalAln&LINK_LOC=blastHomeLink) and the percent identity returned by the BLAST Global Align program was identified. As discussed, above in Section IX, a sequence comparison over just the region where two sequences overlap can be used to determine percent identity according to the '681 patent (EX1001, 127:33–128:31 (stating that two sequences may be aligned over a specified region or over the entire region for maximum correspondence)). I also determined the percent identity of the two aligned sequences using just the region where the query and SEQ ID NO: 72 overlapped using the BLAST Global Align program. This percent identity using just overlapping regions was determined by removing the number of nucleotides from the “overhang” regions of the subject sequence from the percent identity comparison value returned by the BLAST Global Align program for the full length sequence comparison. For example, the alignment of the H/PF/2013 Sequence and SEQ ID NO: 72 returns a percent identity of 99% (10,617/10,773) with zero gaps. EX1020, 3. SEQ ID NO: 72 is longer than the H/PF/2013 Sequence by 156 nucleotides, which yields a percent identity of 100% (10,617/10,617) when those

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GenBank entry for H/PF/2013 and reported a sequence of 10,807 nucleotides long. EX1084. This update simply characterizes an inherent property of the H/PF/2013 strain, and such a sequence still has greater than 99% identity to SEQ ID NO: 72. EX1098.

167. Accordingly, a POSA employing the H/PF/2013 ZIKV strain from Baronti, which comprises a sequence corresponding to the H/PF/2013 Sequence in its RNA genome, to make a ZIKV vaccine would have obtained a vaccine comprising ZIKV having a genome comprising a RNA sequence corresponding to a DNA sequence having at least 80% identity, specifically, 99% identity, to SEQ ID NO: 72.

- e. A POSA would have had a reason to make a ZIKV vaccine capable of stimulating a MN_{50} of greater than 15 in at least 70% of vaccinated subjects because a POSA would have known that higher titers would provide a higher level and longer periods of protection.**

168. As discussed in Section VII.E, flavivirus vaccines, such as

nucleotides are removed from the alignment. A table showing a summary of all alignments and percent identities discussed in this declaration is provided in Appendix A, attached hereto. The exhibits cited for each alignment discussed herein are true and correct copies of the alignments I obtained when performing each alignment according to the foregoing.

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Srivastava's JE-PIV, were known in the art to stimulate high neutralizing antibody titers after administering two doses.

169. A POSA would have known from general knowledge in the art that “[g]enerally speaking, protection is achieved when the neutralizing antibody titre in the serum of the animal at the time of virus challenge is relatively high.”

EX1042, 707–709; EX1045, 1. A POSA, therefore, would have had a reason to make a ZIKV vaccine capable of stimulating high MN₅₀ titers, e.g. > 1000 when following Srivastava's technology, so that the vaccine would provide protection and do so over a longer period of time.

170. As discussed above in ¶133, a POSA developing a ZIKV vaccine using the H/PF/2013 ZIKV strain, which would comprise a sequence corresponding to the H/PF/2013 Sequence in its RNA genome, and using the vaccine technology of Srivastava would have had a reason to employ Thomas's MN₅₀ assay to assess the neutralizing antibody titers stimulated by the resulting vaccine, because it had been used to assess titers for a flavivirus vaccine and was a rapid 4-day assay. A POSA knowing that Thomas's MN₅₀ assay has a “precision ... estimated to range from 39% to 59%” would have had a reason to use a MN₅₀ of greater than 15 as the baseline titer to evidence seroconversion after vaccination because a 59% variability rate applied to the minimum neutralizing antibody titer of 10, which had been used to assess the occurrence of seroconversion by other

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flavivirus vaccines and is just above the limit of detection in Thomas's MN₅₀ assay, would be titer of 15.9 $((10 \times 0.59) + 10 = 15.9)$. See ¶68, above; EX1164, 86.

171. Thus, by using a baseline MN₅₀ titer of greater than 15, a POSA would have had greater confidence that a ZIKV vaccine made following Srivastava's vaccine technology had actually stimulated the production of neutralizing antibodies. Therefore, the lowest end of the claimed range starts just slightly above the limit of detection, but there is no upper limit to the claimed range, and it therefore includes titers in the hundreds and thousands.

172. A POSA would have also sought to make a vaccine that stimulated high titers in greater than 70% of vaccinated subjects, so as to obtain protection in as much of the vaccinated population as possible. See ¶131, above. In fact, IXIARO reflects this practice by using a two-dose regimen that "conferred essentially 100% seroconversion already 7 days after the second immunization," instead of using a single-dose regimen that resulted in 60% seroconversion when IXIARO was administered using a higher dose than normal or 20% seroconversion when administered using a single dose of the standard dosage. EX1059, 2188–2192.

173. Thus, a POSA would have had a reason to make a ZIKV vaccine capable of stimulating a MN₅₀ of greater than 15 in greater than 70% of vaccinated

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subjects because a POSA would have known that (1) higher titers have a higher potential for protecting against disease; (2) neutralizing antibody titers can wane overtime and higher titers would provide longer periods of protection; and (3) high titers stimulated in a higher percentage of the vaccinated population would have been desirable because it would have afforded broader coverage in the relevant population.

- f. A POSA would have had a reason to use a MN assay to determine a vaccine's ability to stimulate neutralizing antibodies because it would have provided higher throughput and a lesser degree of interassay variability than the traditional PRNT assay.**

174. As discussed in ¶73, PRNT₅₀ assays for assaying the neutralizing antibody titers stimulated by vaccines were known to be “labor intensive and therefore not readily amenable to high throughput, making it difficult to use for large-scale surveillance and vaccine trials.” EX1043, 126; *see also* EX1164, 86 (stating that PRNT has “limitations, such as its low throughput, unacceptable labor-intensive nature, and high degree of interassay variability”). To improve upon assays for determining neutralizing antibody titers, Thomas reported a MN₅₀ assay that Thomas discloses—and a POSA would have understood—as providing higher throughput, less interassay variability, and sensitivity in detecting neutralizing antibody titers compared to PRNT assays. EX1164, 86. A POSA making a ZIKV vaccine using the H/PF/2013 ZIKV strain, which would comprise

a sequence corresponding to the H/PF/2013 Sequence in its RNA genome, therefore, would have had a reason to employ Thomas's MN₅₀ assay when determining the resulting vaccine's ability to stimulate neutralizing antibody titers in vaccinated subjects. This is because it would have provided for higher throughput, less interassay variability, and detection of neutralizing antibody titers of the resulting ZIKV vaccine.

g. A POSA would have had a reasonable expectation of success in arriving at the claimed ZIKV vaccine.

175. A POSA combining the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas would have had a reasonable expectation of success in arriving at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would comprise an RNA genome corresponding to a nucleic acid having at least 80%, specifically, 99% identity, to SEQ ID NO: 72, and would be capable of stimulating a MN₅₀ titer of greater than 15 in at least 70% of vaccinated subjects after 2–3 doses.

176. This is because, first, Srivastava discloses a successful vaccine technology for making an immunogenic and protective purified inactivated vaccine for JEV, a flavivirus like ZIKV. *See* Section X.D.1.b, above. Srivastava discloses that its JE-PIV stimulated neutralizing antibody titers > 1000 in 100% of mice after two doses. For example, at a dose of 800 ng, Srivastava's JE-PIV stimulated a GMT of 3,842 and a range of 1,280–7,781. EX1163, 4562, Table 3. Srivastava

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also stated that its technology was based on one used to make a vaccine for the flavivirus DENV, which stimulated neutralizing antibody titers > 100 after two to three doses. *Id.*, 4558 (citing EX1028); EX1028, 1179. Baronti further discloses the known H/PF/2013 strain, which a POSA would have substituted for JEV in Srivastava's vaccine technology. Thomas, moreover, disclosed a MN_{50} that detected neutralizing antibodies stimulated by a flavivirus vaccine, and which provides high-throughput and less interassay variability. EX1164, 86.

177. Second, a POSA would have been aware of the general knowledge in the art that Tauber disclosed that IXIARO, which Srivastava's JE-PIV became, stimulated (1) a GMT of 244, (2) titers ≥ 81 –160 in $\sim 90\%$ of subjects, and (3) titers as high as 19,783. EX1049, 1850. A POSA also would have been aware of the general knowledge in the art that disclosed multiple other purified inactivated flavivirus vaccines that stimulated high neutralizing antibody titers in greater than 70% of subjects when administered in two or three doses. For instance, Putnak observed neutralizing antibody titers ranging from 350–2,500 in 100% of mice and from 100–1,230 in 82% of vaccinated rhesus monkeys after administering two doses of its purified inactivated DENV-2 vaccine. EX1028, Table 4. Putnak also observed titers in the range of 100–1,680 in 88% of monkeys after a third dose. *Id.*, Table 5. Watanaveeradej observed titers after two doses of a DENV vaccine in the range of 139–2,430 in 83% of vaccinated subjects when assessed for neutralizing

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antibodies to each serotype. EX1021, Table 6. Martinez observed neutralizing antibody titers in 100% of subjects and as high as 1,868 for its purified inactivated DENV-1 vaccine, with titers in the range of 107–1,868 in 70% of subjects administered two 5 µg doses. EX1024, 456, Table 3.

178. Given that Srivastava’s JE-PIV and other flavivirus vaccines caused seroconversion and stimulated high neutralizing antibody titers in at least 70% of subjects after two doses, a POSA would have reasonably expected to achieve a MN_{50} of greater than 15 in at least 70% of subjects, including humans and mice, after two doses when using Srivastava’s vaccine technology to make a ZIKV vaccine comprising the H/PF/2013 ZIKV strain. And a POSA would have determined such titers using an MN_{50} assay, such as Thomas’s MN_{50} assay. A POSA would have further had a reasonable expectation of success from the general knowledge in the art that disclosed that other flavivirus vaccines also stimulated high neutralizing antibody titers in at least 70% of subjects.

179. Third, the WHO Zika Pipeline directly stated that ZIKV vaccines can be made “building on the same technologies that have been successfully used to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).” EX1143, 5. A POSA would also have been aware of the general knowledge in the art that directly stated it was expected that a ZIKV vaccine could be successfully developed using other flavivirus vaccine

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technologies. In fact, as reflected in the *Time* magazine article Sifferlin published, it was generally known in the art that Dr. Fauci stated that when making vaccines for viruses that are similar, such as ZIKV and other flaviviruses, “you can translate the technologies that you’ve developed to hasten the end game goal of what you want for a virus like Zika.” EX1162, 1. Similarly, the field also believed there was likely “a good shot at success” in making a vaccine against ZIKV. EX1052, 543. The art, moreover, reported that “[v]accine pioneer Stanley Plotkin” said he did not “see any technical issues [in making a ZIKV vaccine] such as the ones that obviously exist for vaccines against HIV, tuberculosis, and many other agents.” *Id.*

180. Additionally, a POSA would have reasonably expected some degree of efficacy with a purified inactivated ZIKV vaccine made following the vaccine technology of Srivastava but comprising the H/PF/2013 ZIKV strain. Efficacy would have been expected for a ZIKV vaccine made following Srivastava’s technology because, as discussed above, a POSA would have reasonably expected such a vaccine to stimulate the production of neutralizing antibodies greater than 15, as measured by a MN₅₀ assay. A POSA would have reasonably expected titers greater than 15 to afford some amount of protection given that the art correlated titers greater than 10 with being protective for the generally very similar JEV and YFV. See ¶68, above. Srivastava, moreover, disclosed that its JE-PIV that stimulated neutralizing antibody titers > 1000 was efficacious EX1163, 4557,

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4563, Table 3. Thus, a POSA would likewise expect some degree of efficacy for the closely related ZIKV when made into a vaccine using Srivastava's vaccine technology. Further, as discussed above in ¶179, the art stated that it was expected that a ZIKV vaccine made using existing flavivirus technology would be successful.

181. A POSA, therefore, would have had a reasonable expectation of successfully making a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which comprises a sequence corresponding to the H/PF/2013 Sequence in its RNA genome, that would be capable of stimulating a MN_{50} of greater than 15 in at least 70% of vaccinated subjects after 2–3 doses when combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas.

182. Accordingly, for the reasons outlined above in ¶¶140–181 and after considering any objective indicia of nonobviousness, which are addressed below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claim 1 would have been obvious in view of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas.

2. Claims 2, 3, 24, and 25

183. Claim 2 depends from claim 1 and recites that the “ MN_{50} is greater than 20, 25, 30, 35, 40 or 45.” EX1001, 445:42–43. Claim 3 depends from claim 1

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and recites that the “vaccine is capable of stimulating a MN_{50} titer greater than 15 in at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects.” *Id.*, 445:44–47. Claims 24 and 25 depend from claim 2 and recite that the vaccine is capable of stimulating a MN_{50} greater than 50, 55, 60, 65, 70, 75, 80, or 85 (claim 24) and greater or equal to 90 (claim 25). *Id.*, 448:19–22.

184. As discussed in Section X.D.1 for claim 1, a POSA combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas would have had a reason to arrive at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would be capable of stimulating a MN_{50} titer of greater than 15 in at least 70% of vaccinated subjects, and would have had a reasonable expectation of success in doing so.

185. As discussed in X.D.1.e, a POSA would have sought to obtain high titers in as much of the vaccinated population as possible. Consequently, a POSA would have also had a reason to make a vaccine that could stimulate a MN_{50} of much greater than 15 in at least 75% of vaccinated subjects (**claim 3**) because the ZIKV vaccine would have provided protection to more of the vaccinated population.

186. A POSA also would have had a reasonable expectation of success in achieving a MN_{50} titer of greater than 15 in at least 75% percent of subjects

(**claim 3**) for the same reasons outlined for at least 70% of subjects for claim 1, to maximize protection in the vaccinated population. See Section X.D.1.g.

187. Also as discussed in X.D.1.e, a POSA would have sought to make a vaccine that stimulated high MN₅₀ titers because neutralizing antibody titers can wane over time, and by making a vaccine that stimulated a high titer, a POSA would have expected better protection and longer protection over time. Consequently, a POSA would have also had a reason to develop a ZIKV vaccine that could stimulate a MN₅₀ of much greater than 90 (**claims 2, 24, and 25**).

188. A POSA also would have had a reasonable expectation that a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would comprise a RNA sequence corresponding to the H/PF/2013 Sequence in its RNA genome, would have been capable of stimulating a MN₅₀ titer of greater than 90 (**claims 2, 24, and 25**). Srivastava discloses that its JE-PIV stimulated titers in the range of 1,280–7,781 in 100% of mice when administered at 800 ng and 168–3,026 in 100% of mice when administered at 160 ng. EX1163, Table 3. Similarly, IXIARO, which Srivastava's JE-PIV became, stimulated titers greater than 161 in approximately at least 75% of subjects when dosed at 6 µg. EX1049, 1851.

189. Accordingly, in addition to the reasons outlined above for claim 1 and after considering any objective indicia of nonobviousness, which are addressed

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below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claims 2, 3, 24, and 25 would have been obvious.

3. Claims 4 and 21–23

190. Claim 4 depends from claim 1 and recites that the ZIKV comprises an E protein having one of 56 amino acid sequences or a variant amino acid sequence having at least 95% identity to any one of those 56 amino acid sequences. EX1001, 445:48–53. Claims 21–23 depend from claim 1 and recite that the RNA genome is a variant nucleic acid having at least 90%, 95%, or 99% identity to SEQ ID NO: 72, respectively. *Id.*, 448:10–18.

191. As discussed in Section X.D.1 for claim 1, a POSA combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas would have had a reason to arrive at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would be capable of stimulating a MN₅₀ titer of greater than 15 in at least 70% of vaccinated subjects, and would have had a reasonable expectation of success in doing so.

192. The translation of the H/PF/2013 Sequence's ORF is 100% identical to the translation of the ORF in SEQ ID NO: 72,²⁷ which is SEQ ID NO: 73.

²⁷ The translation of the ORF of the H/PF/2013 Sequence was obtained from the GenBank entry for KJ776791.1. EX1019, 1–2.

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EX1001, 121–122, 129:46–51; EX1071, 3–6 (showing a BLAST alignment of the translation of the H/PF/2013 Sequence’s ORF and SEQ ID NO: 73). The portion of SEQ ID NO: 73 corresponding to the E protein is SEQ ID NO: 47. EX1001, 111–112, 129:52–56; *see also* EX1009 (showing a BLAST alignment of SEQ ID NO: 73 and SEQ ID NO: 47). SEQ ID NO: 47 is one of the E protein sequences claimed in claim 4.

193. A POSA, therefore, would have arrived at the vaccine of claim 4 in view of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas. A POSA also would have had a reasonable expectation of success in doing so because the H/PF/2013 Sequence and SEQ ID NO: 72 encode the same E protein, and because the E protein is a surface protein, a POSA would have expected it to be an immunogenic antigen that would stimulate high titers of neutralizing antibodies. EX1080, 2337; EX1048, 25–26.

194. A POSA would also have had a reason to arrive at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would comprise an RNA genome having at least 90%, 95%, and 99% identity to SEQ ID NO: 72 when the RNA genome comprises a sequence corresponding to the H/PF/2013 Sequence. This is because, as discussed in Section X.D.1.d, the H/PF/2013 Sequence is 99% identical to SEQ ID NO: 72, and 100% identical when compared using just the regions where the two sequences completely

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overlap. A POSA, therefore, would have had a reasonable expectation of success in arriving at the ZIKV vaccine of claims 21–23 for the same reasons discussed in Section X.D.1.g, for claim 1.

195. Accordingly, in addition to the reasons outlined above for claim 1 and after considering any objective indicia of nonobviousness, which are addressed below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claims 4 and 21–23 would have been obvious.

4. Claims 5–9

196. Claims 5–9 depend either directly or indirectly from claim 1. Claim 5 recites that the vaccine’s virus is inactivated by, *inter alia*, chemical inactivation. EX1001, 445:54–56. Claim 6 recites contacting the virus with the chemical inactivation agent for longer than is required to completely inactivate the ZIKV as measured by plaque assay. *Id.*, 445:57–61. Claim 7 recites contacting the ZIKV with formaldehyde, and claim 8 recites contacting with the formaldehyde for between 2–10 days. *Id.*, 445:62–67. Claim 9 recites that the chemical inactivation is performed at about 4° C or about 22° C. *Id.*, 446:33–35.

197. As discussed in Section X.D.1 for claim 1, a POSA combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas would have had a reason to arrive at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would be capable of

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stimulating a MN₅₀ titer of greater than 15 in at least 70% of vaccinated subjects, and would have had a reasonable expectation of success in doing so when following the technology of Srivastava and substituting to ZIKV for JEV.

198. A POSA following the vaccine technology of Srivastava to generate a purified inactivated ZIKV vaccine would have used formalin, i.e., aqueous formaldehyde (EX1087, 695), to chemically inactivate the ZIKV virions because Srivastava used formaldehyde to inactivate its JEV (**claims 5 and 7**). EX1163, 4559. Formalin inactivation of virions, including flaviviruses, was also a well-known inactivation process in the art. EX1087, 695, 698. Srivastava also teaches performing the formalin inactivation at 22 °C (EX1163, 4559, 4561) (**claim 9**) for ten days (*id.*) (**claim 8**), which was longer than was required to completely inactivate JEV as measured by a plaque assay because Srivastava observed complete inactivation after the sixth day of the inactivation process as measured by a plaque assay (*id.*, 4561, Table 2) (**claim 6**).

199. A POSA, therefore, would have had a reason to replicate Srivastava's virion inactivation process when making a ZIKV vaccine because it would have also been expected to completely inactivate the ZIKV virions as measured by a plaque assay, given that JEV and ZIKV are closely related flaviviruses. A POSA would have also sought to inactivate the ZIKV virions longer than is required to completely inactivate the virions because doing so would have provided an added

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assurance of safety in making a ZIKV vaccine suitable for administration to the elderly, infants, and immunocompromised and pregnant individuals. See ¶76. Srivastava's process, moreover, resulted in a highly immunogenic and effective vaccine. See Section X.D.1.b.

200. A POSA would also have had a reasonable expectation of success in making a purified formalin-inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain. This is because Srivastava discloses that its vaccine technology worked for JEV and it was also based on a successful formalin-inactivated virus process used with DENV. EX1163, Table 3, 4558 (citing EX1028); EX1028, 1179. And the art suggested that a ZIKV vaccine could successfully be made using technologies that had worked successfully for other flaviviruses, such as Srivastava's purified formalin-inactivated JEV vaccine. See ¶179, above.

201. Accordingly, in addition to the reasons outlined above for claim 1 and after considering any objective indicia of nonobviousness, which are addressed below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claims 5–9 would have been obvious.

5. Claims 10, 11, and 15

202. Claim 10 depends from claim 1 and recites that the vaccine comprises an adjuvant. EX1001, 446:36–37. Claims 11 depends from claim 10 and recites that the adjuvant is an aluminum salt (claim 11). *Id.*, 446:38–39. Claim 15 depends

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from claim 1 and recites that the vaccine comprises one or more pharmaceutically acceptable excipients. *Id.*, 446:53–55.

203. As discussed in Section X.D.1 for claim 1, a POSA combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas would have had a reason to arrive at a purified inactivated ZIKV vaccine comprising the H/PF/2013 ZIKV strain, which would be capable of stimulating a MN₅₀ titer of greater than 15 in at least 70% of vaccinated subjects, and would have had a reasonable expectation of success in doing so when following the technology of Srivastava and substituting to ZIKV for JEV.

204. A POSA following the vaccine technology of Srivastava to generate a purified inactivated ZIKV vaccine would have added aluminum hydroxide, which is an aluminum salt (**claim 10**). This is because Srivastava used aluminum hydroxide as an adjuvant. EX1163, 4560. And aluminum hydroxide was also a well-known adjuvant commonly used in vaccines before March 18, 2016. EX1083, 1:6–17.

205. A POSA would have also included thimerosal in PBS, pharmaceutically acceptable excipients, when following the vaccine technology of Srivastava but for ZIKV. This is because Srivastava used thimerosal in PBS in its JEV vaccine. EX1163, 4560. A POSA would also have known that thimerosal was a common vaccine additive that would have helped prevent microbial

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contamination of the vaccine during storage. EX1022, 149. And PBS would serve as a buffer that aid in maintaining the vaccine formulation's pH and stability.

EX1118, 294–295.

206. Moreover, Srivastava used both aluminum hydroxide and thimerosal in PBS to make a successfully immunogenic and efficacious vaccine for JEV. EX1163, 4560, Table 3. A POSA, consequently, would also have had a reasonable expectation of success in using aluminum hydroxide and thimerosal in PBS, especially in light of ZIKV and JEV being closely related and the art suggesting that a ZIKV vaccine could successfully be made using technologies that had worked successfully for other flaviviruses, such as Srivastava's vaccine technology.

207. Accordingly, in addition to the reasons outlined above for claim 1 and after considering any objective indicia of nonobviousness, which are addressed below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claims 10, 11, and 15 would have been obvious.

E. Claim 12 would have been obvious over the WHO Zika Pipeline, Srivastava, Baronti, H/PF/2013 Sequence, Thomas, and Möhlen.

208. Claim 12 depends from claim 11 and recites that the adjuvant is an aluminum salt with less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV. EX1001, 446:38–44.

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209. As discussed above in Sections X.D.1 and X.D.5 for claims 1 and 11, a POSA would have arrived at using aluminum hydroxide in a purified inactivated ZIKV vaccine when combining the teachings of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas and would have done so with a reasonable expectation of success.

210. A POSA making a ZIKV vaccine would have also had a reason to combine the teachings of Möhlen with the WHO Zika Pipeline, Srivastava, Baronti, H/PF/2013 Sequence, and Thomas. This is because Möhlen discloses that the presence of heavy metals, such as copper, in aluminum salt adjuvants can affect the stability of viral antigens in inactivated virus vaccines. EX1083, 5:1–5. Möhlen discloses that heavy metals, such as copper, can react with residual sulfite that was used to neutralize the formaldehyde in formaldehyde-inactivated virus vaccines and cause oxidative degradation of the antigen. *Id.*, 11:14–22, 16:1–11. A POSA would have also known from the general knowledge in the art that metal ions, such as copper, if present in biopharmaceutical drug products, such as a vaccine, can result in contamination that causes physicochemical degradation of the active molecule, e.g., antigen, over long-term storage. EX1077, 1173, 1177.

211. A POSA developing a formalin-inactivated ZIKV vaccine comprising aluminum hydroxide that may require long-term storage, therefore, would have

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had a reason to use aluminum hydroxide having less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV.

212. Möhlen is directed “to aqueous compositions comprising a protein and an aluminium-salt, said composition comprising less than 350 ppb heavy metal based on the weight of the aqueous composition.” EX1083, Abstract. Möhlen states that “[a]luminium adjuvants have been used in practical vaccination for more than half a century. They induce early, high-titre, long-lasting protective immunity.” *Id.*, 1:11–13. Möhlen further discloses that the aluminum component “is an important source for the heavy metal in the aqueous [pharmaceutical] composition.” *Id.*, 9:12–16.

213. Möhlen found “that the stability of a protein component (e.g. as such or within a complex such as e.g. *a virus* or other pathogen) in the context of an aqueous composition that also comprises aluminium salt and a reactive compound such as e.g. *a sulphite* is critically dependent on the content of heavy metals.” *Id.*, 5:1–5.

214. Möhlen further discloses that:

antigen degradation in aqueous compositions, such as
[an] immunogenic composition, comprising heavy metal
ions present in an aluminium salt, *such as aluminium
hydroxide*, might be explained with an underlying
degradation pathway assuming free-radicals such as e.g.

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free-radicals of sulphite. Formation of free radicals can be catalysed by heavy metal ions present in, for instance, aluminium hydroxide and this effect (in case of a certain amount of heavy metal as indicated according to the invention) could be the underlying root cause mechanism for stability issues as identified.

Id., 11:14–22.

215. Möhlen discloses that inactivated virus vaccines “are likely to comprise one or more reactive compounds” because of the presence of residual sulfite, such as sodium bisulfite, in the vaccine composition that is used to neutralize any residual formaldehyde that was used during inactivation of the virus.

Id., 16:1–11. Möhlen thus teaches that “the absence of heavy metals or presence below levels as identified by the present invention, is of particular relevance when the aqueous composition of the invention comprises an inactivated virus.” *Id.*, 16:8–11.

216. Möhlen discloses that “the stability of aqueous compositions comprising a JEV protein, an aluminium-salt and comprising less than 350 ppb heavy metal based on the weight of the aqueous composition, *and in particular wherein the amount of Cu is less than 3 ppb based on the weight of the aqueous composition, is increased as compared to aqueous composition comprising more*

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than 350 ppb of heavy metal and *more than 3 ppb of Cu.*” *Id.*, 33:24–30; *see also id.*, Tables 12–15. Möhlen further discloses that:

The amount of Cu [i.e., copper] in a composition or method of the inventions is in a particularly preferred embodiment less than 1,25 ppb [i.e., 1.25 ppb] based on the weight of the aqueous composition. In a particularly preferred embodiment said aqueous composition comprises Cu at a level that is below the detection limit of the method for the detection of copper.

Id., 27:27–28:2.

217. Möhlen discloses that “[m]ethods for measuring the level of one or more heavy metals in an aqueous solution are known in the art,” including “mass spectrometry, such as inductively-coupled-plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (F-AAS), and/or graphite furnace atomic absorption spectrometry (GF-AAS).” *Id.*, 28:18–25.

218. Srivastava discloses that the JEV in its vaccine is inactivated using formaldehyde, and the formaldehyde is then neutralized using sodium bisulfite. EX1163, 4559. A POSA following Srivastava’s vaccine technology for ZIKV, therefore, would have had a reason to ensure that the level of copper in the vaccine’s aluminum hydroxide adjuvant was below the limit of detection, including being below 1.25 ppb based on the weight of the composition, as a matter of quality assurance. Doing so would have reduced that chance that

degradation of the ZIKV antigen would occur as a result of oxidative degradation during long-term storage of the ZIKV antigen due to any residual bisulfite that may be present in the vaccine formulation. EX1083, 11:14–22, 16:1–11.

219. A POSA, moreover, would have known from Möhlen that well-known techniques such as “mass spectrometry, such as inductively-coupled-plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (F-AAS), and/or graphite furnace atomic absorption spectrometry (GF-AAS)” were available for assessing levels of copper in the vaccine composition. *Id.*, 28:18–25.

220. A POSA, therefore, would have had a reasonable expectation of success in making a ZIKV vaccine having less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV because aluminum hydroxide was a commonly-used adjuvant in vaccines. *Id.*, 1:11–13. Möhlen, moreover, discloses techniques to measure copper levels, which would have allowed a POSA to determine if the aluminum hydroxide used results in copper levels above 1.25 ppb. *Id.*, 28:18–25. A POSA, therefore, would simply have needed to change the lot of aluminum hydroxide used if copper levels were too high. Indeed, Möhlen discloses assaying lots of aluminum hydroxide for heavy metals, including copper, and finding lots with both high and low amounts of heavy metals. *Id.*, 26:7–13.

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221. Accordingly, in addition to the reasons outlined above for claim 1 and 11, as well as after considering any objective indicia of nonobviousness, which are addressed below for claims 1–12, 15, and 21–25 in Section X.F, it is my opinion that claim 12 would have been obvious over the WHO Zika Pipeline, Srivastava, Baronti, H/PF/2013 Sequence, Thomas, and Möhlen.

F. No objective evidence supports non-obviousness.

222. Before reaching my opinions regarding obviousness laid out above, I also considered whether there were any objective indicia of nonobviousness that support the patentability of the invention. I have been informed by counsel that (1) unexpectedly superior results as compared with the closest prior art, (2) long-felt but unmet need, (3) commercial success, (4) failure of others, (5) skepticism of skilled POSAs before the invention, (6) copying of the invention, (7) industry praise for the invention, and (8) licenses showing industry respect for the invention are examples of objective indicia of nonobviousness. I have also been informed by counsel that evidence on near-simultaneous invention of the alleged invention claimed in the '681 patent can be evidence of the obviousness of the claims.

223. I am not aware of any evidence of commercial success, failure of others, copying, skepticism, praise, or licenses showing industry respect for the alleged invention of the '681 patent claims. I address below the '681 patent's allegations of unexpected superior results, as well as there being (1) evidence of

near-simultaneous invention; and (2) no evidence of long-felt need or failure of others.

1. No unexpectedly superior results

224. The '681 patent alleges that the data regarding immunogenicity of the claimed vaccine “indicate that immunogenicity was [unexpectedly] higher than the recently reported inactivated Zika virus vaccine candidate” of Larocca et al. (EX1006). EX1001, 137:32-34. I disagree, and a POSA would disagree.

225. In making its claim of unexpected results, Valneva compared (1) the MN₅₀ titer of 90 that it obtained with the '681 patent's single example of a purified inactivated ZIKV vaccine dosed once with (2) the MN₅₀ titer of 15 observed in Larocca et al. from administering a purified inactivated ZIKV vaccine also dosed once. *Id.*, 136:22–27.

226. This is improper, however, because a proper comparison of the two vaccines requires that both vaccines be tested in the *same* assay in the *same* lab, or using a validated assay (which the MN₅₀ assay is not, as explained in ¶72). See, e.g., EX1047, 5209 (stating that PRNT “[t]est results between laboratories are ... not readily comparable” due to varying protocols and “[i]n order to achieve lab-to-lab comparability, tests need either to be standardized [i.e., validated] or a calibrated international reference serum needs to be developed;” a POSA would understand MN assays to have at least the same issues given both PRNT and MN

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assays rely on collecting sera, pre-mixing virions and sera, and cell culture techniques). And while the '681 patent states that it used Larocca's MN₅₀ assay, differences between cell culture conditions, for example, such as cell medium supplements, incubation temperatures, and microtiter plate well sizes can lead to differences in results. EX1044, 963; EX1047, 5209. As such, separate MN₅₀ assays performed in separate labs, even if performed according to the same protocol, used to compare two vaccines in the manner Valneva has done are not sufficiently informative as to whether one vaccine is unexpectedly more immunogenic than another vaccine. *Id.*

227. Moreover, a MN₅₀ titer of 90 is not a marked improvement over a MN₅₀ of titer 15, when each is obtained from a single dose of a vaccine and especially when neutralizing antibody titers for other flavivirus vaccines were disclosed in the art as reaching into the thousands. See Section VII.E, above. Valneva's observed MN₅₀ of 90 compared to Larocca's observed MN₅₀ of 15 is merely a minor difference, not a difference in kind.

228. I also understand that, when assessing objective indicia, evidence that arises after the filing date of a patent can be used to evidence an inherent property of a product disclosed in the prior art. A POSA would have understood from an August 4, 2016 paper from Peter Abbink, Rafael Larocca, myself, and others that Larocca's vaccine stimulated MN₅₀ titers into the thousands when administered in

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two doses. EX1062 (“Abbink”). Abbink reported on the MN₅₀ titers of the same purified inactivated ZIKV vaccine from Larocca after the vaccine was administered twice to rhesus monkeys. EX1062, 1129. Abbink reported median log₁₀ MN₅₀ antibody titers of 3.66, i.e., an MN₅₀ of 4,570,²⁸ and 100% of the vaccinated monkeys had titers in the thousands. *Id.*, 1129, FIG. 1B.

229. A POSA, therefore, would have understood Larocca’s vaccine could stimulate neutralizing antibody titers in the thousands after two doses, as the prior art reported other flavivirus vaccines to be capable of doing. *See* Section VII.E. A POSA, therefore, would understand Larocca’s vaccine could stimulate titers much higher than 15 (and 90). Thus, the ’681 patent’s claim of unexpectedly superior results, in that its vaccine was purportedly more immunogenic than Larocca’s, is incorrect.

230. I further understand that unexpected results must be compared to the closest prior art, which is the reference having the most in common with the claimed invention. I understand that Larocca is not prior art to the challenged claims as of their March 18, 2016 date, thus Larocca cannot be the closest prior art. As discussed above, Srivastava and various other purified inactivated flavivirus

²⁸ Abbink reports neutralizing antibody titers as determined by MN₅₀ in values of log₁₀. A log₁₀ MN₅₀ titer of 3.66 is equal to a MN₅₀ titer of 4,570, i.e., 10^{3.66}.

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vaccines were reported to stimulate neutralizing antibody titers, including by MN_{50} , of greater than 90 in at least 75% of subjects.

231. I also understand that unexpected results must be reasonably commensurate with the scope of the claims. As discussed below in Section XI.B.1.b.i, claims 1–12, 15, and 21–25 encompass myriad ZIKV vaccines. But Valneva has only presented data for a single ZIKV vaccine falling within the scope of the claims. EX1001, 136:22–27. But as discussed in XI.B.1.b.ii, genomic variations can result in ZIKV virions that could be less immunogenic than naturally occurring ZIKV strains. Thus, given the large scope of variant ZIKV virions encompassed by claims 1–12, 15, and 21–25, the immunogenicity of the single working example disclosed in the '681 patent, which uses a naturally occurring ZIKV strain, does not indicate that all of the possible ZIKV vaccines encompassed by the claims will behave similarly.

232. In view of ¶¶224–230, it is my opinion that Valneva has failed to show unexpectedly superior results for the full scope of any claim.

2. Near-simultaneous invention

233. I understand that evidence of near-simultaneous invention of the alleged invention claimed in the '681 patent can be evidence of the obviousness of the claims by (1) evidencing the level of skill in the art; and (2) constituting objective evidence that POSAs understood the problem and the solution to it.

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234. Larocca's purified inactivated ZIKV vaccine is such evidence of near-simultaneous invention of a ZIKV vaccine comprising ZIKV having an RNA genome corresponding to a sequence with at least 80% identity to SEQ ID NO: 72 and capable of stimulating a MN_{50} of greater than 90 in 100% of subjects.

235. Larocca's data with its single-dose administration was published online on June 28, 2016. EX1006, 477. I am the senior author on Larocca and my research group collaborated with those at WRAIR in developing the vaccines and obtaining the data disclosed in Larocca. We submitted our data and manuscript to the publisher by May 30, 2016. *Id.* Based on my recollection, I believe the foregoing to be an accurate reflection of the timing of our submission to the publisher and its publication. To my knowledge, we began working on our ZIKV vaccine around January 2016. We, therefore, rapidly arrived at our ZIKV vaccine, discussed below in ¶236, within five months of when development began and within at most 2.5 months of Valneva, a very short period of time in this field. By this time, we had data showing our ZIKV vaccine disclosed in Larocca conferred complete protection in mice and stimulated a MN_{50} titer of 15 when dosed once. *Id.*, 476, Table 1, FIG. 4.

236. Our purified inactivated whole ZIKV vaccine in Larocca comprises a Puerto Rican strain of ZIKV (PRVABC59). *Id.*, 479. The Puerto Rican strain has an RNA genome comprising a sequence corresponding to a DNA sequence having

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98% identity to SEQ ID NO: 72, and having 99% identity when compared using just the regions where the two sequences completely overlap. EX1075 (showing a BLAST alignment of the PRVABC59 sequence to SEQ ID NO: 72). The E protein of the Puerto Rican strain is SEQ ID NO: 40 in the '681 patent (which is claimed in claim 4). EX1076 (showing a BLAST alignment of the translation of the PRVABC59 ORF to SEQ ID NO: 40). Our purified inactivated ZIKV vaccine in Larocca also comprises ZIKV virions inactivated by incubating the virions in formalin for 7 days at 22 °C. EX1006, 479. Our purified inactivated ZIKV vaccine in Larocca is also formulated with aluminum hydroxide as the adjuvant. *Id.*, 476.

237. As discussed above in ¶228, Peter Abbink, Rafael Larocca, I, and others published a follow-up study with our purified inactivated ZIKV vaccine from Larocca. Our data with Larocca's purified inactivated ZIKV vaccine administered using a two-dose schedule published on August 4, 2016 according to the publication. EX1062, 1132. We submitted the data and manuscript for Abbink to the publisher on July 21, 2016. *Id.* Based on my recollection, I believe the foregoing to be an accurate reflection of the timing of our submission to the publisher and its publication.

238. Our data in Abbink demonstrated that Larocca's purified inactivated ZIKV vaccine had median $\log_{10}$ MN₅₀ antibody titers of 3.66, i.e., MN₅₀ of 4,570 ($10^{3.66} = 4,570$), and 100% of the vaccinated monkeys had titers in the thousands.

Id., 1129, FIG. 1B. This data indicates that we near-simultaneously invented a ZIKV vaccine capable of stimulating MN₅₀ neutralizing antibody titers greater than 15 in at least 70% of vaccinated subjects, and further demonstrates the obviousness of claims 1–12, 15, and 21–25.

3. No long-felt, but unmet, need

239. I understand that a showing of a long-felt but unmet need requires three factors. First, the need must have been a persistent one that was recognized by those of ordinary skill in the art. Second, the long-felt need must not have been satisfied by another before the invention. Third, the invention must in fact satisfy the long-felt need.

240. There was no persistent unmet need in the art for a ZIKV vaccine before March 18, 2016. In fact, ZIKV was not for many years or decades recognized as a global threat that necessitated a vaccine. In particular, only until the outbreaks in French Polynesia and Brazil during 2013–2015, which were linked to cases of fetal defects and neurological disorders, did a ZIKV vaccine become of interest. The WHO identified eighteen different programs that were actively developing or considering projects to make ZIKV vaccines by March 3, 2016. EX1143, 5. ZIKV vaccines, therefore, were rapidly developed once the field mobilized to work on a vaccine in late-2015 to early-2016, as evidenced by at least our work published in Larocca and Abbink. Indeed, my work with others on ZIKV

vaccines that was eventually published in Larocca was submitted for publication on May 30, 2016, which is a very short amount of time in this field once a work on a vaccine is initiated, which we began in January 2016. *See* ¶235, above.

4. No failure of others

241. I understand that evidence that others in the art tried and failed to develop inventions similar to a patentee's can be relevant to whether a claimed invention is nonobvious.

242. Larocca and the follow-up studies of Abbink also demonstrate that there was no failure of others in making a ZIKV vaccine that was immunogenic and capable of stimulating MN₅₀ titer of greater than 15 in at least 70% of vaccinated subjects. *See* ¶¶235–237, above. Moreover, the lack of any approved ZIKV vaccine today is not a result of technical difficulties or failure to achieve a ZIKV vaccine that could stimulate sufficient neutralizing antibody titers, but rather it is likely due to the reduced demand for a ZIKV vaccine due to reduction in ZIKV transmissions. EX1099, 2582 (noting that ZIKV outbreaks have currently ceased and have been unpredictable).

G. My conclusions with respect to obviousness

243. In view of ¶¶140–242 above, it is my opinion that (1) claims 1–11, 15, and 21–25 would have been obvious over the combination of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, and Thomas; (2) claim 12

would have been obvious over the combination of the WHO Zika Pipeline, Srivastava, Baronti, the H/PF/2013 Sequence, Thomas, and Möhlen; and (3) no objective indicia support non-obviousness of the claims. Additionally, evidence of near-simultaneous invention of the alleged invention claimed in 1–12, 15, and 21–25 further demonstrates obviousness of the challenged claims.

XI. BASIS OF MY ANALYSIS REGARDING ANTICIPATION

244. As discussed below in Section X.B, it is my opinion that claims 1–15 and 21–25 of the '681 patent are not entitled to a priority date earlier than December 23, 2016. As also discussed below, it is my opinion that claims 1–15 and 21–25 are anticipated by International Publication WO 2017/109225 A1 (“WO '225”; EX1008), published on June 29, 2017.

A. My understanding of the law of anticipation

245. It is my understanding from counsel that a prior art reference anticipates a claim if it (i) discloses each and every element recited in the claim, arranged as claimed and (ii) enables a POSA to make and use the subject matter that anticipates the claim. I set forth my understanding of the enablement requirement in ¶¶248–250, below. I understand that for the purposes of anticipation the prior art reference only need to enable a single embodiment that anticipates the claims at issue, whereas a priority application must enable the full scope of the subject claims. I additionally understand that a reference can

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anticipate even if it does not expressly spell out all the limitations arranged or combined as in the claim, if a POSA, reading the reference would at once envisage the claimed arrangement or combination.

B. Claims 1–15 and 21–25 are not entitled to a priority date of December 23, 2016.

246. As stated above in ¶13, I understand that the '681 patent claims priority to a series of patent applications, including the '664 PCT, filed on December 23, 2016. As discussed in Section X.B, above, I understand that for a patent to gain the benefit of the filing date of an earlier application (also known as claiming priority to an earlier application), the earlier application must both (i) enable and (ii) provide adequate written description of the subject matter later claimed in the patent as of the earlier application's filing date.

247. I understand that for a patent application to satisfy the written description requirement, the application's specification must convey clearly to those skilled in the art that, as of the filing date sought (here, I understand it to be December 23, 2016 for the '664 PCT), the inventor was in possession of the invention claimed. I understand that the purpose of the written description requirement is to ensure that the scope of the right to exclude others, as set forth in the claims, does not overreach the scope of the inventor's contribution in the specification to the art as described.

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248. It is my understanding that to satisfy the written description requirement for a claim to a functionally defined genus (a genus that is claimed by the function that it performs), as is the case for claims 1–15 and 21–25, the specification must (i) show that the inventor had possession of a sufficient number of representative species to show that he or she invented the full scope of the claimed genus; or (ii) disclose structural features common to the members of the genus so that one of skill in the art can visualize or recognize the members of the genus. I understand that demonstrating possession requires a precise definition of the invention. It is also my understanding that, for a functionally-defined genus, the disclosure of a known or disclosed correlation between function and structure in either the specification or known in the art can also be used to show that the inventor was in possession of a genus claim. I understand that, alternatively, one can determine possession by looking for blaze marks in the specification that single out particular species, rather than simply pointing to the genus. I understand that in the context of the written description analysis, the reference to “blaze marks” is an analogy to an old tradition in the woods to marking trails by making blaze marks on the trees; the inquiry is meant to help determine which trees—i.e., species—the claim is claiming.

249. It is my understanding from counsel that an enabling disclosure must allow a POSA to make and use the full scope of the invention without undue

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experimentation as of the filing date sought. I also understand that enablement is analyzed by evaluating the so-called *Wands* factors. I understand from counsel that these factors are: (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the level of one of ordinary skill; (5) the level of predictability in the art; (6) the amount of direction provided by the inventor; (7) the existence of working examples; and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. I understand that one has to consider all the evidence related to each of these factors, and that a conclusion of non-enablement must be based on the evidence as a whole.

250. I understand that the determination that “undue experimentation” would have been needed to make and use the claimed invention is not a single, simple factual determination. Rather, it is a conclusion reached by weighing all the above noted factual considerations. I understand, however, that if a POSA would still need to discover undisclosed embodiments by trial and error or by another method that would require substantial time and effort, such experimentation would be undue. And I understand that when a claim states certain structural requirements and also require performance of some function (e.g., efficacy for a certain purpose), undue experimentation can include undue experimentation in identifying, from among the many concretely identified embodiments that meet the structural requirements, the embodiments that satisfy the functional requirement.

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1. The '664 PCT does not provide written description support for claims 1–15 and 21–25 of the '681 patent.

251. Based on my review of the '664 PCT, the '664 PCT would *not* have conveyed to a POSA that the inventors had possession of the vast genus of ZIKV vaccines encompassed by claims 1–15 and 21–25, i.e., the myriad ZIKV vaccine candidates defined by function discussed below in Section XI.B.1.b.i, when they filed the '664 PCT. My analysis in this respect is set forth in detail below.

a. As of December 23, 2016, aspects of ZIKV structure and function ZIKV were unpredictable.

i. A POSA would have understood that to make the claimed vaccines, ZIKV has to replicate in production cells.

252. A POSA would have recognized that in order to make the claimed ZIKV vaccines, ZIKV must be able to replicate in production cell lines. This is because of the technology that was used in 2016 to make flavivirus vaccines, both in the field and by the inventors of the '681 patent. Briefly, early in the development of flavivirus vaccines (circa the 1950s), researchers used mouse brains for a vaccine antigen source. EX1041, 1328. But “[b]ecause of a potential problem in the use of mouse brains for a vaccine antigen source, efforts to develop cell culture-derived vaccines have been made.” *Id.*, 1329. This is why, by the end of the 20th century, researchers started using the “non-tumorigenic cell line—Vero—derived from the kidney of an African green monkey” for vaccine manufacture. EX1178, 1336. By December 2016, the arsenal of cell lines used for

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vaccine production included many others, e.g., HER (human embryonic retinoblasts), HeLa, CHO (Chinese hamster ovary), HEK293 (human embryonic kidney cells), RK13 (rabbit kidney cells), BHK-21 (hamster kidney cells), etc. *Id.*, Table 2. Importantly, a POSA would have known that by December 23, 2016, cell culture-based methods were the main technology used for producing viral vaccines.

253. A non-comprehensive review of the flavivirus vaccines developed by December 23, 2016 illustrates a wide-spread use of cell culture-based²⁹ technology:

Flavivirus	Technology	Year
DENV	Vero cell culture	1996 (EX1028)
DENV	Vero cell culture	2015 (EX1024)
JEV	Vero cell culture	2001 (EX1163)
JEV	Vero cell culture	2012 (EX1051)
YFV	Vero cell culture	2009 (EX1179)
YFV	Vero cell culture	2010 (EX1036)
YFV	Vero cell culture	2015 (EX1180)

254. The inventors of the '681 patent also point to the use of cell culture-based technology for practicing their invention: “[i]n some embodiments, the Zika

²⁹ The first JEV vaccine was a mouse brain-derived inactivated vaccine developed in Japan in 1954, but as I explained, the technology had changed by 2016.

EX1041, 1328.

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virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-aHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.” EX1001, 7:1–7. And propagating ZIKV in Vero cells is the ’681 patent’s preferred embodiment. *Id.*, 7:10–11. Indeed, the one and only example in the ’681 patent describes using Vero cell culture-based technology to prepare their vaccine, taking “the JEV process platform ... as a basis.” EX1008, 75:24–25, 76:16–82:16.

255. A POSA would have known that cell culture-based process for a viral vaccine production generally includes the following steps: i) introducing viral genomes into production cells by infection or transfection to initiate the virus replication phase; ii) allowing the virus to replicate in the cells and infect neighboring cells; iii) harvesting the culture once a peak in virion concentration has been reached; and iv) downstream processing (e.g., virion inactivation, virion purification, final product formulation). EX1163, 4558-4560. This process requires that the virus can replicate in the production cells. EX1163, 4559; EX1028, 1177. This generally means that the virus has to have the form of a mature virion, as I explained in Section VII.C.

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ii. As of December 23, 2016, aspects of formation of ZIKV virions capable of replication were not fully known.

256. A POSA would have known that ZIKV's ability to replicate is a function of a combination of, e.g., proper virion assembly, maturation, entry into a cell, release of genome into cytosol, and production of viral proteins. EX1048, 23–28; EX1092, 379–380. But as of December 23, 2016, there were “significant gaps of knowledge in the assembly and maturation processes” of ZIKV, and these processes were “largely a black box.” EX1091, 2; EX1092, 378. Even as of 2020, the field knew “little about the viral and host factors involved in the structural transition from immature to mature” ZIKV. EX1091, 2; EX1092, 378. In fact, much knowledge about ZIKV structure and function accumulated only after December 23, 2016.

257. For example, it was well after 2016 that the field identified the detailed aspects of how the NS2A protein functioned as “to be a central hub in the [virion] packaging process.” EX1092, 378. And only in 2019 (i.e., after the filing date of the '664 PCT) Zhang identified previously unknown single-amino-acid NS2A mutants that were “completely defective in viral RNA synthesis,” mutants that “impair[ed] both viral RNA synthesis and virion production,” and mutants that “block[ed] virion assembly.” EX1056, 591–592. The figure below (adapted from

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EX1056, Figure 1B) illustrates the effects of various NS2A mutations on ZIKV production:

Class	NS2A Mutations	Phenotypes
I	D4A, E22A, K31A, G47A, D53A, E67A, H76A, E122A, R146A, D148A, R163A, K186A, K188A, D210A	Mutants do not or weakly affect viral production
II	K56A, F83A, K84A, R86A, P87A, R96A, R102A, D124A, R207A	Mutants dramatically reduce viral production
III	G12A, N130A	Mutants abolish viral RNA synthesis
IV	D7A, R140A, R222A	Mutants abolish virus production by affecting RNA synthesis and virion assembly/release
V	K25A/K26A/R27A, G71A, D73A, E103A, R171A, K192A, K193A	Mutants abolish virion assembly

Based on the observed phenotype, the NS2A mutants in the above table would have had a compromised replication ability. Notably, those mutants were unknown as of December 23, 2016.

258. Similarly, in 2020, DiNunno discovered single-amino-acid Zika mutants that failed to replicate in Vero cells. EX1091, 5. Using alanine substitution of various E protein residues, DiNunno identified E protein mutants F449A, F453A, H446A, W474A, and F497A, all of which “were lethal for ZIKV.” *Id.*, 4, 5. In doing so, DiNunno, identified “critical” residues of ZIKV’s E protein that are “necessary [for ZIKV] to function in mammalian [] cells.” *Id.*, 4–5. A POSA in December 23, 2016, therefore, would not have had the knowledge of these critical residues for ZIKV replication.

259. Even as of 2022, the field did not know the identity of all “the key residu[e]s” of the E protein involved in ZIKV entry into cells, and thus its ability replicate in the production cells. EX1094, 1605. For example, in 2022 Ma

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discovered four E protein mutants that “nearly abolished the infectivity” of ZIKV: Mut 9 (A268/L269A/E270A), Mut 10 (A271/E272A/M273A), Mut36 (E366A/N367A/S368A), and Mut 37 (K369A/M370A/M371A). *Id.*, 1612; FIG. 6. Ma determined that residues A₂₆₈L₂₆₉E₂₇₀A₂₇₁E₂₇₂M₂₇₃ of Zika E protein play “critical roles” in viral entry into a host cell. *Id.*, 1618. While Ma’s 2022 work “advance[d] understanding of the mechanism of flavivirus entry,” to a December 2016 POSA the key E residues crucial for ZIKV cell entry (and ultimately its ability to replicate inside production cells) would have “remain[ed] elusive.” *Id.*, 1605, 1619.

260. Thus, as of December 23, 2016, the field lacked critical knowledge concerning ZIKV replication and infectivity. But as the post-2016 discoveries showed, even “single amino acid mutations have been shown to have [a] significant impact on ZIKV infection phenotypes.” EX1089, 14. Aspects of ZIKV structure and function, therefore, were unpredictable as of December 23, 2016.

b. Claim 1

261. Independent claim 1 recites “[a] Zika virus vaccine comprising a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72” that is capable of stimulating a MN₅₀ virus titer of “greater than 15 in at least 70% of vaccinated subjects.” EX1001, 445:34–41. For the reasons I provide

below, a POSA would have concluded that Valneva did not have possession of the myriad ZIKV vaccines claimed in claim 1 when, on December 23, 2016, it filed the '664 PCT.

i. Claim 1 encompasses millions of ZIKV vaccine candidates defined by function—an unwieldy number

262. A POSA would have understood that claim 1 recites a genus of ZIKV vaccines using functional language. Specifically, claim 1's language that the vaccine is "capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects" has a large scope. *Id.*, 445:37–41. I understand that this limitation is considered to be a "functional limitation"—a limitation that recites a feature by what it *does* rather than by what it *is*. Here, this limitation serves a purpose of claiming a vaccine by its function: the ability to stimulate a particular neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects. Thus, a POSA would have understood that claim 1 contains functional language.

263. The functionally-claimed genus of claim 1 encompasses an unwieldy number of vaccines comprising Zika viruses having variant RNA genomes. Specifically, claim 1 recites, in part, "a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 *or a variant nucleic acid having at least 80% identity to SEQ ID No: 72.*" EX1001, *Id.*, 445:34–

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41 (emphasis added). According to the '681 patent, the sequence identity can be present between “sequences or subsequences ... over a specified region or over the entire sequence.” *Id.*, 127:33–50. In other words, the claimed nucleic acid sequences are not required to be identical to SEQ ID NO: 72 over any particular region and able to overlap with SEQ ID NO: 72 provided that the specified percent identity is maintained.

264. SEQ ID NO: 72 has 10,773 nucleotides. *Id.*, 405 (Sequence Listing). A POSA would have understood that it would be impossible for a human to calculate the number of variant nucleic acids that have at least 80% identity to a sequence of this length without extensive computer power. Indeed, claim 1 does not specify which nucleotide(s) of the 10,773 nucleotide-long sequence (or nucleobases, which are the components of nucleotides that would be varied when making variants) should be substituted with another nucleotide(s) to meet the “at least 80%” level of identity limitation. As such, any of the nucleotides in SEQ ID NO: 72 can be substituted, as long as at least 80% identity is maintained. Further, nothing in the claim limits the nucleic acids to those corresponding to naturally occurring viruses. Therefore, the number of the claimed nucleic acid variants would be unwieldy. To illustrate this point, I calculated the number of variant sequences that are at least 80% identical to a nucleic acid sequence that is just five nucleotides in length (I will refer to it as a “5-mer”). A substitution of a single

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nucleobase in the 5-mer will result in a sequence that is 20% different from the 5-mer $((1/5) \times 100 = 20\%)$, which makes it 80% identical to the 5-mer. Because there are 4 different naturally occurring DNA nucleobases (adenine (A), guanine (G), thymine (T), and cytosine (C)), each given nucleobase in the 5-mer can be switched to any of the other three nucleobases. Thus, the number of sequences that are 80% identical to the 5-mer is $3 \times 5 = 15$. It would have been within a POSA's skill to perform these calculations in December of 2016.

265. I performed similar calculations with a longer sequence: a 20-mer. A POSA would have been able to perform the same calculations in December of 2016. A substitution of four nucleobases in the 20-mer will result in a sequence that is 20% different from the 20-mer $((4/20) \times 100 = 20\%)$, which makes it 80% identical to the 20-mer. I determined the number of possible different 20-mer sequence combinations (i.e., variants) having 1, 2, 3, or 4 substitutions using the following combinatorial math equation (see EX1131, 209–210), where S is the number of substitutions:

$$3^S(20! \div (S! (20 - S)!))$$

According to my calculations, the number of sequences that are at least 80% identical to the 20-mer is 424,995 (the sum of 60 sequences with 1 substitution; 1,710 sequences with 2 substitutions; 30,780 sequences with 3 substitutions; and

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392,445 sequences with 4 substitutions).³⁰ Because the length of SEQ ID NO: 72 (10,773 nt) is more than 500-times longer than that of a 20-mer ($10,773/20 = 538$), the number of variants that are at least 80% identical to the 20-mer shows that the corresponding number for SEQ ID NO: 72 would be unwieldy, given the use of factorial math in the calculation to determine the number of different sequence combinations. And a POSA would have needed the aid of a computer/algorithm to calculate the exact number (although the POSA would have expected the number to be extremely large).

266. I understand that Dr. Scott Bailey calculated the number of variant nucleic acid sequences for a 10,773 nucleotide-long nucleic acid having the

³⁰ The calculation are shown below:

1 substitution: $3^1 * (20! / (1! * (20 - 1)!)) = 3 * (2,432,902,008,176,640,000 / (1 * 121,645,100,408,832,000)) = 60;$

2 substitutions: $3^2 * (20! / (2! * (20 - 2)!)) = 9 * (2,432,902,008,176,640,000 / (2 * 6,402,373,705,728,000)) = 1,710;$

3 substitutions: $3^3 * (20! / (3! * (20 - 3)!)) = 27 * (2,432,902,008,176,640,000 / (6 * 355,687,428,096,000)) = 30,780;$

4 substitutions: $3^4 * (20! / (4! * (20 - 4)!)) = 81 * ((2,432,902,008,176,640,000 / (24 * 20,922,789,888,000)) = 392,445.$

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percent identity recited in claim 1 (and dependent claims 2, 3, 5-15, 24, and 25) and claims 21-23. A POSA seeking to determine the number of variants would work with someone with expertise in bioinformatics, like Dr. Bailey, as part of a multidisciplinary team. I reviewed Dr. Bailey's declaration (EX1078), and conclude that his analysis is the type of information that experts like myself would reasonably rely upon in forming their opinions.³¹ The number of nucleic acid variants Dr. Bailey calculated as being at least 80% identical to SEQ ID NO: 72 is expectedly unwieldy: $3.88 \times 10^{3,366}$. EX1078, ¶23. To put these numbers in context, the number of these variant nucleic acid sequences is vastly more than the number of atoms in the observable universe, which is estimated to be 3.28×10^{80} . EX1103. And Dr. Bailey's calculations are in line with my calculations on just the 20-mer.

267. Further, due to the degeneracy of the genetic code, those $3.88 \times 10^{3,366}$ nucleic acid variants could encode many millions of polyproteins, according to Dr. Bailey. *Id.*, ¶¶47–49.

³¹ Relying on my own expertise, I was able to form my opinions vis-à-vis written description and enablement without reliance on Dr. Bailey's declaration. However, Dr. Bailey's declaration confirms and builds upon my analysis regarding the millions of variants encompassed by the language of the claims, and thus it further supports my opinions vis-à-vis written description and enablement.

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268. For example, I understand that nucleic acid variants having up to 5 nucleotide substitutions in *just the first 33 nucleotides* of the open-reading frame of SEQ ID NO: 72 potentially yield 6,032,020 full-length amino acid sequences. *Id.* And because each of these amino acid sequences, or polyproteins, is what makes up a ZIKV virion, the many millions of the polyproteins could represent a similarly unwieldy number of different candidate ZIKV vaccines.

269. In sum, accounting for the many different variant and virus sequences, which would include sequences that would encode virions that do not replicate, and vaccine types, claim 1 represents an enormous genus of candidate ZIKV vaccines.

ii. The '664 PCT does not demonstrate to a POSA that the inventors possessed the large genus of claimed ZIKV vaccines on December 23, 2016

270. A POSA would have understood that the '664 PCT does not demonstrate that the inventors possessed the unwieldy number of ZIKV vaccines encompassed within claim 1 of the '681 patent as of December 23, 2016. As described above, claim 1 encompasses millions of vaccine candidates that meet the structural limitations of claim 1 (the claimed genome variants). But the '664 PCT contains minimal to no details about which of the unwieldy number of vaccine candidates would meet the functional limitation of being able to stimulate a neutralizing antibody titer, which implies a virus sequence capable of replicating in

cell lines and stability to be produced as a vaccine. And given the unpredictability in the art, the few naturally occurring ZIKV sequences disclosed in the '664 PCT would not have convinced a POSA that other nucleic acid variants would perform similarly to the disclosed sequences.

a) The '664 PCT provides only one working example of a ZIKV vaccine encompassed by claim 1; all disclosed ZIKV sequences are closely related to the example

271. A POSA would have agreed that the disclosure of the '664 PCT is not commensurate in scope with the large breadth of claim 1 vis-à-vis variant nucleic acid sequences. The '664 PCT provides a single working example of a ZIKV vaccine: a vaccine made from a naturally occurring ZIKV having RNA corresponding to the DNA sequence provided by SEQ ID NO: 72. EX1008, Example 1. To the extent that the '664 PCT also discloses twelve sequence variants (*see id.*, Sequence listing, SEQ ID NO: 2–13; referred to here as “the disclosed variants”), there are no working or prophetic examples featuring those variants. And in any event, as a POSA would have recognized, all of those variants are naturally occurring and closely related to the ZIKV represented by SEQ ID NO: 72. *See* Appendix A. Besides the disclosed variants, the '664 PCT does not provide any other DNA variants, nor does it demonstrate that the inventors had made or otherwise possessed ZIKV vaccines comprising a ZIKV having an RNA

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genome corresponding to a variant DNA having at least 80% identity to SEQ ID NO: 72, as recited in claim 1.

272. The single working example in the '664 PCT discloses a ZIKV vaccine made from a naturally occurring ZIKV. Specifically, it uses the ZIKV strain H/PF/2013, originally isolated in 2013, from the European Virus Archive. EX1008, 76:16–23. The H/PF/2013 strain has an RNA sequence corresponding to the DNA sequence of SEQ ID NO: 72. To make the vaccine from the H/PF/2013 ZIKV, the inventors used Vero cell culture-based process, which, as I described, was a standard methodology for making flavivirus vaccines as of December 23, 2016. *Id.*, Example 1. A POSA would have understood that the inventors made only one vaccine, and that only this vaccine was shown to be capable of stimulating neutralizing antibodies. *Id.*, 84:20–28, 85:19–20, 85:25–28.

273. The '664 PCT also discloses additional sequences, SEQ ID NOs: 2–69, but those sequences do not add to the diversity of the exemplified ZIKV vaccine. A POSA would have recognized that only SEQ ID NOs: 2–13 correspond to “a variant nucleic acid having at least 80% identity to SEQ ID NO: 72,” as required by claim 1. *See* Appendix A, attached hereto. A POSA would have further

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recognized that SEQ ID NOs: 2–13³² were all “isolated from infected subjects,” and therefore encode naturally occurring ZIKV. Importantly, a POSA would have appreciated that being isolated from the subjects, ZIKVs encoded by SEQ ID NOs: 2–13 were all *a priori* infectious (i.e., the subjects were “infected” with the virus) and therefore would be able to replicate in production cells. EX1177, 602.

274. Further, according to sequence alignments that I performed using BLAST, as discussed in footnote 26, above, SEQ ID NOs: 2–10 and 13 (the Asian strains) are all at least 97% identical to SEQ ID NO: 72, and most of them are >98% identical to SEQ ID NO: 72 (an Asian strain). See Appendix A. SEQ ID NOs: 11 and 12 (the African strains) are >88% identical to SEQ ID NO: 72, and 99% identical to each other. *Id.* Notably, the ’664 PCT does not demonstrate the ability of a ZIKV vaccine comprising a ZIKV having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 2–13 to stimulate neutralizing antibodies. In any event, a POSA would have concluded that ZIKV encoded by SEQ ID NO: 2–13 are all naturally occurring viruses.

³² SEQ ID NOs: 2–10 and 13 correspond to Asian ZIKV strains, while SEQ ID NOs: 11 and 12 correspond to African ZIKV strains. EX1001, 21–104.

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b) The disclosed variants, and ZIKV vaccines made therefrom, are not representative of the large genus claimed

275. A POSA would have concluded that the variant sequences disclosed in the '644 PCT are not representative of the vast genus encompassed by claim 1 of the '681 patent. As I explained, the disclosed sequences (SEQ ID NOs: 2–13 and 72) of the '644 PCT all correspond to naturally occurring, infectious viruses. But claim 1 does not encompass vaccine candidates made *only* from the naturally occurring viruses. Indeed, claim 1 encompasses millions of vaccines comprising ZIKV virions having variant genomes. For example, the candidates that meet structural requirements of claim 1 would include all of the NS2A mutants identified by Zhang in 2019.³³ As I show in Appendix B, Zhang's mutants are all more than 98% identical to SEQ ID NO: 72 when the sequence are aligned across their full length. The candidates would also include DiNunno's E protein mutants (first identified in 2020). As I show in Appendix B, DiNunno's mutants are 88%

³³ The sequences in Zhang 2019 or DiNunno 2020 were compared with SEQ ID 72 with or without the additional point mutations described in Zhang 2019 or DiNunno 2020. *See* Appendix B. I also determined the percent identity of variant sequences having each of the point mutations described in Zhang 2019 and DiNunno 2020 introduced into SEQ ID NO: 72. *See id.*

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identical to SEQ ID NO: 72. For the reasons discussed below, the naturally occurring ZIKVs corresponding to SEQ ID NOs: 2–13 and 72 cannot be representative of the variants of, e.g., Zhang and DiNunno.

276. As I explained, a POSA would have understood that viruses corresponding to SEQ ID NOs: 2–13 and 72 are naturally occurring and thus are able to replicate in production cells. As I also explained, a POSA would have known that in order to make the claimed ZIKV vaccines, ZIKV must be able to replicate (so that it could be propagated in a cell culture). But the mutants discovered by Zhang and DiNunno had a compromised replication ability. EX1056, 591–592; EX1091, 5. If tried, a POSA would not be able to make the claimed vaccine from Zhang and DiNunno mutants using the cell culture-based method disclosed in the '664 PCT. SEQ ID NOs: 2–13 and 72, therefore, are not representative of all vaccine candidates encompassed by claim 1.

277. A POSA would also recognize that the '664 PCT provides no blaze marks for determining which nucleic acid variants would produce a ZIKV vaccine that stimulates antibody titers as claimed, and which would not. Indeed, besides listing examples of specific variants having at least 80% identity to SEQ ID NO: 72 (e.g., SEQ ID NOs: 2–13), the '664 PCT does not disclose specific nucleic acid (or corresponding amino acid) modifications that can be introduced into ZIKV's genome so as to result in ZIKV that could be used to manufacture a

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vaccine using the cell culture-based method disclosed in the '664 PCT. But as explained above, even “single amino acid mutations have been shown to have [a] significant impact on ZIKV infection phenotypes.” EX1089, 14; *see also* EX1056, FIG. 1B; EX1091, 5. The '664 PCT does not provide information sufficient to establish that a POSA would have understood how to identify the species of ZIKV that can be grown and made into a vaccine capable of stimulating neutralizing antibodies, as claim 1 requires. And given the unpredictability in the relevant art in December of 2016, the mere disclosure that a ZIKV corresponds to “a variant genome that is at least 80% ... identical to any one of SEQ ID NOs: 2–13 or 72,” (EX1008, 56:48–51) does not show that the inventors were in possession of the unwieldy number of such vaccines when they filed the '664 PCT.

278. In sum, a POSA would have recognized that the '664 PCT's disclosure does not represent the enormous genus of vaccines claimed in claim 1, because its limited examples were isolated from human subjects and thus are not representative of nearly any ZIKV vaccine candidate encompassed by claim 1.

c) The '664 PCT fails to describe a structure-function correlation within the claimed genus, which was not known in the art as of December 23, 2016

279. The '664 PCT does not disclose structural features common to the claimed genus of ZIKV vaccines (i.e., to ZIKV's genomic sequences encoding proteins) that would permit a POSA to achieve the claimed function. As I

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explained, the claimed vaccine must comprise virions capable of replication that can be “a live virus, a chimeric virus, an attenuated live virus, a modified live virus, or a recombinant live virus;” or it can be an inactivated virus. EX1001, 6:45–50; 131:9–26. The vaccine also must be capable of stimulating the claimed neutralizing antibodies titers. The ’664 PCT fails to disclose structural features common to the claimed ZIKV vaccines having genomes capable of producing virions that can be replicated in production cells, and also fails to disclose a way to distinguish those genomes capable of producing a virus that can be replicated (for a cell-culture production) from the genomes incapable of doing so.

280. For example, the ’664 PCT discloses that “the Zika virus of the current invention comprises the sequence of SEQ ID NO: 72 or, preferably, a sequence with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 72.” EX1008, 76:30–32. First, the recited sequence identities allow for a huge variability, and encompass non-functional variants. For example, as I show in Appendix B, both Zhang’s NS2A and DiNunno’s E protein mutants (identified after 2016) satisfy the structural requirement of being at least 80% identical to SEQ ID NO: 72, yet they could not be made into a vaccine, as described above. Further, the ’664 PCT does not explain which structural feature(s) of the nucleic acid sequences makes them capable of producing ZIKV capable of replication, or why. Similarly, the ’664 PCT discloses

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that “the Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E-protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.” *Id.*, 77:5–8. Again, the ’664 PCT does not explain which variant sequences would provide the same result—specifically, form a ZIKV virion that can replicate in production cells, and therefore does not shed much light on a correlation between the sequence variants and the function. Nor was this correlation established in the art by December 23, 2016, as I explained in detail above.

281. Thus, a POSA would have recognized that the art as of December 23, 2016 did not provide a structure-function correlation for the genus of vaccines claimed in claim 1 of the ’681 patent. And the ’664 PCT does not explain which structural characteristics should be modified, and in what way, to arrive at the full scope of the claimed ZIKV vaccines capable of stimulating a neutralizing antibody titer substantially higher than 90 in at least 70% of a variety of vaccinated subjects. As such, the ’664 PCT describes only a starting point and an invitation for further research into which of the unwieldy number of possible ZIKV vaccines meeting the structural limitations of claim 1 are capable of stimulating the claimed neutralizing antibody titers.

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282. In sum, a POSA reading the '664 PCT in light of the general knowledge in the art as of December 23, 2016 would have concluded that the inventors did not possess the vast genus of ZIKV vaccines claimed in claim 1 of the '681 patent when they filed the '664 PCT. Therefore, it is my opinion that claim 1 of the '681 patent is not entitled to the December 23, 2016, filing date of the '664 PCT.

c. Claims 2, 3, 24, and 25

283. Claim 2 specifies that the vaccine is capable of stimulating a MN₅₀ titer “greater than 20, 25, 30, 35, 40 or 45.” EX1001, 445:42–43. Claim 3 recites that the Zika virus vaccine is capable of stimulating a MN₅₀ titer greater than 15 in at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects. *Id.*, 445:44–47. Claims 24 and 25 depend from claim 2 and recite that the vaccine is capable of stimulating a MN₅₀ greater than 50, 55, 60, 65, 70, 75, 80, or 85 (claim 24) and greater or equal to 90 (claim 25). *Id.*, 448:19–22.

284. I understand that claims 2, 3, 24, and 25 also include all of the requirements of independent claim 1 from which they depend. And claims 24 and 25 also include the limitations of claim 2.

285. A POSA would have recognized that claims 2, 3, 24, and 25 do not specify further requirements regarding the sequence of the variant nucleic acids. To the extent that claims 2, 3, 24, and 25 recite MN₅₀ values that are different from

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those in claim 1, the scope of each claim 2, 3, 24, and 25 as a whole remains unwieldy because no additional requirements are imposed vis-à-vis nucleic acid sequences.

286. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production cells, so as to be used to make a vaccine meeting the claims' functional limitations. Thus, for the same reasons discussed in claim 1, the inventors did not possess the vast genus of ZIKV vaccines claimed in claims 2, 3, 24, and 25 of the '681 patent when they filed the '664 PCT. Therefore, it is my opinion that claims 2, 3, 24, and 25 of the '681 patent are not entitled to the December 23, 2016 filing date of the '664 PCT.

d. Claim 4

287. Claim 4 requires that the Zika virus comprises “an E protein having an amino acid sequence provided by any one of SEQ ID NOs: 14–69, *or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14–69* and able to pack a virulent Zika virus.” EX1001, 445:48–53 (emphasis added). I understand that claim 4 also includes all of the requirements of independent claim 1 from which it depends.

288. A POSA would have recognized that claim 4 has an enormous breadth. As I explained above, SEQ ID NO: 72 encodes a ZIKV's genome that is

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translated into a polyprotein that is cleaved to produce ten proteins, the E protein being one of them. EX1082, 14. While claim 4 recites specific E protein sequences (and variants thereof), it does not narrow the enormous variability of other ZIKV proteins (encoded by the millions of variants of SEQ ID NO: 72 of claim 1). Thus, because claim 4 does not specify further requirements regarding the variant sequences outside of the E protein, claim 4 encompasses an unwieldy number of vaccine candidates due to variations allowed in the remaining nine ZIKV proteins (as explained for claim 1) and non-coding regions of the genome.

289. In any event, the limitation “a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14–69” alone encompasses a tremendous number of E protein variants. Estimating the number of E protein candidates encompassed by claim 4 would be difficult without an aid of a computer, although I would expect that number to be extremely large. Thus, similar to my calculations for the 5-mer and the 20-mer nucleic acid variants, I calculated the number of different polypeptides that are at least 95% identical to a polypeptide sequence having 20 amino acids. Because there are 20 (twenty) different naturally occurring amino acids, each given amino acid in the 20-mer polypeptide can be substituted by any 19 (nineteen) other amino acids. Thus, the number of sequences that are 95% identical to the 20-mer polypeptide is $19 \times 20 =$

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380 because a sequence having 95% identity to the 20-mer can have 1 substitution.

It would have been within a POSA's skill to perform these calculations.

290. I performed similar calculations with a longer sequence: an 80-mer polypeptide. A POSA would have been able to perform the same calculations. The number of sequences that are at least 95% to an 80-mer polypeptide will contain as few as 1 substitution to as many as 4 substitutions $((4/80) \times 100 = 5\%)$. I determined the number of possible different 80-mer sequence combinations having 1, 2, 3, or 4 substitutions using the following combinatorial math equation, where S is the number of substitutions:

$$19^S(80! \div (S!(80 - S)!))$$

According to my calculations, the number of sequences that are 95% identical to the 80-mer is 206,677,764,900 (the sum of 1,520 sequences with 1 substitution; 1,140,760 sequences with 2 substitutions; 563,535,440 sequences with 3 substitutions; and 206,113,087,180 sequences with 4 substitutions).³⁴

³⁴ The calculations are shown below:

1 substitution: $19^1 * (80! / (1! * (80 - 1)!)) = 19 * (7.1569457046263802294811533723187 \times 10^{118} / (1 * 8.9461821307829752868514417153983 \times 10^{116})) = 1,520;$

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291. Because the lengths of SEQ ID NOs: 14–69 are ~6.25-times longer than that of the 80-mer ($498/80 = 6.225$ (SEQ ID NOs: 14, 15, and 69);³⁵ $500/80 = 6.25$ (SEQ ID NOs: 27–29);³⁶ $504/80 = 6.3$ (SEQ ID NOs: 16–26 and 30–68)³⁷), the number of sequences 95% identical to the 80-mer shows that the corresponding number would be unwieldy for the 56 different claimed SEQ ID NOs (SEQ ID

2 substitutions: $19^2 * (80! / (2! * (80 - 2)!)) = 361 * (7.1569457046263802294811533723187 \times 10^{118} / (2 * 1.1324281178206297831457521158732 \times 10^{115})) = 1,140,760;$

3 substitutions: $19^3 * (80! / (3! * (80 - 3)!)) = 6,859 * (7.1569457046263802294811533723187 \times 10^{118} / (6 * 1.4518309202828586963407078408631 \times 10^{113})) = 563,535,440;$

4 substitutions: $19^4 * (80! / (4! * (80 - 4)!)) = 130,321 * ((7.1569457046263802294811533723187 \times 10^{118} / (24 * 1.8854947016660502549879322608611 \times 10^{111})) = 206,113,087,180.$

³⁵ The number of amino acids in SEQ ID NOs: 14, 15, and 69 is 498.

³⁶ The number of amino acids in SEQ ID NOs: 27–29 is 500.

³⁷ The number of amino acids in SEQ ID NOs: 16–26 and 30–68 is 504.

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NOs: 14–69) given the use of combinatorial math in the calculation to determine the number of different sequence combinations.

292. I also understand that Dr. Bailey used a computer algorithm to calculate the minimal number of E protein candidates encompassed by claim 4. I understand from Dr. Bailey’s Declaration that the minimal number of E protein candidates is at least 2.17×10^{74} . EX1078, ¶24. This is still an unwieldy number.

293. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production cells, so as to be used to make a vaccine meeting the claim’s functional limitations. Nor would a POSA have known, as of December 23, 2016, which variant amino acid sequences that are at least 95% identical to any one of SEQ ID NOs: 14–69 is able to pack a virulent Zika virus, as required by claim 4. Thus, the inventors did not possess the vast genus of ZIKV vaccines claimed in claim 4 of the ’681 patent when they filed the ’664 PCT. Therefore, claim 4 of the ’681 patent is not entitled to the December 23, 2016, filing date of the ’664 PCT.

e. Claims 5–9

294. Claim 5 depends from claim 1 and further specifies that the Zika virus “is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.” EX1001, 445:54–56. Claim 6 depends from claim 5 and further specifies that “the chemical inactivation comprises contacting the Zika virus with a

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chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.” *Id.*, 445:57–61. Claim 7 depends from claim 5 and further specifies that “the chemical inactivation comprises contacting the Zika virus with formaldehyde.” *Id.*, 445:62–64. Claim 8 depends from claim 7 and further specifies that “the chemical inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.” *Id.*, 445:65–67. Claim 9 depends from claim 5 and further specifies that “the chemical activation is performed at about +4° C. or about +220° C.” *Id.*, 446:33–35.

295. A POSA would have recognized that claims 5–9 do not specify further requirements regarding the sequence of the variant nucleic acids.

296. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production cells, so as to be used to make a vaccine meeting the claims’ functional limitations. Thus, for the same reasons discussed in claim 1, the inventors did not possess the vast genus of ZIKV vaccines claimed in claims 5–9 of the ’681 patent when they filed the ’664 PCT. Therefore, claims 5–9 of the ’681 patent are not entitled to the December 23, 2016, filing date of the ’664 PCT.

f. Claims 10–15

297. Claims 10–14 specify the Zika virus vaccine further comprises “an adjuvant.” EX1001, 446:36–52. Claim 11 depends from claim 10 and further

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specifies that “the adjuvant is an aluminium salt adjuvant.” *Id.*, 446:38–39. Claim 12 depends from claim 11 and further specifies that the “aluminium salt adjuvant is aluminium hydroxide with less than 1.25 parts per billion (ppb) copper (Cu) based on a final pharmaceutical composition comprising the Zika virus.” *Id.*, 446:40–44. Claim 13 depends from claim 10 and further specifies that “the adjuvant comprises a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).” *Id.*, 446:45–48. Claim 14 depends from claim 13 and further requires that “the peptide comprises the sequence KLKL₅KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)₁₃ (SEQ ID NO: 70).” *Id.*, 446:49–52. Claim 15 depends from claim 1 and specifies that the Zika virus vaccine further comprises “one or more pharmaceutically acceptable excipients.” *Id.*, 446:53–55.

298. A POSA would have recognized that claims 10–15 do not specify further requirements regarding the sequence of the variant nucleic acids.

299. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production cells, so as to be used to make a vaccine meeting the claims’ functional limitations. Thus, for the same reasons discussed in claim 1, the inventors did not possess the vast genus of ZIKV vaccines claimed in claims 10–15 of the ’681 patent when

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they filed the '664 PCT. Therefore, claims 10–15 of the '681 patent are not entitled to the December 23, 2016, filing date of the '664 PCT.

g. Claims 21–22

300. Claims 21 depends from claim 1 and specifies that the “variant nucleic acid has at least 90% identity to SEQ ID NO: 72.” EX1001, 448:11–13. Claim 22 depends from claim 1 and specifies that the “variant nucleic acid has at least 95% identity to SEQ ID NO: 72.” *Id.*, 448:14–16.

301. Based on my calculations for the 5-mer and the 20-mer, I predict that the number of nucleic acid sequences encompassed by each of claim 21 and 22 is still unwieldy. I also understand that Dr. Bailey calculated the precise number of nucleic acid sequences that are at least 90% and at least 95% identical to SEQ ID NO: 72. The number of sequences is unwieldy:

Claim 21: There are $4.45 \times 10^{2,032}$ variant DNA sequences that are at least 90% identical to SEQ ID NO: 72.

Claim 22: There are $7.91 \times 10^{1,182}$ variant DNA sequences that are at least 95% identical to SEQ ID NO: 72.

EX1078, ¶43.

302. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production cells, so as to be used to make a vaccine meeting the claims' functional limitations.

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Thus, for the same reasons discussed in claim 1, the inventors did not possess the vast genus of ZIKV vaccines claimed in claims 21 or 22 of the '681 patent when they filed the '664 PCT. Therefore, it is my understanding that claims 21–22 of the '681 patent are not entitled to the December 23, 2016, filing date of the '664 PCT.

h. Claim 23

303. Claim 23 depends from claim 1 and specifies that the “variant nucleic acid has at least 99% identity to SEQ ID NO: 72.” EX1001, 448:17–19. Based on my calculations for the 5-mer and the 20-mer, above, I conclude that the number of nucleic acid sequences encompassed by claim 23 is still unwieldy.

304. I also understand that Dr. Bailey calculated the precise number of nucleic acid sequences that are at least 99% identical to SEQ ID NO: 72. The number of sequences is unwieldy: 1.57×10^{310} . EX1078, ¶43.

305. Claim 23 encompasses variants that a *post*-2016 POSA would have predicted to not function as claimed. As I show in Appendix B, all Zhang’s NSA2 mutants that had compromised ability to replicate (mutants in Classes II–V) are about 98% identical to SEQ ID NO: 72 when aligned over their entire length. Thus, Zhang’s mutants do not meet the structural requirements of “at least 99% identity to SEQ ID NO: 72” when analyzed in this way.³⁸ However, the amino acids that

³⁸ I note that when the sequences are compared over the overlapping region, which

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are mutated in Zhang's NSA2 mutants in Classes II–V are conserved between Zhang's strain FSS13025 and the NSA2 protein encoded by SEQ ID NO: 72. EX1191 (sites of Zhang's mutants highlighted); *see also* Appendix B. Notably, making any one of those mutations in SEQ ID NO: 72 would result in variants that meet the structural requirements of “at least 99% identity to SEQ ID NO: 72.” *See* Appendix B. The same also holds for making any one of DiNunno's mutants in SEQ ID NO: 72, which also occur in amino acids that are conserved between DiNunno's strain MR-766 and the E protein encoded by SEQ ID NO: 72. EX1192 (sites of DiNunno's mutants highlighted); *see also* Appendix B. Because of the amino acid conservations, a POSA would have predicted that the same mutations, made in SEQ ID NO: 72 (and, ultimately, in the NS2A protein or E protein), would result in similarly-compromised ZIKV variants. Yet the '664 PCT does not shed much light on a correlation between the sequence variants and the function, as I explained for claim 1. Nor was this correlation established in the art by December 23, 2016, as I explained in detail above.

306. For the same reasons discussed for claim 1, a POSA would not have known the full scope of variants that would be capable of replicating in production

is also disclosed by the patent to be a possibility to determine percent identity of sequences, the limitation of “at least 99%” is still met.

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cells, so as to be used to make a vaccine meeting the claims' functional limitations. Thus, for the same reasons discussed in claim 1, the inventors did not possess the vast genus of ZIKV vaccines claimed in claim 23 of the '681 patent when they filed the '664 PCT. Therefore, it is my understanding that claim 23 of the '681 patent is not entitled to the December 23, 2016, filing date of the '664 PCT.

2. The '664 PCT does not enable the full scope of claims 1–15 and 21–25 of the '681 patent.

307. As noted, I understand that the so-called *Wands* factors are used to assess whether a patent application's disclosure is enabling. Based on my review of the '664 PCT, the '664 PCT does not enable a POSA to make and use the full scope of the invention of claims 1–15 and 21–25 of the '681 patent without undue experimentation. My analysis of the *Wands* factors is set forth in detail below.

a. Claim 1

i. The enormous breadth of claim 1, which includes vaccine candidate that could not be made, weighs against enablement.

308. As I explained above in ¶¶262–269, claim 1 encompasses an unwieldy number of ZIKV vaccine candidates. I refer to that analysis for the purposes of addressing “the breadth of the claim” *Wands* factor.

309. As I also explained above, claim 1 encompasses embodiments that meet structural limitations of the claim but do not function as claimed. Indeed, the candidates that meet structural requirements of claim 1 would include ZIKV

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mutants that do not replicate and thus cannot be used to make vaccines, as identified by Zhang in 2019, as well as those identified by DiNunno in 2020. See Appendix B. But a POSA would not have been able to use either Zhang's or DiNunno's mutants to make the claimed vaccines because the mutants lacked infectivity and thus could not be grown in a cell culture. EX1056, 591–592; EX1091, 5. Thus, at least those non-functional embodiments meet structural, but not functional, limitations of claim 1.

ii. The nature of the invention and the high level of unpredictability in the art establish a need for making and screening each claimed variant to make and use the full scope of the invention, weighing against enablement

310. A POSA would have recognized that the claimed vaccine candidates would need to be made and screened in order to practice the claimed invention. The subject matter of the '681 patent relates to Zika vaccines comprising *any* Zika virus variant, as long as it is at least 80% identical to SEQ ID NO: 72. As I discuss in Section XI.B.1.a, ZIKV's ability to replicate in production cells was a requirement for making the claimed vaccines, yet aspects of ZIKV assembly and replication were not fully known as of December 2016. In fact, Barnard called the process "largely a black box" as late as 2020. EX1092, 378. As the post-2016 discoveries showed, even "single amino acid mutations have been shown to have [a] significant impact on ZIKV infection phenotypes." EX1089, 14. Notably, a

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number of specific ZIKV protein mutants with a compromised ability to replicate were discovered only after December 23, 2016. EX1056, FIG. 1B; EX1091, 5; EX1094, 1605. Thus, whether a given ZIKV variant can produce a polyprotein that will yield the proteins required for proper viral assembly, RNA replication, and packaging, was difficult to predict as of December 23, 2016, and required testing to confirm.

311. Also, mutating a viral genome and growing and isolating the resulting virus to make vaccines is itself a process that generally requires extensive and iterative experimentation and optimization. EX1100, 74 (“Current methodologies [as of 2013] for construction of infectious cDNA clones are time-consuming, unpredictable and laborious processes frequently associated with undesirable mutations or unstable/toxic clones in bacteria.”).

312. In sum, due to there being “significant gaps” in the aspects concerning ZIKV replication as of December 23, 2016 (EX1091, 2), a POSA would be left needing to systematically test each ZIKV variant, using multiple replicates, to make and use the full scope of the claimed ZIKV vaccines.

313. To the extent that the art had reported one ZIKV vaccine candidate by December 23, 2016 (Larocca 2016), Larocca’s vaccine contained “purified inactivated virus (PIV) [] derived from the Puerto Rico PRVABC59 strain.” EX1006, 475–476. PRVABC59 is a naturally occurring ZIKV strain that is closely

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related to the H/PF/2013 strain tested in the '644 PCT—it has 98% identity to SEQ ID NO: 72 and is already disclosed as SEQ ID NO: 4 in the '664 PCT. See Appendix A. Thus, Larocca would not have provided a POSA with any additional guidance vis-à-vis the effects of genetic modifications on ZIKV replication in production cells, and therefore suitability for a vaccine, across the full scope of claim 1 of the '681 patent.

iii. Level of ordinary skill in the art

314. While the ordinary level of skill in the fields relevant to the '681 patent (e.g., infectious diseases, virology, immunology, vaccinology) is relatively high, given the unpredictability of the art, even a highly skilled POSA would need to engage in an undue experimentation to practice the full scope of claim 1 of the '681 patent. This is particularly true given the “significant gaps” in knowledge concerning ZIKV’s replication as of December 23, 2016. EX1091, 2; EX1092, 378. Against this backdrop, a POSA would have needed significant guidance and working examples as of December 23, 2016, to be able to make and use the full scope of vaccines comprising a ZIKV having as little as 80% identity to SEQ ID NO: 72, such as those claimed in the '681 patent.

iv. Little to no direction and only one working example provided by the '664 PCT specification weighs against enablement.

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315. As discussed in ¶¶271–274 above, the '664 PCT provides only one working example of a ZIKV vaccine falling within the scope of claim 1. The one working ZIKV vaccine was made from the ZIKV having an RNA genome corresponding to the DNA sequence of SEQ ID NO: 72. EX1001, 84–86. Other than this single exemplified vaccine, the '664 PCT does not disclose any other example that shows that variant DNA sequences having at least 80% identity to SEQ ID NO: 72 could encode a ZIKV RNA that could be translated into ZIKV proteins to form a stable and immunogenic ZIKV.

316. The '664 PCT also fails to correlate any aspects of the structure of ZIKV—e.g., the DNA sequences encoding ZIKV RNA—with their purported immunogenicity. See ¶¶279–282, above. Nor does the '664 PCT identify what types of variations in those DNA would produce stable (mature and infectious) ZIKV that can be grown, purified and formulated into a vaccine product. Given the an unwieldy number of possible vaccine candidates encompassed by claim 1, the one exemplified vaccine does not provide sufficient information regarding which vaccine candidates would stimulate the claimed neutralizing antibody titer and which would not.

- v. The quantity of experimentation to determine which members of the broadly claimed genus would have the required functionality weighs in favor of non-enablement**

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317. Given the extremely limited disclosures of the '644 PCT, as well as the state of the art as of December 23, 2016, a POSA would find the quantity of experimentation needed to make and test the broad scope of ZIKV vaccines encompassed by claim 1 of the '681 patent to be immense. The '644 PCT does not identify key structural features of the myriad ZIKV variants that would need to be included in the variants to achieve the claimed immunologic function.

318. For example, to practice the full scope of claim 1, a POSA would have needed to confirm that the millions of claimed DNA sequences can encode RNAs that produce stable virions. This would require, for example, a POSA to make each and every variant sequence, clone all sequences into expression vectors, introducing the vectors into the production cells, determine whether all the ZIKV strains can replicate so that they can be grown in a cell culture, develop growth and purification protocols for all the viable viruses, and express and purify all the various ZIKVs. Making and using the full scope of ZIKV vaccines as of December 23, 2016, therefore, would have been an impossibly laborious and time-intensive undertaking.

319. Further, a POSA would need to generate and test a sufficient number of variant sequences within the scope of claim 1 to demonstrate that the broad range of the sequences claimed in the '681 patent all function to make stable and immunogenic ZIKVs. A POSA would have to conduct a significant trial and error

process to determine which of the claimed variants would function, as claimed.

Given the wide breadth of the claims, this trial-and-error process would require many tests and a great amount of time and energy to make and use the full scope of the claimed vaccines.

vi. Weighing the *Wands* factors, undue experimentation would be required to practice the full scope of claim 1

320. In summary, weighing each of the *Wands* factors, a POSA would have concluded that the '644 PCT would not have enabled a POSA to make and use the full scope of claim 1 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. As described, a POSA would have concluded that claim 1 of the '681 patent encompasses an unwieldy number of ZIKV vaccine candidates, not all of which can be used to make a vaccine. A POSA would have also concluded that the '644 PCT discloses only a single working example of such a vaccine. Thus, a POSA seeking to produce the full scope of the claim, would have been forced to make and screen an unwieldy number of ZIKV vaccine candidates to determine which embodiments of the '681 patent's claim 1 have the claimed function, and consequently to make and use the full scope of the claimed invention. And although the level of skill in the art is high, this does not outweigh the unpredictability in the art, the lack of guidance and working examples provided in the '644 PCT, and the quantity of experimentation required. Accordingly, a

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POSA would not have been able to make and use the full scope of claim 1 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. Therefore, claim 1 of the '681 patent is not entitled to the December 23, 2016 filing date of the '664 PCT.

b. Claims 2, 3, 24, and 25

321. As I explained above, a POSA would have recognized that claims 2, 3, 24, and 25 do not specify further requirements regarding the sequence of the variant nucleic acids. To the extent that claims 2, 3, 24, and 25 recite MN₅₀ values that are different from those in claim 1, the scope of each claim 2, 3, 24, and 25 as a whole remains enormous because no additional requirements are imposed vis-à-vis nucleic acid sequences.

322. Thus, for the same reasons described for claim 1—e.g., a POSA would be forced to make and screen millions of ZIKV vaccine candidates to identify operable ZIKV vaccine embodiments—a POSA would not be able to make and use the full scope of claims 2, 3, 24, and 25 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. Therefore, claims 2, 3, 24, and 25 of the '681 patent are not entitled to the December 23, 2016 filing date of the '664 PCT.

c. Claim 4

323. As described above, the scope of claim 4 is enormous. A POSA would have to make and screen millions of E proteins to identify proteins that are able to pack a ZIKV virion to then identify operable ZIKV vaccine embodiments. Thus, a POSA would not be able to make and use the full scope of claim 4 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. Therefore, claim 4 is not entitled to the December 23, 2016 filing date of the '664 PCT.

d. Claims 5–15

324. As I explained above, a POSA would have recognized that claims 5–15 do not specify further requirements regarding the sequence of the variant nucleic acids.

325. Thus, for the same reasons described for claim 1—e.g., a POSA would be forced to make and screen millions of ZIKV vaccine candidates to identify operable ZIKV vaccine embodiments—a POSA would not be able to make and use the full scope of claims 5–15 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. Therefore, claims 5–15 of the '681 patent are not entitled to the December 23, 2016 filing date of the '664 PCT.

e. Claims 21–23

326. As explained, the scope of each of claim 21 and 22 is enormous and encompass variants that the post-filing art identified as non-functional vis-à-vis their ability to replicate in production cells. EX1056, 591–592. And as I also explained above, claim 23 encompasses variants that a *post*-2016 POSA would have predicted to not function as claimed.

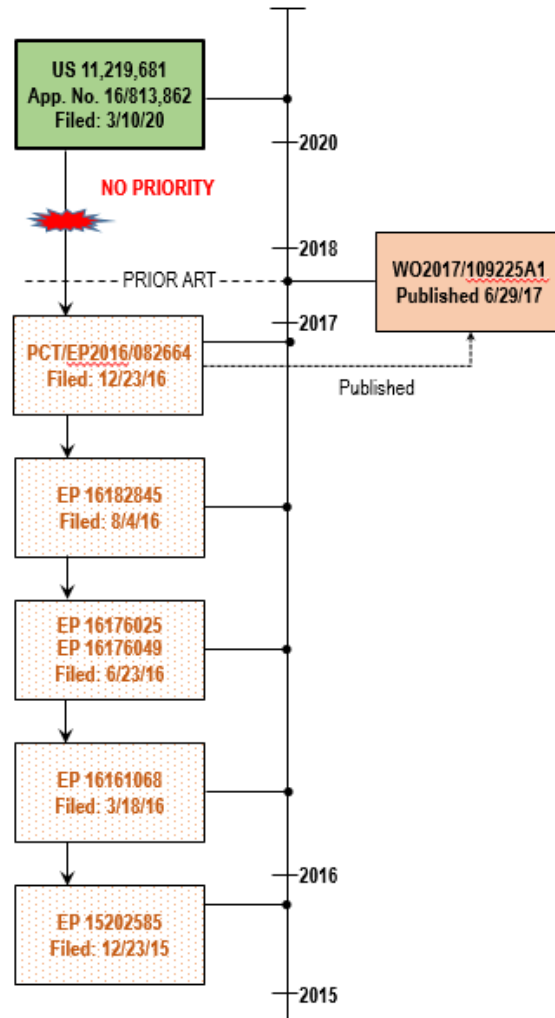
327. Thus, for the same reasons described for claim 1—e.g., a POSA would be forced to make and screen millions of ZIKV vaccine candidates to identify operable ZIKV vaccine embodiments—a POSA would not be able to make and use the full scope of claims 21-23 of the '681 patent without undue experimentation as of the filing date of the '644 PCT. Therefore, claims 21–23 of the '681 patent are not entitled to the December 23, 2016 filing date of the '664 PCT.

C. Claims 1–15 and 21–25 are anticipated by WO '225.

328. WO '225 (EX1008) discloses and enables each and every limitation of claims 1–15 and 21–25, arranged as claimed. WO '225 published on June 29, 2017, which is more than one year before the March 10, 2020 filing date of the '681 patent. I understand that if the '681 patent does not receive the benefit of the December 23, 2016 filing date of the '664 PCT for the reasons discussed above in Section XI.B, then WO '225 qualifies as prior art to claims 1–15 and 21–25 of the

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'681 patent. The figure below illustrates when WO '225 published in relation to the March 10, 2020 filing date of the '681 patent:



329. I understand that a prior art publication, like WO '225, is presumed enabled in this proceeding. Nevertheless, WO '225 discloses a species of the challenged claims in its single working example and that example, along with the disclosures of WO '225, provides sufficient detail to enable a POSA to make and use that single working example without the need for undue experimentation in light of the general knowledge in the art. The disclosures of WO '225 related to the

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single working example disclosed in WO '225 would have been enabling to a POSA before March 10, 2020, because WO '225 discloses methods of propagating the disclosed known H/PF/2013 strain in Vero cells, inactivating the ZIKV with formaldehyde, purifying the inactivated ZIKV with protamine sulfate and sucrose gradient purification, and formulating the purified inactivated ZIKV with aluminum hydroxide and PBS. EX1008, 76:20–82:35. Those techniques were also all well-known in the art. *See* Section VII.E (discussing various purified inactivated flavivirus vaccines).

330. The discussion below shows where WO '225 provides a single working example that falls within each of claims 1–15 and 21–25 of the '681 patent and, therefore, anticipates claims 1–15 and 21–25 of the '681 patent. WO '225 has the same text as the '664 PCT and discloses only a single embodiment that falls within the challenged claims. This common text, however, does not adequately describe or enable the full scope of claims 1–15 and 21–25 of the '681 patent, for the reasons discussed above in Section XI.B.

1. Claims 1–3 and 21–25 of the '681 patent

331. The claim chart below lists representative disclosures from WO '225 that teach each element of claim 1 of the '681 patent.

Claim 1 of the '681 patent	Disclosures of WO '225 (EX1008)
A Zika virus vaccine comprising a Zika virus	“Disclosed herein are virus vaccines and compositions comprising an inactivated Zika virus,

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Claim 1 of the '681 patent	Disclosures of WO '225 (EX1008)
<p>having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72,</p>	<p>and related methods of producing said vaccines and compositions.” (2:27–28.)</p> <p>“Zika Virus Strain H/PF/2013 was originally isolated from a 51-year-old woman (accession number KJ776791.1, also SEQ ID NO: 13 herein) from French Polynesia. A sample was obtained from the European Virus Archive (EVAg; Ref-SKU: 001v-EVA1545). Based on this material, a research master seed bank (rMSB) was prepared on Vero cells as the cell substrate and the genomic sequence was checked by sequencing.... The sequence obtained from the rMSB by use of these primers is provided by SEQ ID NO: 72. There was 100% overlap of the sequence with the published sequence of Zika Virus Strain H/PF/2013 (SEQ ID NO: 13). However, we sequenced additional regions 5' (an additional 40 bp) and 3' (an additional 160 bp) represented in SEQ ID NO: 72. In a preferred embodiment, the Zika virus of the invention comprises SEQ ID NO: 72.” (76:16–27 (emphasis added).)</p>
<p>wherein said Zika virus vaccine is capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects, wherein the neutralizing antibody titer is determined using a microneutralization assay (MN₅₀).</p>	<p>“In particular, the invention is directed to a virus vaccine comprising an optimally inactivated virus particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability.” (5:25–28 (emphasis added).)</p> <p>“One intraperitoneal immunizations of 200 µL were administered. A dose titration (12 µg, 3 µg, 1 µg, 0.33 µg, 0.11 µg, 0.037 µg and 0.012 µg, equivalent to the protein amount in IXIARO) of inactivated Zika virus formulated with aluminium</p>

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Claim 1 of the '681 patent	Disclosures of WO '225 (EX1008)
	hydroxide (Al(OH) ₃) at a final concentration of 0.7%. Three weeks after immunization, blood was collected and immune sera were prepared.” (84:23–27.) “Another neutralization assay was performed using the microneutralization assay as described by Larocca, et al. (2016, Nature doi:10.1038/nature18952). It was found that the inactivated Zika virus of the current invention had an MN50 (microneutralization) titer of 90 at 1 µg of inactivated purified virus. ” (85:25–28 (emphasis added).)

332. WO '225 is directed to “Zika virus vaccines and compositions and methods of producing and administering said vaccines to subjects in need thereof.” EX1008, Abstract. WO '225 discloses a single working example of a ZIKV vaccine containing the H/PF/2013 ZIKV strain, which has an RNA genome comprising a sequence corresponding to the DNA sequence in SEQ ID NO: 72. *Id.*, 75:20–85:28.

333. WO '225 discloses harvesting H/PF/2013 ZIKV virions propagated in Vero cells, purifying the virions using protamine sulfate followed by sucrose density centrifugation, and then inactivating the virions using formalin. *Id.*, 77:10–82:16. WO '225 then discloses formulating the ZIKV vaccine into a composition comprising the purified inactivated H/PF/2013 ZIKV virions, PBS, and aluminum hydroxide. *Id.*, 82:18–27.

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334. WO '225 discloses administering the inactivated H/PF/2013 ZIKV vaccine to mice via intraperitoneal immunization using “[a] dose titration (12 µg, 3 µg, 1 µg, 0.33 µg, 0.11 µg, 0.037 µg and 0.012 µg, equivalent to the protein amount in IXIARO).” *Id.*, 84:20–25. WO '225 discloses that “[t]hree weeks after immunization, blood was collected and immune sera were prepared.” *Id.*, 84:26–27. WO '225 discloses assaying the sera for neutralizing antibodies using a MN₅₀ assay and determining that the vaccine stimulated an MN₅₀ of 90 when administered at a dose of 1 µg. *Id.*, 84:25–28.

335. WO '225 additionally discloses that it is directed to “a virus vaccine comprising an optimally inactivated particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability.” *Id.*, 5:26–28. As discussed above in ¶170, a MN₅₀ of greater than 15 would be indicative of seroconversion.

336. WO '225, therefore, discloses a ZIKV vaccine comprising the H/PF/2013 ZIKV strain with an RNA genome comprising a sequence corresponding to the DNA sequence provided by SEQ ID NO: 72, wherein the ZIKV vaccine is capable of stimulating a MN₅₀ of greater than 15 (*viz.*, a MN₅₀ of 90) in at least 70% of vaccinated subjects, as required by claim 1.

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337. Thus, WO '225 discloses each and every element of claim 1, arranged as claimed. A POSA would also be able to follow the disclosures of WO '225 to make and use the disclosed single working example without undue experimentation because it relies on the known H/PF/2013 ZIKV strain, which WO '225 discloses the sequence of and the virions as being obtained from the European Virus Archive depository, and known methods to produce the ZIKV vaccine.³⁹ See ¶329, above.

338. Claims 2, 24, and 25 recite that the ZIKV vaccine stimulates a MN₅₀ of greater than 20, 25, 30, 35, 40, or 45 (claim 2); 50, 55, 60, 65, 70, 75, 80, or 85 (claim 24); and greater or equal to 90 (claim 25). EX1001, 445:42–43, 448:19–22. As discussed above for claim 1, WO '225 discloses that its single working example of a ZIKV vaccine comprising an RNA genome corresponding to the DNA sequence of SEQ ID NO: 72 is capable of stimulating a MN₅₀ of 90 in at least 70%

³⁹ While it is my opinion that the '664 PCT, which published as WO '225, does not enable the full scope of the '681 patent claims (see Section XI.B.2, above), there is sufficient disclosure provided in the specification of WO '225 and general knowledge in the art to follow the methods of WO '225 to enable its single working example.

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of vaccinated subjects. Thus, for the same reasons discussed above for claim 1, WO '225 anticipates claims 2, 24, and 25.

339. Claim 3 recites that the ZIKV vaccine is capable of stimulating a MN_{50} titer greater than 15 in at least, among others, 75% of subjects. *Id.*, 445:44–47. As discussed above for claim 1, WO '225 discloses that it is directed to “a virus vaccine comprising an optimally inactivated particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability.” EX1008, 5:26–28. A MN_{50} of greater than 15 would be indicative of seroconversion. See ¶170, above. WO '225, therefore, discloses that its single working example of a ZIKV vaccine is capable of stimulating a MN_{50} titer greater than 15 in at least 75% of subjects. As such, in addition to the reasons discussed for claim 1, WO '225 anticipates claim 3.

340. Claims 21–23 recite that the ZIKV vaccine's RNA genome corresponds to SEQ ID NO: 72 or a variant nucleic acid sequence that has at least 90%, 95%, and 99% identity to SEQ ID NO: 72, respectively. EX1001, 448:11–19. As discussed above, the ZIKV vaccine in the single working example of WO '225 uses the H/PF/2013 strain that has an RNA genome comprising a sequence corresponding to the DNA sequence of SEQ ID NO: 72. EX1008,

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76:20–25. As such, WO '225 anticipates claims 21–23 for the same reasons discussed above for claim 1.

2. Claim 4

341. The claim chart below lists representative disclosures from WO '225 that teach each element of claim 4.

Claim 4 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 1, wherein the Zika virus comprises an E protein having an amino acid sequence provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.	See discussion for claim 1, above. “[T]he Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.” (77:5–8 (emphasis added).)

342. WO '225 discloses that its single working example uses the H/PF/2013 ZIKV strain, which has an E protein having the amino acid sequence of SEQ ID NO: 47. EX1008, 77:5–8. SEQ ID NO: 47 is one of the SEQ ID NOs recited in claim 4. As such, WO '225 anticipates claim 4 for the same reasons discussed above for claim 1.

3. Claims 5–9

343. The claim chart below lists representative disclosures from WO '225 that teach each element of claims 5–9.

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Claim 5 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 1, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.	See discussion for claim 1, above. <p>“The purified viral solution was inactivated by incubation with 0.02% formaldehyde over a period of ten days in a 22°C controlled-temperature incubator. The formaldehyde is neutralized by addition of sodium metabisulphite on the tenth day.” (81:10–12 (emphasis added).)</p>

Claim 6 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.	See discussion for claim 5, above. <p>Table 7 of WO '225 discloses that at the start of the inactivation process, the H/PF/2013 ZIKV particles formed 3.4×10^8 plaque forming units. On day 5 of the inactivation process, no plaques could be detected. No plaques could be detected on day 10, as well. This indicates that the ZIKV has been contacted with a chemical inactivation agent for longer than is required to completely inactivate the ZIKV when assessed with the plaque assay of WO '225.</p>

Claim 7 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 5, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.	See discussion for claim 5, above.

Claim 8 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 7, wherein the chemical inactivation comprises contacting the Zika virus	See discussion for claim 7, above.

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Claim 8 of the '681 patent	Disclosures of WO '225 (EX1008)
with formaldehyde for between 2-10 days.	

Claim 9 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 5, wherein the chemical activation is performed at about +4° C. or about +22° C.	See discussion for claim 5, above.

344. In its single working example, WO '225 discloses chemically inactivating the H/PF/2013 ZIKV virions by incubating the virions in formaldehyde for ten days at +22° C (**claims 5 and 7–9**). EX1008, 81:10–12. WO '225 also reports that the ZIKV virions were completely inactivated on day 5 of the inactivation protocol when assessed with the plaque assay of WO '225 because no plaque forming units above the limit of detection were observed. *Id.*, Table 7. The inactivation process in WO '225 that inactivates the ZIKV virions for ten days thus is a chemical inactivation process that comprises contacting the ZIKV with a chemical inactivation agent for longer than is required to completely inactivate the ZIKV when assessed with the plaque assay of WO '225 (**claim 6**).

345. Thus, WO '225 discloses each and every element of claims 5–9, arranged as claimed. Before March 10, 2020, a POSA would also have been able to follow the disclosures of WO '225 to make and use the disclosed single working

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example without undue experimentation because it provides sufficient disclosure for a POSA to implement the procedures disclosed in the single working example along with the general knowledge in the art. As such, in addition to the reasons discussed above for claim 1, WO '225 anticipates claims 5–9.

4. Claims 10–15

346. The claim chart below lists representative disclosures from WO '225 that teach each element of claims 10–15.

Claim 10 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 1, further comprising an adjuvant.	See discussion for claim 1, above. “ZikaV NIV day10 (Zika peak ~36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (-5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP).” (82:29–32 (emphasis added).)

Claim 11 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 10, wherein the adjuvant is an aluminium salt adjuvant.	See discussion for claim 10, above.

Claim 12 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 11, wherein said aluminium salt adjuvant is aluminium hydroxide with less than	See discussion for claim 11, above. “A preferred aluminium salt is the aluminium hydroxide with reduced Cu content, e.g. lower

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Claim 12 of the '681 patent	Disclosures of WO '225 (EX1008)
1.25 parts per billion (ppb) copper (Cu) based on a final pharmaceutical composition comprising the Zika virus.	than 1,25ppb based on the weight of the Zika composition.” (20:19–21.)

Claim 13 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 10, wherein the adjuvant comprises a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).	See discussion for claim 10, above. “In some embodiments, the adjuvant is comprised of two components. In some embodiments, the 2-component adjuvant comprises an antibacterial peptide and a TLR9 agonist . In some embodiments, the antibacterial peptide is provided by the amino acid sequence KLKL₅KLK (SEQ ID NO: 71) . In some embodiments, the TLR9 agonist is a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN) . In some embodiments, the I-ODN comprises the nucleic acid sequence (dIdC)₁₃ (SEQ ID NO: 70).... In some embodiments, the adjuvant is IC31®, i.e. KLKL₅KLK (SEQ ID NO: 71) and the nucleic acid sequence (dIdC)₁₃ (SEQ ID NO: 70), in combination with an aluminium salt such as aluminium hydroxide. ” (20:24–33 (emphasis added).)

Claim 14 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 13, wherein the peptide comprises the sequence KLKL ₅ KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC) ₁₃ (SEQ ID NO: 70).	See discussion for claim 13, above.

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Claim 15 of the '681 patent	Disclosures of WO '225 (EX1008)
The Zika virus vaccine according to claim 1, further comprising one or more pharmaceutically acceptable excipients.	See discussion for claim 1, above. “ZikaV NIV day10 (Zika peak-36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (-5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP).” (82:29–32 (emphasis added).)

347. In its single working example, WO '225 discloses that the ZIKV vaccine comprises aluminum hydroxide as an adjuvant (**claims 10 and 11**) and a pharmaceutically acceptable excipient, phosphate buffered saline (PBS) (**claim 15**). EX1008, 82:29–32.

348. WO '225 also discloses that “[a] preferred aluminium salt is the aluminium hydroxide with reduced Cu content, e.g. lower than 1,25ppb based on the weight of the Zika composition.” *Id.*, 20:19–21. WO '225 further discloses combining aluminum hydroxide with “IC31®, i.e. KLKL₅KLK,” an antibacterial peptide, “and the nucleic acid sequence (dIdC)₁₃,” an I-ODN. *Id.*, 20:24–33. While the disclosures related to copper content and the IC31 adjuvant in WO '225 are found outside the single working example, WO '225 makes clear that it contemplates using aluminum hydroxide with the claimed copper content and IC31 adjuvant in combination with aluminum hydroxide. *Id.*, 20:19–33. In fact, the single working example discloses using aluminum hydroxide as the adjuvant in the

***Inter Partes Review of U.S. Patent No. 11,219,681
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vaccine. *Id.*, 82:29–32. WO '225 further states that its invention includes using IC31 and aluminum hydroxide in combination and it is preferred to use aluminum hydroxide with copper content than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV. *Id.*, 20:19–33. A POSA, therefore, would immediately and readily envisage using the claimed (1) aluminum hydroxide with less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV (**claim 12**); and (2) IC31 adjuvant (**claims 13 and 14**) in combination with aluminum hydroxide in the single working example WO '225 discloses.

349. As such, WO '225 discloses each and every element of claims 10–15, arranged as claimed. Before March 10, 2020, a POSA would also have been able to follow the disclosures of WO '225 to make and use the disclosed single working example without undue experimentation, because it relies on the known H/PF/2013 ZIKV strain and known methods to produce the ZIKV vaccine. *See* ¶329, above. Moreover, aluminum hydroxide and IC31 were well-known adjuvants before March 10, 2020, and could have been readily used by a POSA. EX1083, 1:6–17; EX1066, Abstract. A POSA, moreover, would have had knowledge from the art regarding how to measure the level of copper in aluminum hydroxide to assure that the level of copper is less than 1.25 ppb copper based on a final pharmaceutical composition comprising the ZIKV. *See* ¶217, above.

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350. As such, in addition to the reasons discussed above for claim 1, WO '225 anticipates claims 10–15.

XII. CONCLUSION

351. As an expert in the field of virology and vaccine research and development, it is my opinion that (1) claims 1–12, 15, and 21–25 cannot claim priority to the December 23, 2015 filing date of EP '585 because it does not mention ZIKV at all, and thus they would have been obvious in view of the prior art that was available before March 18, 2016; and (2) claims 1–15 and 21–25 cannot claim priority to the December 23, 2016 filing date of the '664 PCT because the '664 PCT does not provide written description or enablement support for the claims, and thus they are anticipated by WO '225 before the March 10, 2020 filing date of the '862 application that became the '681 patent.

APPENDIX A

***Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)***

Sequence of interest	Sequence aligned to	% identity	% identity of overlapping regions	Exhibit No.
SEQ ID NO: 2	SEQ ID NO: 72	99%	99%	EX1105
SEQ ID NO: 3	SEQ ID NO: 72	99%	99%	EX1106
SEQ ID NO: 4	SEQ ID NO: 72	98%	99%	EX1107
SEQ ID NO: 5	SEQ ID NO: 72	99%	99%	EX1108
SEQ ID NO: 6	SEQ ID NO: 72	99%	99%	EX1109
SEQ ID NO: 7	SEQ ID NO: 72	99%	99%	EX1110
SEQ ID NO: 8	SEQ ID NO: 72	98%	98%	EX1111
SEQ ID NO: 9	SEQ ID NO: 72	99%	99%	EX1112
SEQ ID NO: 10	SEQ ID NO: 72	98%	99%	EX1113
SEQ ID NO: 11	SEQ ID NO: 72	89%	89%	EX1114
SEQ ID NO: 12	SEQ ID NO: 72	89%	89%	EX1115
SEQ ID NO: 13	SEQ ID NO: 72	99%	100%	EX1116
SEQ ID NO: 11	SEQ ID NO: 12	99%	99%	EX1117
H/PF/2013 (KJ776791.1)	SEQ ID NO: 72	99%	100%	EX1020
H/PF/2013 (KJ776791.1) ORF translation	SEQ ID NO: 73	100%	N/A	EX1071
PRVABC59 (KU501215.1)	SEQ ID NO: 72	98%	99%	EX1075
PRVABC59 (KU501215.1) ORF translation	SEQ ID NO: 40	100%	N/A	EX1075

APPENDIX B

***Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)***

I. Percent identity analysis for Zhang's Class II, III, IV, and V mutants

Class	NS2A Mutations	Phenotypes
I	D4A, E22A, K31A, G47A, D53A, E67A, H76A, E122A, R146A, D148A, R163A, K186A, K188A, D210A	Mutants do not or weakly affect viral production
II	K56A, F83A, K84A, R86A, P87A, R96A, R102A, D124A, R207A	Mutants dramatically reduce viral production
III	G12A, N130A	Mutants abolish viral RNA synthesis
IV	D7A, R140A, R222A	Mutants abolish virus production by affecting RNA synthesis and viron assembly/release
V	K25A/K26A/R27A, G71A, D73A, E103A, R171A, K192A, K193A	Mutants abolish virion assembly

Analysis 1: % sequence identity with SEQ ID 72 for introducing Zhang's Class II, III, IV, and V mutations into SEQ ID NO: 72

The following numbers of nucleotide changes in SEQ ID NO: 72 would yield the following sequence identities:

- 1 nucleotide difference: 10772/10773 = 99.99% identity
- 2 nucleotide differences: 10771/10773 = 99.98% identity
- 6 nucleotide differences: 10767/10773 = 99.94% identity

To perform the analyses below, I identified the codon in SEQ ID NO: 72 that encodes the amino acid being mutated.

Class II

- K56A⁴⁰ would be an AAG → GCG mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- F83A would be a TTC → GCC mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

⁴⁰ Zhang numbered the amino acids of the mutants by their location in the ZIKV

NS2A protein. EX1056, FIG. 1.

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- K84A would be an AAA → GCA mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- R86A would be an AGA → GCA mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- P87A would be a CCA → GCA mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- R96A would be an AGA → GCA mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- R102A would be a CGT → GCT mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- D124A would be GAC → GCC mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- R207A would be AGG → GCG mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

Class III

- G12A would be a GGA → GCA mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- N130A would be an AAT → GCT mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

Class IV

- D7A would be a GAT → GCT mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- R140A would be a CGA → GCA mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- R222A would be an AGG → GCG mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

Class V

- K25A/K26A/R27A would be AAG/AAG/AGA → GCG/GCG/GCA mutations in SEQ ID NO: 72 (6 nucleotide difference), yielding a sequence having 99.94% identity to SEQ ID NO: 72
- G71A would be a GGA → GCA mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- D73A would be a GAT → GCT mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- E103A would be GAA → GCA mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- R171A would be an AGA → GCA mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- K192A would be an AAG → GCG mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- K193A would be an AAG → GCG mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

Analysis 2: % sequence identity of Zhang's Class II, III, IV, and V mutants compared to SEQ ID NO: 72

Zhang used ZIKV strain FSS13025 (GenBank accession no. KU955593.1). EX1056, 588 (citing reference [43] (Shan; EX1101) as the source of the cDNA clone Zhang used); EX1101, 892 (identifying GenBank sequence used)

The sequence for ZIKV strain FSS13025 (10,807 nucleotides) is about 98% identical to SEQ ID NO: 72. EX1190.

The following numbers of nucleotide changes in the FSS13025 sequence would yield the following sequence identities when aligned to SEQ ID NO: 72

- 1 nucleotide difference: $10619/10807 = 98.26\%$ identity

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Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)***

- 2 nucleotide differences: 10618/10807 = 98.25% identity
- 6 nucleotide differences: 10614/10807 = 98.21% identity

To perform the analyses below, I identified the codon in the FSS13025 sequence that encodes the amino acid being mutated.

Class II

- K56A would be an AAG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- F83A would be a TTC → GCC mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- K84A would be an AAA → GCA mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- R86A would be an AGA → GCA mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- P87A would be a CCT → GCT mutation in the FSS13025 sequence (1 nucleotide difference), yielding a sequence having 98.26% identity to SEQ ID NO: 72
- R96A would be an AGA → GCA mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- R102A would be a CGT → GCT mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- D124A would be GAC → GCC mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72

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- R207A would be AGG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72

Class III

- G12A would be a GGA → GCA mutation in the FSS13025 sequence (1 nucleotide difference), yielding a sequence having 98.26% identity to SEQ ID NO: 72
- N130A would be an AAT → GCT mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72

Class IV

- D7A would be a GAT → GCT mutation in the FSS13025 sequence (1 nucleotide difference), yielding a sequence having 98.26% identity to SEQ ID NO: 72
- R140A would be a CGA → GCA mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- R222A would be an AGG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72

Class V

- K25A/K26A/R27A would be AAG/AAG/AGA → GCG/GCG/GCA mutations in the FSS13025 sequence (6 nucleotide difference), yielding a sequence having 98.21% identity to SEQ ID NO: 72
- G71A would be a GGA → GCA mutation in the FSS13025 sequence (1 nucleotide difference), yielding a sequence having 98.26% identity to SEQ ID NO: 72
- D73A would be a GAT → GCT mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72

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- E103A would be GAG → GCG mutation in the FSS13025 sequence (1 nucleotide difference), yielding a sequence having 98.26% identity to SEQ ID NO: 72
- R171A would be an CGG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 98.25% identity to SEQ ID NO: 72
- K192A would be an AAG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- K193A would be an AAG → GCG mutation in the FSS13025 sequence (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

II. Percent identity analysis for DiNunno's mutants

Analysis 1: % sequence identity with SEQ ID 72 for introducing DiNunno's mutations into SEQ ID NO: 72

The following numbers of nucleotide changes in SEQ ID NO: 72 would yield the following sequence identities:

- 1 nucleotide difference: 10772/10773 = 99.99% identity
- 2 nucleotide differences: 10771/10773 = 99.98% identity

To perform the analyses below, I identified the codon in SEQ ID NO: 72 that encodes the amino acid being mutated.

Mutants

- H446A⁴¹ would be CAT → GCT mutation in SEQ ID NO: 72 (2 nucleotide

⁴¹ DiNunno numbered the amino acids of the mutants by their location in the ZIKV E protein. EX1091, FIG. 1.

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difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

- F449A would be a TTC → GCC mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- F453A would be a TTT → GCT mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72
- W474A would be an TGG → GCG mutation in SEQ ID NO: 72 (1 nucleotide difference), yielding a sequence having 99.99% identity to SEQ ID NO: 72
- F497A would be a TTC → GCC mutation in SEQ ID NO: 72 (2 nucleotide difference), yielding a sequence having 99.98% identity to SEQ ID NO: 72

Analysis 2: % sequence identity of DiNunno's mutants compared to SEQ ID NO: 72

DiNunno used ZIKV strain MR-766 (GenBank accession no. AY632535.2).
EX1091, 2

The sequence for ZIKV strain MR-766 (10,794 nucleotides) is about 89% identical to SEQ ID NO: 72. EX1115 (aligning SEQ ID NO: 12 (AY632535.2) to SEQ ID NO: 72).

The following numbers of nucleotide changes in the MR-766 sequence would yield the following sequence identities when aligned to SEQ ID NO: 72

- 1 nucleotide difference: $9583/10810 = 88.65\%$ identity
- 2 nucleotide differences: $9582/10810 = 88.64\%$ identity

To perform the analyses below, I identified the codon in the MR-766 sequence that encodes the amino acid being mutated.

Mutants

- H446A would be a CAC → GCC mutation in the MR-766 sequence (2 nucleotide difference), yielding a sequence having 88.64% identity to SEQ ID NO: 72

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- F449A would be a TTT → GCT mutation in the MR-766 sequence (2 nucleotide difference), yielding a sequence having 88.64% identity to SEQ ID NO: 72
- F453A would be a TTC → GCC mutation in the MR-766 sequence (2 nucleotide difference), yielding a sequence having 88.64% identity to SEQ ID NO: 72
- W474A would be an TGG → GCG mutation in the MR-766 sequence (1 nucleotide difference), yielding a sequence having 88.65% identity to SEQ ID NO: 72
- F497A would be a TTC → GCC mutation in the MR-766 sequence (2 nucleotide difference), yielding a sequence having 88.64% identity to SEQ ID NO: 72

APPENDIX C

***Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)***

Claim 1 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
A Zika virus vaccine	<p style="text-align: center;"><u>WHO Zika Pipeline</u></p> <p>“As part of the broader response to Zika WHO has initiated an emergency research and development plan.... [T]he R&D Blueprint aims to develop and implement a roadmap for R&D preparedness, and to enable roll-out of an emergency R&D response as early and as efficiently as possible for emerging pathogens for which there are no, or insufficient, preventive and curative solutions. In December 2015 WHO convened a workshop to identify a short-list of pathogens to be prioritized immediately. Zika was identified as a serious risk, necessitating further action as soon as possible. The emergency research and development plan has been tailored to the current state of understanding of Zika fever and addresses research and development needs for novel means of vector control, diagnostics, vaccines, and therapeutics.” (2.)</p> <p>“Zika virus (ZIKV) belongs to the family of flaviviridae and to the antigenic complex Spondweni. It is also closely related to the four dengue viruses, as evidenced by strong serologic cross-reactivity. Genotyping and geographical analysis have revealed an African and an Asian lineage, but sequence homology is high and the clinical and serologic relevance is unclear. It is assumed that a ZIKV vaccine can be developed building on the same technologies that have been successfully used to develop human flavivirus vaccines (Yellow Fever, Tick-Borne Encephalitis, Japanese Encephalitis, Dengue).” (5.)</p> <p>“WHO has conducted a landscape analysis of approaches taken by commercial, governmental, academic and any other known entities towards the development of ZIKV vaccine candidates.... The landscape analysis revealed 18 active programmes (including 5 academic groups and 15 commercial entities, some working in collaborative partnership)....” (<i>Id.</i>)</p>

**Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)**

Claim 1 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
	<p>“WHO has initiated a consultative process to develop a target product profile for a Zika vaccine for use in the emergency context and targeted at the protection of women in child-bearing age and pregnant women.” (<i>Id.</i>)</p> <p style="text-align: center;"><u>Srivastava</u></p> <p>“Japanese encephalitis virus (JEV) is a member of the flavivirus family and the causal agent of Japanese encephalitis (JE).” (4557.)</p> <p>“A second generation, purified, inactivated vaccine (PIV) against Japanese encephalitis (JE) virus was produced and tested in mice where it was found to be highly immunogenic and protective. The JE-PIV was made from an attenuated strain of JE virus propagated in certified Vero cells, purified, and inactivated with formalin.” (Abstract.)</p> <p>“The JE-PIV described in the present report was produced using methods originally developed for making the dengue-2 PIV.... The JE-PIV was tested in mice where it was found to be highly immunogenic and protective.” (4558.)</p> <p>“[Vero] cells have several desirable features: (1) they can be maintained in serum-free medium while supporting the growth of JE viruses to high titer; (2) the cell culture supernatants can be harvested several times for making one, large production lot; and (3) the absence of serum products increases the potential safety of the vaccine and enables purification to be accomplished by simple, efficient and inexpensive methods.” (4564.)</p> <p>“[T]he results presented here suggest that the processes and materials used for production of the new JE-PIV will result in a product that is safe, effective and economical. The manufacturing methods are sufficiently robust,</p>

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Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)**

Claim 1 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
	demonstrating good scalability, efficiency, and product yield.” (<i>Id.</i>)
<p>comprising a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72,</p>	<p style="text-align: center;"><u>Baronti</u></p> <p>“ZIKA strain H/PF/2013 isolated onto Vero cells from the serum of the patient was made available by the European Virus Archive (EVA).” (1.)</p> <p>“Viral RNA extracted from the cell culture supernatant at passage 3 was used for next-generation sequencing ... following nonspecific amplification; 137,311 reads produced a 10,617-nucleotide (nt)-long consensus contig, including the virus complete open reading frame (ORF) sequence (10,272 nt).” (<i>Id.</i>)</p> <p>“Maximum likelihood phylogenetic reconstruction ... indicated that it belonged to the Asian lineage, sharing common ancestorship and ca. 99.9% nt and aa identities with isolates circulating in southern Asia and the Pacific islands in the late 2000s.... This points to the spread of the Asian lineage, which has been suggested to originate from the introduction of ZIKV in Southeast Asia around 1945.” (<i>Id.</i>)</p> <p>“The virus genome sequence described here has been deposited in the GenBank database under the accession no. KJ776791.” (<i>Id.</i>)</p> <p style="text-align: center;"><u>The H/PF/2013 Sequence</u></p> <p>ZIKV strain H/PF/2013 polyprotein gene, having GenBank accession no. KJ776791. When aligned to SEQ ID NO: 72 by BLAST, the H/PF/2013 Sequence has 99% identity to SEQ ID NO: 72 and 100% identity when just the overlapping regions are aligned.</p>
<p>wherein said Zika virus vaccine is capable of stimulating a neutralizing</p>	<p style="text-align: center;"><u>Srivastava</u></p> <p>“The ability of the JE-PIV to elicit virus-neutralizing antibodies in mice was determined. Groups of ten adult</p>

**Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)**

Claim 1 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)																																																		
antibody titer greater than 15 in at least 70% of vaccinated subjects,	<p>outbred mice were immunized with graded doses of JE-PIV, lot 0574, or with licensed JE vaccine (JE-Vax). Animals were given two inoculations of vaccine 4 weeks apart and blood was obtained 2 weeks following the second inoculation. Sera were tested for JE virus-neutralizing antibodies (Table 3). The dose of vaccine required to elicit detectable titers (PRNT₅₀ > 1:10) of neutralizing antibody in 50% of vaccinated animals (i.e. the Immunizing Dose 50%, ID₅₀) was calculated by Probit. The ID₅₀ for JE-PIV was 4 ng (95% CI 2–7 ng), which was less than the ID₅₀ of 15 ng (95% CI 14–17 ng) for JE-Vax. The lowest dose of the JE-PIV tested that elicited neutralizing antibody in 100% of vaccinated mice was 32 ng.” (4562–4563.)</p> <p>Table 3 Virus neutralizing antibody titers (PRNT₅₀) in mice vaccinated with JE PIV lot 0574 or commercial JE Vax</p> <table><tr><th>Vaccine</th><th>Dose (ng)</th><th>PRNT₅₀ GMT</th><th>PRNT₅₀ Range</th><th>Seroconversion rate</th><th>Immunizing dose 50% (ID₅₀)</th></tr><tr><td rowspan="5">JE PIV</td><td>800</td><td>3842</td><td>1280–7781</td><td>10/10</td><td rowspan="5">4 ng (2–7 ng, 95% CI)</td></tr><tr><td>160</td><td>1373</td><td>168–3026</td><td>10/10</td></tr><tr><td>32</td><td>113</td><td>34–563</td><td>10/10</td></tr><tr><td>6</td><td>ND^b</td><td>ND</td><td>7/10</td></tr><tr><td>1</td><td>ND</td><td>ND</td><td>1/10</td></tr><tr><td rowspan="5">JE Vax^a</td><td>1200</td><td>733</td><td>283–3754</td><td>10/10</td><td rowspan="5">15 ng (14–17, 95% CI)</td></tr><tr><td>240</td><td>117</td><td>18–863</td><td>10/10</td></tr><tr><td>48</td><td>90</td><td>55–150</td><td>7/10</td></tr><tr><td>10</td><td>ND</td><td>ND</td><td>6/10</td></tr><tr><td>2</td><td>ND</td><td>ND</td><td>1/10</td></tr></table> <p>^a For JE-Vax, the virus protein concentration as reconstituted from the vial was 6 micrograms (6000 ng) per ml from information supplied by the manufacturer. ^b Not determined.</p> <p>Table 3 shows the JE-PIV vaccine stimulated titers in 100% of vaccinated mice in the range of 34–563, 168–3026, and 1280–7781 when given two 32 ng, 160 ng, and 800 ng doses given four weeks apart, respectively. (Table 3.)</p> <p style="text-align: center;"><u>Thomas</u></p> <p>“The microneutralization test that we used was developed at the WRAIR Pilot Bioproduction Facility.... This new assay was shown to be specific and sensitive for the detection of anti-DENV neutralizing antibodies (e.g., limit of the blank < 1:3.3; limit of detection < 1:7; limit of quantification ≤ 1:10 for all four serotypes). The precision of the assay was estimated to range from 39% to 59% depending on serotype.” (86.)</p>	Vaccine	Dose (ng)	PRNT ₅₀ GMT	PRNT ₅₀ Range	Seroconversion rate	Immunizing dose 50% (ID ₅₀)	JE PIV	800	3842	1280–7781	10/10	4 ng (2–7 ng, 95% CI)	160	1373	168–3026	10/10	32	113	34–563	10/10	6	ND ^b	ND	7/10	1	ND	ND	1/10	JE Vax ^a	1200	733	283–3754	10/10	15 ng (14–17, 95% CI)	240	117	18–863	10/10	48	90	55–150	7/10	10	ND	ND	6/10	2	ND	ND	1/10
Vaccine	Dose (ng)	PRNT ₅₀ GMT	PRNT ₅₀ Range	Seroconversion rate	Immunizing dose 50% (ID ₅₀)																																														
JE PIV	800	3842	1280–7781	10/10	4 ng (2–7 ng, 95% CI)																																														
	160	1373	168–3026	10/10																																															
	32	113	34–563	10/10																																															
	6	ND ^b	ND	7/10																																															
	1	ND	ND	1/10																																															
JE Vax ^a	1200	733	283–3754	10/10	15 ng (14–17, 95% CI)																																														
	240	117	18–863	10/10																																															
	48	90	55–150	7/10																																															
	10	ND	ND	6/10																																															
	2	ND	ND	1/10																																															

**Inter Partes Review of U.S. Patent No. 11,219,681
Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)**

Claim 1 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
<p>wherein the neutralizing antibody titer is determined using a microneutralization assay (MN50).</p>	<p style="text-align: center;"><u>Thomas</u></p> <p>“The microneutralization test that we used was developed at the WRAIR Pilot Bioproduction Facility to overcome many of the PRNT’s limitations, such as its low throughput, unacceptable labor-intensive nature, and high degree of interassay variability. This new assay was shown to be specific and sensitive for the detection of anti-DENV neutralizing antibodies” (86.)</p> <p>“Antibodies to each DENV type were measured at the Pilot Bioproduction Facility, WRAIR, using a 96-well quantitative microneutralization assay (MN50) performed in Vero cells (World Health Organization [WHO], National Institute for Biological Standards and Control [NIBSC]-011038011038) with an initial input of 50 plaque-forming units (PFU) each DENV-2, DENV-3, and DENV-4 and 100 PFU DENV-1.... Seven threefold serial dilutions from 1:3.3 to 1:2,430 were tested per sample. The MN50 titers were automatically processed by an EXCEL spreadsheet, which used a log midpoint linear regression program model to derive the MN50 titer. The MN50 titer is the reciprocal of the serum dilution that neutralizes $\geq 50\%$ of dengue virus, leading to a reduction of 50% of the optical density measured by enzyme-linked immunosorbent assay on fixed cells after 4 days of incubation. Seropositivity was defined as a titer $\geq 1:10$.” (75.)</p>
Claim 2 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
<p>The Zika virus vaccine according to claim 1, wherein said MN₅₀ is greater than 20, 25, 30, 35, 40, or 45.</p>	<p>See claim 1, discussed above.</p>

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Declaration of Dan H. Barouch, M.D., Ph.D. (Exhibit 1002)**

Claim 3 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein the Zika virus vaccine is capable of stimulating a MN50 titer greater than 15 in at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects.	See discussion for claim 1, above.

Claim 4 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein the Zika virus comprises an E protein having an amino acid sequence provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.	<p>See discussion for claim 1, above.</p> <p style="text-align: center;"><u>Baronti</u></p> <p>“Viral RNA extracted from the cell culture supernatant at passage 3 was used for next-generation sequencing ... following nonspecific amplification; 137,311 reads produced a 10,617-nucleotide (nt)-long consensus contig, including the virus complete open reading frame (ORF) sequence (10,272 nt).” (1.)</p> <p>“The virus genome sequence described here has been deposited in the GenBank database under the accession no. KJ776791.” (<i>Id.</i>)</p> <p style="text-align: center;"><u>The H/PF/2013 Sequence</u></p> <p>The translation of the H/PF/2013 Sequence’s ORF is 100% identical to the translation of the ORF in SEQ ID NO: 72, which is SEQ ID NO: 73. The portion of SEQ ID NO: 73 corresponding to the E protein is SEQ ID NO: 47.</p>

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Claim 5 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.	<p>See discussion for claim 1, above.</p> <p style="text-align: center;"><u>Srivastava</u></p> <p>“The virus was inactivated with 0.05% formalin at a constant temperature of 22 °C, conditions established by previous experiments (data not shown).” (4561.)</p>

Claim 6 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)																																																				
The Zika virus vaccine according to claim 5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.	<p>See discussion for claim 5, above.</p> <p style="text-align: center;"><u>Srivastava</u></p> <p>“The virus was inactivated with 0.05% formalin at a constant temperature of 22 °C, conditions established by previous experiments (data not shown). Samples were removed daily from the vaccine, neutralized with sodium bisulfite, and tested for residual live virus by inoculation of Vero cells (Table 2). A total of 48 h after addition of formalin, the vaccine was re-filtered and transferred to a fresh flask. No infectious virus was recovered after 72 h and the incubation was continued for an additional 168 h to ensure complete inactivation.” (4561.)</p> <p>Table 2 Formalin inactivation kinetics of the purified vaccine virus</p> <table><tr><th>Sample</th><th>Time post-inactivation (h)</th><th>Plaques detected by Vero cell-amplified virus assay</th><th>Residual virus titer by direct assay on Vero cells, PFU/ml</th></tr><tr><td>1</td><td>0</td><td>Positive</td><td>2.4 × 10⁸</td></tr><tr><td>2</td><td>24</td><td>Positive</td><td>2.8 × 10³</td></tr><tr><td>3</td><td>36</td><td>Positive</td><td>2.8 × 10²</td></tr><tr><td>4</td><td>48</td><td>Positive</td><td>5.8 × 10¹</td></tr><tr><td>5</td><td>60</td><td>Positive</td><td>5</td></tr><tr><td>6</td><td>72</td><td>Negative</td><td>None</td></tr><tr><td>7</td><td>84</td><td>Negative</td><td>None</td></tr><tr><td>8</td><td>108</td><td>Negative</td><td>None</td></tr><tr><td>9</td><td>132</td><td>Negative</td><td>None</td></tr><tr><td>10</td><td>156</td><td>Negative</td><td>None</td></tr><tr><td>11</td><td>180</td><td>Negative</td><td>None</td></tr><tr><td>12</td><td>240</td><td>Negative</td><td>None</td></tr></table> <p>(Table 2.)</p>	Sample	Time post-inactivation (h)	Plaques detected by Vero cell-amplified virus assay	Residual virus titer by direct assay on Vero cells, PFU/ml	1	0	Positive	2.4 × 10 ⁸	2	24	Positive	2.8 × 10 ³	3	36	Positive	2.8 × 10 ²	4	48	Positive	5.8 × 10 ¹	5	60	Positive	5	6	72	Negative	None	7	84	Negative	None	8	108	Negative	None	9	132	Negative	None	10	156	Negative	None	11	180	Negative	None	12	240	Negative	None
Sample	Time post-inactivation (h)	Plaques detected by Vero cell-amplified virus assay	Residual virus titer by direct assay on Vero cells, PFU/ml																																																		
1	0	Positive	2.4 × 10 ⁸																																																		
2	24	Positive	2.8 × 10 ³																																																		
3	36	Positive	2.8 × 10 ²																																																		
4	48	Positive	5.8 × 10 ¹																																																		
5	60	Positive	5																																																		
6	72	Negative	None																																																		
7	84	Negative	None																																																		
8	108	Negative	None																																																		
9	132	Negative	None																																																		
10	156	Negative	None																																																		
11	180	Negative	None																																																		
12	240	Negative	None																																																		

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Claim 7 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 5, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.	See discussion for claim 5, above.
Claim 8 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 7, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.	See discussion for claim 5, above. <p style="text-align: center;"><u>Srivastava</u></p> <p>“The virus was inactivated with 0.05% formalin at a constant temperature of 22 °C, conditions established by previous experiments (data not shown). Samples were removed daily from the vaccine, neutralized with sodium bisulfite, and tested for residual live virus by inoculation of Vero cells (Table 2). A total of 48 h after addition of formalin, the vaccine was re-filtered and transferred to a fresh flask. No infectious virus was recovered after 72 h and the incubation was continued for an additional 168 h to ensure complete inactivation.” (4561.)</p>
Claim 9 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 5, wherein the chemical activation is performed at	See discussion for claim 5, above.

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Claim 9 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
about +4° C. or about +22° C.	

Claim 10 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, further comprising an adjuvant.	<p>See discussion for claim 1, above.</p> <p style="text-align: center;"><u>Srivastava</u></p> <p>“Three hundred ml of the dialyzed bulk vaccine was transferred aseptically to a clean, sterile, 500 ml bottle (Nalgene). Fifteen ml of aluminum hydroxide (Rehydragel 2% aluminum hydroxide, Reheis) was added to the 300 ml of vaccine.” (4560.)</p> <p>“Animals were inoculated with graded doses of JE-PIV, lot 0475, adjuvanted with 0.1% aluminum hydroxide.” (4561.)</p>

Claim 11 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 10, wherein the adjuvant is an aluminium salt adjuvant.	See discussion for claim 10, above.

Claim 12 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), Thomas (EX1164), and Möhlen (EX1083)
The Zika virus vaccine according to claim 11, wherein said aluminium salt	See discussion for claim 11, above.

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Claim 12 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), Thomas (EX1164), and Möhlen (EX1083)
<p>adjuvant is aluminium hydroxide with less than 1.25 parts per billion (ppb) copper (Cu) based on a final pharmaceutical composition comprising the Zika virus.</p>	<p style="text-align: center;"><u>Srivastava</u></p> <p>“The formalin in the samples was neutralized with sodium bisulfite by addition of one part 3.75% (w/v) sodium metabisulfite to 100 parts of vaccine.” (4559.)</p> <p style="text-align: center;"><u>Möhlen</u></p> <p>“[T]he stability of a protein component (e.g. as such or within a complex such as e.g. a virus or other pathogen) in the context of an aqueous composition that also comprises aluminium salt and a reactive compound such as e.g. a sulphite is critically dependent on the content of heavy metals.” (5:1–5.)</p> <p>“Without being bound to theory, antigen degradation in aqueous compositions, such as immunogenic composition, comprising heavy metal ions present in an aluminium salt, such as aluminium hydroxide, might be explained with an underlying degradation pathway assuming free-radicals such as e.g. free-radicals of sulphite. Formation of free radicals can be catalysed by heavy metal ions present in, for instance, aluminium hydroxide and this effect (in case of a certain amount of heavy metal as indicated according to the invention) could be the underlying root cause mechanism for stability issues as identified as part of the inventive contribution.” (11:14–22.)</p> <p>“Additional or alternative reactive compound may also be present to inactivate any residual formaldehyde in the aqueous solution, such as the reactive compound sulphite. As detailed above, heavy metals are thought to catalyse oxidation of (bi)sulphite and formation of sulphite radicals present in the aqueous composition, which in turn may induce degradation of protein present in the composition. Therefore, compositions comprising inactivated virus or bacteria or immunogenic parts thereof are likely to comprise one or more reactive compounds. Hence, the absence of</p>

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Claim 12 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), Thomas (EX1164), and Möhlen (EX1083)
	<p>heavy metals or presence below levels as identified by the present invention, is of particular 10 relevance when the aqueous composition of the invention comprises an inactivated virus or bacterium or immunogenic part thereof.” (16:1–11.)</p> <p>“It has been observed that the aluminium component is an important source for the heavy metal in the aqueous composition. Thus one way to control the amount of heavy metal in the aqueous composition is to control the amount of heavy metal in the aluminium source used to generate the aqueous composition.” (9:12–16.)</p> <p>“As demonstrated in the Examples, the stability of aqueous compositions comprising a JEV protein, an aluminium-salt and comprising less than 350 ppb heavy metal based on the weight of the aqueous composition, and in particular wherein the amount of Cu is less than 3 ppb based on the weight of the aqueous composition, is increased as compared to aqueous composition comprising more than 350 ppb of heavy metal and more than 3 ppb of Cu.” (33:24–30.)</p> <p>“The amount of Cu in a composition or method of the invention is in a particularly preferred embodiment less than 1,25 ppb based on the weight of the aqueous composition. In a particularly preferred embodiment said aqueous composition comprises Cu at a level that is below the detection limit of the method for the detection of copper.” (27:27–28:2.)</p> <p>“Methods for measuring the level of one or more heavy metals in an aqueous solution are known in the art. Examples of such methods include mass spectrometry, such as inductively-coupled-plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (F-AAS),</p>

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Claim 12 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), Thomas (EX1164), and Möhlen (EX1083)
	<p>and/or graphite furnace atomic absorption spectrometry (GF-AAS).” (28:18–25.)</p> <p>“As demonstrated in the Examples, Aluminium hydroxide (Alum) Lot 4230 was identified to contribute significantly to antigen degradation in JEV vaccine FVL09L37. In Example 3 it is shown that this Alum lot comprises at 10 least the following metals: Cu, Ni, W, Co, Os, Ru, Cd, Ag, Fe, V. Higher levels of Fe, Ni and Cu ions were noted in Alum lot 4230 when compared to other investigated lots. Lot 4230 was the only one where residual Cu ions were detected.” (26:7–13.)</p>

Claim 15 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, further comprising one or more pharmaceutically acceptable excipients.	<p>See discussion for claim 1, above.</p> <p style="text-align: center;"><u>Srivastava</u></p> <p>“A solution of Thimerosol [sic] was prepared by addition of 1 g Thimerosol [sic] powder to 100 ml of sterile PBS. After the Thimerisol [sic] was dissolved the solution was filtered through a 0.22 um nitrocellulose filter unit (Nalgene). A total of 3.15 ml of the 1% Thimerisol [sic] solution was added to the vaccine, which was left to mix on the shaker for an additional 10 min.” (4560.)</p>

Claim 21 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein said variant nucleic	See discussion for claim 1, above.

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Claim 21 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
acid has at least 90% identity to SEQ ID NO: 72.	

Claim 22 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein said variant nucleic acid has at least 95% identity to SEQ ID NO: 72.	See discussion for claim 1, above.

Claim 23 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 1, wherein said variant nucleic acid has at least 99% identity to SEQ ID NO: 72.	See discussion for claim 1, above.

Claim 24 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 2, wherein said MN50 is greater than 50, 55, 60, 65, 70, 75, 80, or 85.	See discussion for claim 2, above.

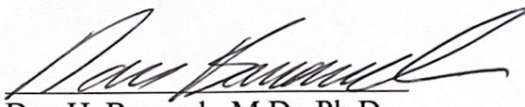
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Claim 25 of the '681 patent	Disclosures of the WHO Zika Pipeline (EX1143), Srivastava (EX1163), Baronti (EX1160), the H/PF/2013 Sequence (EX1019), and Thomas (EX1164)
The Zika virus vaccine according to claim 2, wherein said MN50 is greater or equal to 90.	See discussion for claim 2, above.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Respectfully submitted,



Dan H. Barouch, M.D., Ph.D.

Date: December 15, 2022