Notes on Zika Manufacturing Capacity

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On February 1st, 2016, the World Health Organization (WHO) had declared the Zika virus to be a "public health emergency of international concern".[1] Only 9 month earlier the first reports of Zika virus being transmitted locally, that is via mosquitoes, were emerging from Brazil.[2] The Zika virus carrying mosquitos had quickly reached American shores and by July 2016 the CDC had linked four new Zika cases to local mosquito transmission in Florida.[3] To date there have been 220 locally acquired cases and 4781 travel- associated cases reported in the US.[4] In American territories (ie: Puerto Rico, American Samoa and the US Virgin Islands) the number of locally acquired infections are a staggering 36,498 cases.

The Zika virus is a pathogen from the *Flaviviridae* family. *Flaviviridae* viruses can cause serious illness and include yellow fever virus, japanese encephalitis virus, hepatitis C virus (HCV) and the emerging dengue virus. Although mosquitoes are the primary vector for the Zika virus, it can also be transmitted from person-to-person through sexual contact or from mother-to-child *in utero*. About 80% of individuals infected by Zika are asymptomatic and in rare instance they can develop Guillain–Barré syndrome.[5] When symptoms do occur they present as low grade fever, joint pain, rash, headache and conjunctivitis (pinkeye).[6] However, the most devastating consequence of this viral infection is the development of microcephaly in babies born from women who contracted Zika while pregnant. This birth defect often results in infants having smaller or abnormal brains and partially collapsed skull that can greatly affect the child's proper development, induce seizures, cause intellectual disabilities and limit motor skills.

Currently there are no treatment or vaccines for Zika. However, like HCV, Zika has a positive-sense single-stranded RNA genome and requires an RNA-dependent RNA polymerase (RdRp) to complete its replication cycle. HCV and Zika virus share 80% RdRp sequence homology and scientist found that sofosbuvir inhibited the zika replication in various cell types.[7] Sofosbuvir could be a promising therapeutic candidate for Zika, but ultimately developing a zika vaccine is key to curbing the epidemic. Currently there are at least 28 entities working on different Zika vaccines candidates that are at various stages of developments (table 1). The US government is funding the development of 4 candidates though the National Institutes of Allergy and Infectious Diseases (NIAID) and through the department of defence (DoD). The army's ZPIV vaccine and the NIAID DNA vaccine both

[1] http://www.who.int/emergencies/zika-virus/history/en/
[2] http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=30075=en%20%28accessed%2002%20Feb%202016%29
[3] https://www.cdc.gov/media/releases/2016/p0729-florida-zika-cases.html
[4] https://www.cdc.gov/zika/intheus/maps-zika-us.html (accessed Feb 9)
[5] Lin HH, Huang LM, Wu SC. Zika Virus Molecular Biology and Perspectives for Vaccine Development: A Review. J Nurs Res. 2017 Feb;25(1):3-6.
[6] https://www.cdc.gov/zika/symptoms/symptoms.html
[7] Sacramento CQ *et al*. The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. Sci Rep. 2017 Jan 18;7:40920.

exhibited very promising results in preclinical and animal studies and are currently undergoing clinical trials.

Table 1: Zika virus vaccine candidates (from Lin *et al.*)[5]

Developers	Platform	Antigen	Trial phase
Bharat Biotech	Inactivated	Whole virus	Non-clinical
Bio-Manguinhos / Fiocruz	Recombinant vaccine vector	PrM/E and PrM/E/NS1	Non-clinical
	Inactivated	Whole virus	
	Recombinant subunit (non VLP)	E protein	
	Recombinant subunit VLP (non-fusion)	E protein	
Butantan	Inactivated	Whole virus	Non-clinical
	Live, attenuated target organism	Whole virus	
GeneOne/InOvio	DNA	prME	Phase 1
GeoVax/University of Georgia/CDC	Recombinant viral vector	PrME+NS1	Non-clinical
Hawaii Biotech, Inc.	Recombinant subunit (non VLP)	E protein	Non-clinical
Institut Pasteur Shanghai, Shanghai, China	Recombinant subunit VLP (non-fusion)		Non-clinical
Institut Pasteur, Paris, France	Recombinant vaccine vector	preM/E	Non-clinical
NewLink Genetics	Inactivated	All structural antigens	Non-clinical
	Recombinant subunit VLP (fusion)	prM-E	
NIAID Intramural	Live, attenuated target organism	ZIKV prME	Non-clinical
	Live, attenuated target organism	ZIKV full genome	
Protein Sciences/Sinergium Biotech/ Lab Liomont /UMN	Recombinant subunit (non VLP)	E protein	Non-clinical
Replikins, Ltd and LLC	Peptide	Synthetic peptides	Non-clinical
Sanofi Pasteur	Recombinant viral vector	Zika structural proteins	Non-clinical
Themis Bioscience GmbH	Recombinant vaccine vector		Non-clinical
Valera (Moderna)	mRNA	prME	Non-clinical
Valneva	Inactivated	Whole virus	Non-clinical
Vaxart	Recombinant vaccine vector	Env+	Non-clinical
VRC/NIAID	DNA	PrM and E	Phase 1
WRAIR / BIDMC/Harvard / NIAID / Sanofi Pasteur	Inactivated	Whole virus	Non-clinical

Note. CDC= Centers for Disease Control and Prevention; NIAID= National Institute of Allergy and Infectious Diseases; UMN= University of Minnesota; VRC= Vaccine Research Center; WRAIR=; BIDMC= Beth Israel Deaconess Medical Center; VLP=; prM= pre-membrane; E= envelope genes; NS1= NS1 influenza protein (a viral nonstructural protein); ZIKV=Zika virus; Env= envelope.

The DoD candidate is a purified inactivated virus (PIV) and the authors describe how to produce the vaccine in their August 2016 Nature publication entitled “Vaccine protection against Zika Virus from Brazil”.

> “The ZIKV purified inactivated virus (PIV, also termed ZPIV) vaccine was produced at the Pilot Bioproduction Facility, Walter Reed Army Institute of Research, Silver Spring, MD, USA. The PIV vaccine was based on the Puerto Rican PRVABC59 isolate, which was obtained from the US Centers for Disease Control and Prevention, Fort Collins, CO, USA. The Vero cells used for passage and vaccine production were a derivative of a certified cell line manufactured at The Salk Institute, Swiftwater, PA. After inoculation, virus was collected on days 5 and 7, clarified by centrifugation and depth filter (0.45–0.2 μ m), and treated with benzonase. The viral harvest was concentrated with an ultrafilter followed by purification using Captocore chromatography resin. The purified ZIKV was then inactivated with formalin (0.05%) at 22 °C for 7 days. Following inactivation, formalin was removed by dialysis, and the antigen concentration was adjusted. The final PIV vaccine was assessed for

infectivity by passage in Vero cells followed by plaque assays to demonstrate inactivation." [8]

This protocol is based on one that was developed by the army to produce IXIARO, an inactivated whole-virus Japanese encephalitis vaccine. Vaccines based on purifying a microorganism followed by inactivating them, are among the oldest types of vaccines we still use.

The concept of using inactivated pathogens as prophylaxis was first tried in 1886 by killing cholera virus and immunizing pigeons.[9] The earliest immunization attempt in humans with an inactivated pathogen was in 1897 using typhoid, but the key element to routinely producing such vaccines *in vitro* was discovered by Drs. Enders and Weller in developing techniques for growing poliovirus in cell culture.[9] They were awarded a Nobel prize in 1954 for their work and since, the underlying procedures to developing and producing inactivated viral vaccines haven't derived much from the foundations Enders and Weller established. Though today, techniques are more efficient and the resulting vaccines have a significantly better safety profiles due to more robust inactivation methods.

Generally, the biggest roadblock to developing an inactivated vaccine, is finding a reliable way to grow stocks of the virus in the lab. This would involve finding the proper organism to grow the virus so that is can be harvest, inactivated and turned into a vaccine. Ideally, researchers would try to grow the virus in a cell line, since this it is the most cost effective solution. Unfortunately some viruses are difficult to grow in cell culture rendering the whole process very time consuming and tedious. This is the case for influenza, for example, since it has to be manufactured in fertilized chicken eggs! Fortunately ZPIV can be grown in a standard cell line called vero cells.

Because these techniques are straightforward and relatively standard in a virologist's tool box, many drug companies would be capable of manufacturing inactivated whole virus vaccines, provided they had access to the particular parameters needed to effectively produce the vaccine and test its inactivation. In the United states there are at least 158 companies and facilities that manufacture different types of vaccines (Table 2).

Table 2: Vaccine companies in the USA (adapted from biopharmguy)[10]

Company/ Facilities	Location	Specialty
IGNITE Immunotherapy	Alameda, CA	Oncolytic Virus Vaccines
Dynavax Technologies	Berkeley, CA	DNA Therapeutics
Gritstone Oncology	Emeryville, CA	Personalized cancer vaccines
Novartis Diagnostics	Emeryville, CA	Small Molecules, Vaccines, Biologics
Entest Biomedical	La Mesa, CA	Immuno-therapeutic treatments that address illnesses and maladies in both veterinary and human medicine.
CardioVax	Los Angeles, CA	Small Molecules

[8] Larocca RA et al. Vaccine protection against Zika virus from Brazil.Nature. 2016 Aug 25;536(7617):474-8.
[9] Barbara Sanders, Martin Koldijk, Hanneke Schuitemaker. Vaccine Analysis: Strategies, Principles, and Control. Chapter: Inactivated Viral Vaccines. pp 45-80. Springer-Verlag. 2015
[10] http://biopharmguy.com/links/company-by-location-vaccines.php

ImmunoScience	Pleasanton, CA	HIV diagnostic, vaccine
Juvaris	Pleasanton, CA	Vaccines
Tolerion	Portola Valley, CA	DNA Vaccines for Autoimmune Disorders
Bavarian Nordic	Redwood City, CA	Vaccines
PaxVax	Redwood City, CA	Vaccines
Activate Immunotherapy	San Diego, CA	Biologic Vaccines, small molecules
Adamis Pharmaceuticals	San Diego, CA	Specialty Pharmaceuticals
BioMedicure	San Diego, CA	Cancer Vaccines, Cancer Research Products
Inovio Pharmaceuticals	San Diego, CA	DNA Vaccines
MabVax Therapeutics	San Diego, CA	Small Molecules
MediGene	San Diego, CA	Small Molecules, Oncolytic Viruses
Novartis	San Diego, CA	Small Molecules, Vaccines, Biologics
PaxVax	San Diego, CA	Vaccines
Pfenex	San Diego, CA	Biosimilars, Vaccines
Polynoma	San Diego, CA	Melanoma Vaccine
Vical	San Diego, CA	DNA Delivery, Vaccines
Galena Biopharma	San Ramon, CA	Cancer Vaccines
Meissa Vaccines	South SF, CA	Vaccines
Pfizer	South SF, CA	Biologics
SutroVax	South SF, CA	Conjugate vaccines and protein-based vaccines
Vaxart	South SF, CA	Oral Vaccines
Altravax	Sunnyvale, CA	Vaccines
Novartis	Vacaville, CA	Small Molecules, Vaccines, Biologics
ImmunoCellular Therapeutics	Woodland Hills, CA	Cancer Antigen Vaccines
Sandoz (Novartis)	Broomfield, CO	Small Molecules, Vaccines, Biologics
Vivaldi Biosciences	Fort Collins, CO	Live-Attenuated Flu Vaccines
Celldex Therapeutics	Branford, CT	Small Molecules, Biologics, Vaccines
Protein Sciences	Meriden, CT	Biologics, Vaccines
EpitoGenesis	Storrs, CT	Vaccine Delivery Technologies
Nanotherapeutics	Alachua, FL	Small Molecules, Bone Grafts, Vaccines
TapImmune	Jacksonville, FL	Vaccines
Aurora Biopharma	Miami, FL	Immunotherapeutic treatments
Opko Health	Miami, FL	Biologics, Vaccines, Diagnostics, etc
GeoVax	Atlanta, GA	Vaccines
Inhibikase Therapeutics	Atlanta, GA	Therapeutic Vaccines
Novartis	Duluth, GA	Small Molecules, Vaccines, Biologics
GeneCure Biotechnologies	Norcross, GA	Gene Transfer Technology
CSL Behring	Bradley, IL	Biologics
Pfizer	Andover, MA	Small Molecules, Vaccines, Biologics
Antera Therapeutics	Boston, MA	Peanut Allergy Preventative
Matrivax Research & Development	Boston, MA	Encapsulation of Bacterial Pathogens

Merck	Boston, MA	Small Molecules, Vaccines, Biologics
TremRx	Boston, MA	Vaccines
Affinivax	Cambridge, MA	Vaccine Discovery Technology
Aurora Biopharma	Cambridge, MA	Immunotherapeutic treatments
Genocea Bioscience	Cambridge, MA	Vaccines
Gritstone Oncology	Cambridge, MA	Personalized cancer vaccines
ImmusanT	Cambridge, MA	Peptide therapeutic vaccine
Inhibikase Therapeutics	Cambridge, MA	Therapeutic Vaccines
Medvax Technologies	Cambridge, MA	Cancer vaccines
Neon Therapeutics	Cambridge, MA	Vaccines & T cell therapies
Novartis	Cambridge, MA	Small Molecules, Vaccines, Biologics /Diagnostics
Sanofi Pasteur	Cambridge, MA	Biologics, Vaccines
Sanofi Pasteur	Cambridge, MA	Biologics, Vaccines
Vaxxas Nanopatch	Cambridge, MA	Vaccine patch delivery system
VBI Vaccines	Cambridge, MA	Vaccine Development
Sanofi Pasteur	Canton, MA	Biologics, Vaccines
Alopexx Vaccine (Alopexx)	Concord, MA	Vaccine
Celldex Therapeutics	Fall River, MA	Small Molecules, Biologics, Vaccines
Berg	Framingham, MA	Small Molecules
Agenus	Lexington, MA	Biologics
Xenetic Biosciences	Lexington, MA	biologic drugs, novel oncology therapeutics and vaccinations
Celldex Therapeutics	Needham, MA	Small Molecules, Biologics, Vaccines
OncoPep	North Andover, MA	Cancer vaccine
Selecta Biosciences	Watertown, MA	Synthetic Vaccine Particles
Antigen Express (Generex Biotechnology)	Wellesley, MA	Biologics
Opko Health	Woburn, MA	Biologics, Vaccines, Diagnostics, etc
PharmAthene	Annapolis, MD	Biological and Chemical Weapon Defense
Emergent BioSolutions	Baltimore, MD	Biologic Vaccines
PathoVax	Baltimore, MD	HPV vaccine
Sanofi Pasteur	Bethesda, MD	Biologics, Vaccines
Biofactura	Frederick, MD	Biosimilar production technology, smallpox drug
Biological Mimetics	Frederick, MD	Vaccines, Therapeutic Antibodies Discovered by "Immune Dampening and Refocusing"
Medigen	Frederick, MD	Cancer & Infectious disease vaccines, contract services
MedImmune (AstraZeneca)	Frederick, MD	Small Molecules, Vaccines, Biologics
Altimmune	Gaithersburg, MD	Biologics, Vaccines, Drug Delivery
Emergent BioSolutions	Gaithersburg, MD	Biologic Vaccines
MedImmune (AstraZeneca)	Gaithersburg, MD	Small Molecules, Vaccines, Biologics
Valneva	Gaithersburg, MD	Vaccines (Marketing)
Wellstat Group	Gaithersburg, MD	Group of Many Small Drug and Diagnostic Companies
Biologics Resources	Germantown, MD	Vaccines, Biologics

Neuralstem	Germantown, MD	Stem Cell Technology
SynAm Vaccine	Germantown, MD	Pneumococcal vaccine
Emergent BioSolutions	Rockville, MD	Biologic Vaccines
GlaxoSmithKline	Rockville, MD	Small Molecules, Vaccines, Biologics
Immunomic Therapeutics	Rockville, MD	Vaccines (Lysosomal Associated Membrane Protein)
Integrated BioTherapeutics	Rockville, MD	Vaccines
Sanaria	Rockville, MD	Malaria Vaccine, Reagents for malaria research
NanoBio	Ann Arbor, MI	Topical, Mucosal Vaccines and Therapies
Emergent BioSolutions	Lansing, MI	Biologic Vaccines
GlaxoSmithKline	St. Louis, MO	Small Molecules, Vaccines, Biologics
Immunophotonics	St. Louis, MO	Autologous cancer vaccine
Sequoia Sciences	St. Louis, MO	Antibacterial Small Molecules, Vaccines for UTI
Takeda	Bozeman, MT	Biologics, Vaccines
Heat Biologics	Chapel Hill, NC	Therapeutic Vaccines
Liquidia Technologies	Morrisville, NC	Particle-Based Therapies
GlaxoSmithKline	Research Triangle Park, NC	Small Molecules, Vaccines, Biologics
Annias Immunotherapeutics	RTP, NC	Therapeutic Brain Tumor Vaccine
Pfizer	Sanford, NC	Small Molecules, Vaccines, Biologics
Sandoz (Novartis)	Wilson, NC	Small Molecules, Vaccines, Biologics
NovaDigm Therapeutics	Grand Forks, ND	Fungal & Bacterial Infection Vaccines
Novartis	Lincoln, NE	Small Molecules, Vaccines, Biologics
VaxInnate	Cranbury, NJ	Vaccines
Novartis	East Hanover, NJ	Small Molecules, Vaccines, Biologics
Sandoz (Novartis)	East Hanover, NJ	Small Molecules, Vaccines, Biologics
Celldex Therapeutics	Hampton, NJ	Small Molecules, Biologics, Vaccines
Pfizer	Madison, NJ	Small Molecules, Vaccines, Biologics
Advaxis	North Brunswick, NJ	Listeria Vaccine
GlaxoSmithKline	Parsippany, NJ	Small Molecules, Vaccines, Biologics
Novartis	Parsippany, NJ	Small Molecules, Vaccines, Biologics
Emergent BioSolutions	Princeton, NJ	Biologic Vaccines
Sandoz (Novartis)	Princeton, NJ	Small Molecules, Vaccines, Biologics
Merck	Rahway, NJ	Small Molecules, Vaccines, Biologics
Akorn	Somerset, NJ	Biologics, Vaccines
Agilvax	Albuquerque, NM	VLP vaccines
United Biomedical	Hauppauge, NY	Monoclonals, Vaccines, Biosimilars
DanDrit Biotechnology	New York, NY	Cancer Vaccines
Intellect Neurosciences	New York, NY	Vaccines Discovery platform
Pfizer	New York, NY	Small Molecules, Vaccines, Biologics
Pfizer	Pearl River, NY	Small Molecules, Vaccines, Biologics
Alpha-1 Biologics	Stony Brook, NY	small molecules, peptides, diagnostic, vaccine
Codagenix	Stony Brook, NY	Live-attenuated vaccine design technology

TechnoVax	Tarrytown, NY	VLP vaccines
Najit Technologies	Beaverton, OR	Vaccines
TomegaVax	Beaverton, OR	Cytomegalovirus-based vaccines
UbiVac	Portland, OR	Vaccine/Immunotherapy
GlaxoSmithKline	Collegeville, PA	Small Molecules, Vaccines, Biologics
Pfizer	Collegeville, PA	Small Molecules, Vaccines, Biologics
GlaxoSmithKline	Conshohocken, PA	Small Molecules, Vaccines, Biologics
Immunotope	Doylestown, PA	Immunotherapy for cancer and chronic viral infections
Immunomic Therapeutics	Hershey, PA	Vaccines (Lysosomal Associated Membrane Protein)
CSL Behring	King of Prussia, PA	Biologics
GlaxoSmithKline	King of Prussia, PA	Small Molecules, Vaccines, Biologics
Merck	Lansdale, PA	Small Molecules, Vaccines, Biologics
GlaxoSmithKline	Marietta, PA	Small Molecules, Vaccines, Biologics
GlaxoSmithKline	Montgomery, PA	Small Molecules, Vaccines, Biologics
Merck	North Wales, PA	Small Molecules, Vaccines, Biologics
Avax Technologies	Philadelphia, PA	Vaccines
GlaxoSmithKline	Philadelphia, PA	Small Molecules, Vaccines, Biologics
Inovio Pharmaceuticals	Plymouht Meeting, PA	DNA Vaccines
Molecular Targeting Technologies (MTTI)	West Chester, PA	Diagnostics & Therapeutics
Berg	Nashville, TN	Small Molecules
Vitruvian Biomedical	Addison, TX	Alzheimer's vaccine, diagnostics, enzyme-detection device
Bellicum Pharmaceuticals	Houston, TX	Cellular Therapy
Merck	Elkton, VA	Small Molecules, Vaccines, Biologics
Cascadian Therapeutics	Seattle, WA	Small Molecules & Immunotherapy
EpiThany	Seattle, WA	Cancer Vaccines
Etubics	Seattle, WA	Targeted immunotherapies and vaccine products
Immune Design	Seattle, WA	DNA Vaccines
TRIA Biosciences	Seattle, WA	Vaccines
FluGen	Madison, WI	Vaccines & Adjuvants
Madison Vaccines	Madison, WI	DNA vaccines, companion diagnostic