APPLICATION OF REVERSE GENETICS IN RNA VIRUS VACCINE DEVELOPMENT: A BRIEF REVIEW

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Abstract— **RNA viruses can quickly spread and cause serious, even fatal, illnesses in both humans and animals. Platforms for creating and refining viral mutants for vaccine development have been made available by the introduction of reverse genetics methods for manipulating and studying the genomes of RNA viruses. In this article, we review the effects of RNA virus reverse genetics systems on previous and ongoing initiatives to develop efficient and secure viral treatments and vaccines.**

Keywords— **RNA virus, Vaccine, cDNA, Reverse Genetics**

I. INTRODUCTION

One of the greatest achievements of human creativity, scientific research, and the joint international efforts of the public health community is the development of vaccines. When compared to pre-vaccination rates, the incidence and fatality rates of RNA virus infections including polio, measles, mumps, and rubella have decreased by more than 95% (1). Conventional methods for developing RNA virus vaccines, notwithstanding their historical effectiveness, such as live-attenuation through passaging (forward genetics) or inactivation, may be less effective at producing high-quality candidates than rational targeted mutagenesis (reverse genetics). The generation of vaccines is made easier by targeted alterations and directed attenuation by advancements in recombinant DNA technology and virus reverse genetics, which has revealed important insights into the replication and pathogenicity of RNA viruses. By enabling the investigation of specific genetic alterations in virus genomes, reverse genetics and molecular engineering of viruses have revolutionized the field of virology. The first infectious RNA viral clone to produce poliovirus was discovered in 1981 using cDNA (2). Since then, reverse genetic clones representing all significant virus families have been produced using reverse genetics technology and recombinant virus design. Additionally, these methods and strategies are now the main focus of new initiatives to develop vaccines that combine particular adjustments to the virus- or component-based systems to produce long-lasting immunity in the host without harmful side effects or hazards. Since the conception of successful live-attenuated vaccines, the field of vaccine discovery and development has been shaped by new vaccine preparations utilizing suitable vectors, expression of particular viral proteins or components (subunit vaccines), and the creation of virus-like particles (VLPs). The difficulty of developing a secure, immunogenic platform that promotes long-lasting immunity in the presence of numerous viral systems has led to a remarkable amount of inventiveness and variation in the methods used. High immunogenicity, cheaper costs, and ease of transport and administration are all advantages of using replicating viruses in vaccines, such as live-attenuated or chimeric vector-based platforms. But in immunocompromised hosts, these viruses may not be sufficiently attenuated and may revert to more dangerous characteristics. Subunit, attenuated, or killed microorganism vaccines, on the other hand, offer the advantages of being typically safer and having the ability to provide the most immunogenic components. The expenses, length of development, and lower induced immune responses, however, bring their own difficulties in terms of planning and execution. We explain RNA virus reverse genetics systems and give a summary of current initiatives to apply reverse genetics technology in the creation of secure and reliable vaccines in this review.

II. BIOLOGY OF RNA VIRUS & REVERSE GENETICS DESIGN

In RNA virus reverse genetics, First, RNA-dependent RNA polymerase (RdRp) that is active in RNA viruses produces genomic copies. In addition, almost all RNA virus replication mechanisms lie in the cytoplasm rather than the nucleus of the host cell. For positive-strand RNA viruses, host ribosomes quickly translate the genomic RNA to produce viral protein products following entry and uncoating. Positive-sense RNA virus reverse genetics systems mainly concentrate on the delivery of either delivery of cDNA under the control of a viral transcription promoter such as T7 or CMV or transcribed genomic RNA into the cell cytoplasm because the virus rarely needs to package additional non-structural proteins in the virion (3-10). However, the introduction of the RdRp and other crucial proteins to start genomic replication is frequently necessitated by the employment of extra helper constructions

in negative-strand and double-strand RNA virus reverse genetic systems. Utilizing host polymerases like Pol I and Pol II to synthesize viral RNA and promote the generation of host mRNA is a more modern alternative strategy that has been used in influenza research (11-12). This method has improved the efficiency of recovering recombinant viruses while streamlining the logistics of plasmid transfection (13). The bacterial artificial chromosome (BAC) is another tool that has been used to create infectious RNA virus clones. BAC constructs are single-copy DNA plasmids based on the F-plasmid of bacteria that can insert large DNA segments and allow them to be transcribed under the direction of a transcriptional promoter like T7. These constructs are genetically stable in E. coli. Few RNA viruses alter host gene expression and result in oncogenesis because the majority of RNA virus replication techniques are separated from the host genome and replication apparatus. As a result, host cell transformation is often not taken into consideration by RNA reverse genetic methods. Then again due to decreased genomic stability and decreased fidelity and proofreading activity of the viral RdRp, the majority of RNA viruses have tiny genome sizes, with the majority fewer than 15 kb. Due to their higher versatility in manipulating and changing the virus genome, cDNA genetic clones are frequently used in reverse genetic techniques (14-20).

III. BIOLOGY OF POSITIVE STRAND RNA VIRUS & VACCINE DEVELOPMENT

The genomes of positive-strand RNA viruses are contagious as soon as they enter host cells. Viral RNA is translated into one or more polyproteins by host ribosomes upon entry, which is then processed by either host or viral proteases (21-24). Positive-strand RNA virus clones have been produced using a variety of reverse genetics platforms, but almost all of them aim to incorporate sense genomic RNA transcripts directly or cDNA for transcription by an integrated RdRp. Here, we discuss methods used to create picornavirus and flavivirus infectious clones, as well as how these methods have been and are still being used to create vaccines (25).

1.1. Picornaviruses
A group of non-enveloped, positive-strand RNA viruses known as picornaviruses infects a variety of human and animal hosts. Illnesses caused by human picornaviral pathogens range from the common cold to poliomyelitis. The RNA genome of the picornavirus is surrounded by an icosahedral capsid structure. As two of the most thoroughly investigated picornaviruses to date, poliovirus and rhinovirus serve as model systems for the investigation of RNA virus biology, pathology, and epidemiology. Since there are no non-human primate reservoirs known to exist in nature, significant efforts were made to create and execute a polio vaccine in the 1950s with the goal of eradicating the disease. Increased attempts were made to create and design vaccines for numerous other human infections as a result of the efficacy of the inactivated

(Salk) and live-attenuated (Sabin) vaccines (26). The goal of global immunization campaigns is to eradicate the illness. The development and use of poliovirus vaccines with great success allowed for the evaluation of novel ideas and ways to research and comprehend the biology of RNA viruses. Racaniello and Baltimore's 1981 introduction of a full-length poliovirus cDNA clone and subsequent recovery of infectious virus was a significant advancement (2). Modern reverse genetics techniques for recovering picornaviruses mostly use cDNA-based systems, which are more effective and attributable to developments in the knowledge of and creation of plasmids for gene delivery. Theiler's virus [27], Hepatitis A virus [26], Foot and Mouth Disease virus [28], Swine Vesicular Disease virus [29], and two Echoviruses [30,31] are just a few of the picornaviruses for which reverse genetics techniques have been developed.

Since they were discovered more than 50 years ago, rhinoviruses continue to be the principal cause of the common cold throughout the world. However, overcoming the challenging issue of creating an immunogenic platform that can confer protective immunity to the more than 100 rhinovirus serotypes that are currently prevalent in nature is the key to generating a broadly protective rhinovirus vaccine (32-33). Four different serotype-specific proteins (VP1, VP2, VP3, and VP4) that make the rhinovirus capsid are known to be immunogenic and may cause cross-reactive antibodies (32,34-36). However, creating a polyvalent vaccination with different serotype variants of the immunogenic capsid proteins is likely necessary in order to produce broadly-neutralizing rhinovirus protection (36). The broad immunogenic memory required for effective polyvalent rhinovirus vaccine formulations has not yet been achieved. However, it has been discovered that certain areas of VP0 (the precursor to VP2 and VP4) are conserved throughout A and B group rhinoviruses, and when combined with a TH1-promoting adjuvant, mice had a cross-serotype immunological response (34). The recent development of chimeric or new reverse genetics platforms, which includes a mouse model of infection, and the discovery of conserved epitopes may open up a fresh path for the effective presentation of epitopes from the diversity of rhinoviruses (37-39).

1.2. Flaviviruses
Positive-strand RNA viruses called flaviviruses are enveloped and small that infect a variety of hosts (40-52). Most flaviviruses are transmitted by arthropod vectors because their genomes are significantly smaller which is 10–12 kb in size. Asymptomatic to severe neurological conditions such as encephalitis, meningitis, and myelitis are all caused by flaviviruses (53). The first human viral disease to be identified was the Yellow Fever Virus (YFV), a lethal flavivirus that causes over 30,000 deaths annually (according to the WHO) (54–56). The significance of creating reverse genetic platforms and effective vaccinations has been highlighted by outbreaks and sicknesses linked to flaviviruses. Similar to picornaviruses,

almost all flavivirus reverse genetics platforms use a bacterial artificial chromosome (BAC) platform or in vitro transcription of full-length or ligated cDNA segments of the genome, which are then transfected or electroporated into suitable cells. Many flaviviruses, including YFV (57,58), Dengue Types 1-4 (59-66), JEV (67), Kunjin virus (68), Tick-borne encephalitis virus (TBEV) (69-71), Langat virus (72, 73), West Nile virus (WNV) (6,74), have been the subject of reverse genetics platforms. Recombinant flavivirus technology advancements have been crucial to efforts to develop novel vaccines (75). Developing inactivated or live-attenuated strains for vaccines against flaviviruses was one of the early vaccine initiatives. Using a serially passaged virus that was originally isolated from an African patient, Max Theiler created the safe YFV live-attenuated vaccine 17D in 1937 (76). The current vaccine composition is virtually unchanged from that which was initially created more than 70 years ago, and it offers protective protection for more than 30 years (77–79). JEV was discovered and researched for the first time in the 1930s, and the first inactivated vaccine made from mouse brain was created in Japan in 1954 (53,80).

Worldwide, the dengue virus is endemic to tropical and subtropical regions. There are currently five different dengue virus serotypes known, one of which was discovered in 2013 (53,81). When a secondary infection occurs with a different serotype after an initial infection with one serotype, the illness becomes more severe (82). Therefore, any dengue virus vaccine must either offer protection against all serotypes to the fullest extent possible to prevent setting the stage for an increase in disease from a heterotypic infection, or it must get rid of the immunogenic parts of the virus that lead to an increase in disease severity following heterotypic infections.

IV. BIOLOGY OF NEGATIVE STRAND RNA VIRUS & VACCINE DEVELOPMENT

Genomes and antigenomes of negative-strand RNA viruses are unable to function as mRNA. They must form ribonucleoprotein complexes and be encapsidated with nucleocapsid in order to be a substrate of RdRp (RNPs) (83-85). The antigenomes of negative-strand RNA viruses are generally co-transfected with one or more plasmids containing the nucleocapsid and replicase machinery. They are delivered either as a linearized cDNA or as a plasmid under a T7 promoter. Negative-strand RNA virus reverse genetics systems have developed more slowly than positive-strand viral systems as a result of these biological prerequisites for starting an infection (86). Initially, helper viruses were used to recover viruses in order to create infectious clones because they could provide the viral genes and proteins required for replication. Isolating the desired mutants was challenging with these methods, though. In 1994, the rabies virus was successfully recovered completely from cDNA for the first time (87,88). Despite the biological constraints and difficulties associated with working with negative-strand RNA viruses, a number of successful negative-strand RNA virus vaccines have been developed, and many more are currently undergoing clinical trials at various stages.

1.3. Paramyxoviruses

Numerous illnesses affecting humans and animals are brought on by paramyxovirus which is enveloped, single-stranded RNA viruses with a negative sense. There are known human paramyxoviruses that cause the common cold, measles, mumps, pneumonia, and other illnesses. The genome of a paramyxovirus, which is normally between 15 and 19 kb long, is only carried in a single copy. Paramyxoviruses must insert their replication apparatus, including the RdRp, into the virion during assembly, just like other negative-strand RNA viruses do. Very similar methods are used in the reverse genetic systems of paramyxoviruses. First, a promoter like T7 RNA polymerase is used to clone the full-length genome or antigenome as cDNA, together with helper plasmids that express nucleocapsid and polymerase proteins. Co-transfection of the plasmids into permissive cell lines. However, the majority of contemporary reverse genetics techniques use a T7 cell line or transfect a T7 plasmid. Earlier reverse genetics systems used a co-infection approach with a vaccinia virus-producing T7. Respiratory syncytial virus (RSV) was recently the subject of the first BAC-based reverse genetics system for a negative-strand RNA virus (21). For various paramyxoviruses, including the measles virus (89), mumps virus (90), Hendra virus (91), Nipah virus (92), and RSV (21,93), reverse genetics techniques have been developed. The success of developing paramyxovirus vaccines has varied despite the availability of reverse genetics technologies. In the 1950s, the first paramyxovirus vaccine was developed. A measles virus cultivation system was developed by John Enders, who also cultured the Edmonston strain of an attenuated measles virus (named after the child from which it was isolated). Despite being originally under-attenuated, the Edmonston strain was later modified and helped to successfully license a measles vaccine in 1963 (94). Building on the success of the live-attenuated measles vaccine, Maurice Hilleman was able to cultivate and modify a strain of the mumps he named Jeryl Lynn after his daughter, from whom it was isolated (95). Hilleman later contributed to the creation of the Wistar RA 27/3 strain of the MMR vaccine, a live-attenuated combination vaccination that was originally approved for use in 1971 (96). New efforts to create vaccinations against other deadly RNA viruses have been made in response to the early success of the measles and mumps vaccines. The creation of a vaccine for the respiratory syncytial virus continues to be one of the biggest challenges in the design of viral vaccines (RSV). The first chimpanzee with upper respiratory disease and the presence of respiratory syncytial virus (RSV) was discovered in 1955 (97). Since its discovery, RSV has been acknowledged as the virus that kills most infants globally [98,99]. Over 100,000 hospitalizations a year are solely attributable to RSV upper and lower respiratory infections in the US (98–100]). Despite a significant clinical

burden, there are currently no authorized RSV vaccines, and the only existing therapies are expensive and only provide passive immunity by giving prophylactic antibodies (101–103). Young newborns are the population most vulnerable to RSV infection (98). The optimal RSV vaccine must therefore be immunogenic, genetically stable, and suitable for use in young children.

1.4. Orthomyxoviruses
Orthomyxoviruses are enveloped, negative-sense RNA viruses with genomes that are 12 to 15 kb in size and made up of several linear segments. Similar to paramyxoviruses, numerous orthomyxoviruses, most notably avian influenza and swine influenza, cause respiratory infections in humans and animals. The co-infection of a cell allows for the reassortment of the virus genomes, which is a crucial aspect of orthomyxovirus evolution. Influenza viruses require a functionally active viral ribonucleoprotein complex (RNP), which is made up of the viral genomic RNA, nucleoprotein (NP), and viral RdRp, in order to be transcriptionally active (comprised of PB1, PB2, and PA proteins) (104-112). For the influenza A virus (IAV), the first reverse genetics techniques were created, but these methods used helper viruses that had to be rebuffed in order to retrieve recombinants. Around the turn of the millennium, the first helper virus-free systems were developed (113,114). These initial systems needed co-transfection of eight plasmids expressing the eight viral RNA segments along with four or more plasmids under the control of a Pol II promoter. Depending on the number of viral genomic segments and how the helper protein constructs were organized, different numbers of plasmids had to be co-transfected for recovery, ranging from 10 (which expressed two helper proteins on each of two plasmids using the Pol II promoter and an IRES) to as many as 17 (113-118). However, employing a bidirectional expression system, recent developments in reverse genetics platforms have decreased the total number of plasmids required to 8 or less (11, 12). In this system, viral RNA synthesis is driven by a human Pol I promoter in conjunction with either a murine Pol I terminator or a CMV Pol II promoter, while viral mRNA synthesis is driven by the CMV Pol II promoter (11). However, despite this increased IAV production, only a small number of mammalian cell lines, such as the Vero or Madin-Darby canine kidney (MDCK) cell lines, can produce recombinant viruses for vaccines (119,120). The effectiveness of both uni- and bidirectional methods, however, has been hampered by the poor transfectability of certain cell lines and discrepancies in Pol I and Pol II compatibility. In several of these cell lines, the incorporation of species-specific polymerase promoters has boosted the effectiveness of recovery (119,120). The combination of up to 8 Pol I driven IAV genes on one plasmid and up to 3 Pol II drive genes on another plasmid has recently been developed; this method increases the likelihood that a single cell will receive all necessary plasmids during recovery (121). Influenza vaccination tactics have been transformed by the development of practical reverse genetics systems for analyzing and determining the structure and function of influenza proteins. The present trivalent vaccination has been around for a while, although it used to contain three distinct strains (2 A strains and 1 B strain), which were chosen based on WHO guidelines prior to the upcoming flu season. However, the virus used to produce these vaccines were either inactivated or live-attenuated (via cold adaptation). Tetravalent or quadrivalent vaccines, which may be generated in animal cell cultures rather than chicken eggs, or virus-like particles (VLPs) in cultures of S. frugiperda insect (Sf9) cells are examples of next-generation immunization techniques (122–129). A seasonal influenza vaccine made of pure HA proteins and created utilizing a baculovirus-expression technology was given FDA approval in 2013 (130). These recombinant vaccines were effectively utilized to immunize people between the ages of 18 and 49, and they represent a significant advancement in influenza vaccine design and implementation because they require less time to produce than the traditional egg-based method (131).

V. REVERSE GENETIC APPROACHES FOR RNA VIRUS VACCINE DEVELOPMENT AND SYNTHETIC BIOLOGY

1.5. Challenges and Limitations
The development of novel vaccines and treatments, as well as the enhancement of the use and efficacy of those already on the market, represent a significant frontier of synthetic biology. The present gap in time between vaccine design and manufacture is a significant obstacle to the effective use of vaccines. Seasonal vaccinations against influenza viruses are necessary, but there is little time for development and deployment when the correct formulations are chosen. Reverse genetics technology has made recent strides that could shorten recuperation and production times from months to weeks. For instance, Dormitzer et. al. has shown that it is possible to create a recombinant influenza virus in just five days using the existing reverse genetics technique (132). Rapid techniques to recovering and adapting viruses will be crucial in the public health response as new emerging viruses continue to emerge and pandemics continue to occur. To improve the effectiveness and timing of delivery during epidemic and pandemic outbreaks, common chimeric reverse genetic platforms for quick cloning and production of surface antigens from emerging diseases and pathogens are being developed. Genetic stability is a significant barrier to the creation of successful live-attenuated vaccines. The lower fidelity of the RdRp causes RNA viruses to often have significant mutation rates. However, recent research has demonstrated that bias in codon usage can be used to change virus translation and, as a result, reproduction. Codon-deoptimization, also known as substituting non-preferred codons based on host cell codon use bias into the poliovirus genome, has been demonstrated by a number of research groups to reduce plaque areas and virus yields (133,134). The amount of viral protein can be adjusted without affecting function by altering codons rather than amino

acids. More importantly, the quantity of codon modifications made to the coding sequence severely restricts the possibility of reversion. This strategy represents a promising new direction for the creation of genetically stable, attenuated vaccination platforms.

VI. CONCLUSION

Despite the fact that many human infections have reverse genetics systems, not all of them have led to effective vaccine platforms. New reverse genetic techniques have been developed as a result of significant advances in biotechnology. These approaches could be used to create next-generation vaccinations. Optimizing antigens for display in vaccinations has become a widespread method known as structural vaccinology or structure-based antigen design (135). RSV continues to be a significant barrier to lowering viral childhood morbidity, as was previously indicated. Much effort has been put into analyzing the antigenic regions of the RSV fusion (F) protein and improving F expression constructs for greater immunogenicity since the discovery and use of palivizumab, the only licensed prophylactic inhibitory approach to RSV infection at the moment (105). The generation and identification of powerful neutralizing antibodies with higher neutralizing potencies than palivizumab have been made possible by current structural vaccinology research to assess the pre-fusion and post-fusion antigenic forms of RSV F protein (13). Predictive structural modeling and structure-based antigen design are now realistic choices for new efforts to create vaccines, for which there are now no vaccinations. This is due to the availability of new structures and sequences. The area of virology has been influenced by biotechnology developments just as much as any other branch of research. The science of vaccine creation has undergone a revolution thanks to the ability to create reverse genetics platforms for the analysis and manipulation of RNA viral genomes. Here, we have discussed the reverse genetics of RNA viruses as well as historical and present efforts to create vaccines to protect against several human RNA virus infections. The construction of next-generation vaccines against pathogens for which we already have vaccination techniques as well as those for which we do not will continue to be fuelled by new developments in reverse genetics technologies, adjuvants, non-human models of infection, and surveillance. Current shifts in human demographics and healthcare accessibility are likely to alter cost-benefit calculations and prompt the allocation of fresh funds for research and vaccine development. High efficacy, safety, and stability are the distinguishing characteristics of an effective viral vaccine, despite the constant innovation of vaccine design and use, and will always be a matter of concern.

Conflict of interest
The authors declared no conflicts of interest.

VII. REFERENCES

[1]. Roush SW, Murphy TV, Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. Jama. 2007 Nov 14;298(18), pp.2155-2163.

[2]. Racaniello VR, Baltimore D. Cloned poliovirus complementary DNA is infectious in mammalian cells. Science. 1981 Nov 20;214(4523), pp.916-919.

[3]. Almazán F, DeDiego ML, Sola I, et al. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. MBio. 2013 Sep 10;4(5):e00650-13.

[4]. Almazán F, González JM, Pénzes Z, et al. Engineering the largest RNA virus genome as an infectious bacterial artificial chromosome. Proceedings of the National Academy of Sciences. 2000 May 9;97(10), pp.5516-5521.

[5]. Scobey T, Yount BL, Sims AC, et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. Proceedings of the National Academy of Sciences. 2013 Oct 1;110(40), pp.16157-16162.

[6]. Shi PY, Tilgner M, Lo MK, Kent KA, Bernard KA. Infectious cDNA clone of the epidemic West Nile virus from New York City. Journal of virology. 2002 Jun 15;76(12), pp.5847-5856.

[7]. Thiel V, Herold J, Schelle B, et al. Infectious RNA transcribed in vitro from a cDNA copy of the human coronavirus genome cloned in vaccinia virus. Journal of General Virology. 2001 Jun 1;82(6), pp.1273-1281.

[8]. Yount B, Curtis KM, Baric RS. Strategy for systematic assembly of large RNA and DNA genomes: transmissible gastroenteritis virus model. Journal of virology. 2000 Nov 15;74(22), pp.10600-10611.

[9]. Yount B, Curtis KM, Fritz EA, et al. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. Proceedings of the National Academy of Sciences. 2003 Oct 28;100(22), pp.12995-13000.

[10]. Yount B, Denison MR, Weiss SR, et al. Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59. Journal of virology. 2002 Nov 1;76(21), pp.11065-11078.

[11]. Hoffmann E, Neumann G, Hobom G, et al. "Ambisense" approach for the generation of influenza A virus: vRNA and mRNA synthesis from one template. Virology. 2000 Feb 15;267(2), pp.310-317.

[12]. Hoffmann E, Webster RG. Unidirectional RNA polymerase I–polymerase II transcription system for the generation of influenza A virus from eight plasmids. Journal of General Virology. 2000 Dec 1;81(12), pp.2843-2847.

[13]. Flatz L, Bergthaler A, de la Torre JC, et al. Recovery of an arenavirus entirely from RNA polymerase I/II-driven cDNA. Proceedings of the National Academy of Sciences. 2006 Mar 21;103(12), pp.4663-4668.

[14]. Yun SI, Kim SY, Rice CM, et al. Development and application of a reverse genetics system for Japanese encephalitis virus. Journal of virology. 2003 Jun 1;77(11), pp.6450-6465.

[15]. Choi YJ, Yun SI, Kang SY, et al. Identification of 5′ and 3′ cis-acting elements of the porcine reproductive and respiratory syndrome virus: acquisition of novel 5′ AU-rich sequences restored replication of a 5′-proximal 7-nucleotide deletion mutant. Journal of virology. 2006 Jan 15;80(2), pp.723-736.

[16]. St-Jean JR, Desforges M, Almazán F, et al. Recovery of a neurovirulent human coronavirus OC43 from an infectious cDNA clone. Journal of virology. 2006 Apr 1;80(7), pp.3670-3674.

[17]. DeDiego ML, Alvarez E, Almazán F, et al. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. Journal of virology. 2007 Feb 15;81(4), pp.1701-1713.

[18]. Suzuki R, de Borba L, dos Santos CN, et al. Construction of an infectious cDNA clone for a Brazilian prototype strain of dengue virus type 1: characterization of a temperature-sensitive mutation in NS1. Virology. 2007 Jun 5;362(2), pp.374-383.

[19]. Fan ZC, Bird RC. An improved reverse genetics system for generation of bovine viral diarrhea virus as a BAC cDNA. Journal of virological methods. 2008 May 1;149(2), pp.309-315.

[20]. Rasmussen TB, Reimann I, Uttenthal Å, et al. Generation of recombinant pestiviruses using a full-genome amplification strategy. Veterinary Microbiology. 2010 Apr 21;142(1-2), pp.13-17.

[21]. Hotard AL, Shaikh FY, Lee S, et al. A stabilized respiratory syncytial virus reverse genetics system amenable to recombination-mediated mutagenesis. Virology. 2012 Dec 5;434(1), pp.129-136.

[22]. Bálint Á, Farsang A, Zádori Z, et al. Molecular characterization of feline infectious peritonitis virus strain DF-2 and studies of the role of ORF3abc in viral cell tropism. Journal of virology. 2012 Jun 1;86(11), pp.6258-6267.

[23]. Usme-Ciro JA, Lopera JA, Enjuanes L, et al. Development of a novel DNA-launched dengue virus type 2 infectious clone assembled in a bacterial artificial chromosome. Virus research. 2014 Feb 13;180, pp.12-22.

[24]. Kandolf R, Hofschneider PH. Molecular cloning of the genome of a cardiotropic Coxsackie B3 virus: full-length reverse-transcribed recombinant cDNA generates infectious virus in mammalian cells. Proceedings of the National Academy of Sciences. 1985 Jul;82(14), pp.4818-4822.

[25]. Mizutani SA, Colonno RJ. In vitro synthesis of an infectious RNA from cDNA clones of human rhinovirus type 14. Journal of virology. 1985 Nov;56(2), pp.628-632.

[26]. Cohen JI, Ticehurst JR, Feinstone SM, et al. Hepatitis A virus cDNA and its RNA transcripts are infectious in cell culture. Journal of virology. 1987 Oct;61(10), pp.3035-3039.

[27]. Roos RP, Stein S, Routbort M, et al. Theiler's murine encephalomyelitis virus neutralization escape mutants have a change in disease phenotype. Journal of virology. 1989 Oct;63(10), pp.4469-4473.

[28]. Zibert A, Maass G, Strebel K, et al. Infectious foot-and-mouth disease virus derived from a cloned full-length cDNA. Journal of Virology. 1990 Jun;64(6), pp.2467-2473.

[29]. Inoue T, Yamaguchi S, Saeki T, et al. Production of infectious swine vesicular disease virus from cloned cDNA in mammalian cells. Journal of general virology. 1990 Aug 1;71(8), pp.1835-1838.

[30]. Blackburn RV, Racaniello VR, Righthand VF. Construction of an infectious cDNA clone of echovirus 6. Virus research. 1992 Jan 1;22(1), pp.71-78.

[31]. Zimmermann H, Eggers HJ, Zimmermann A, et al. Complete nucleotide sequence and biological properties of an infectious clone of prototype echovirus 9. Virus research. 1995 Dec 1;39(2-3), pp.311-319.

[32]. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. Science. 2009 Apr 3;324(5923), pp.55-59.

[33]. Waman VP, Kolekar PS, Kale MM, et al. Population structure and evolution of Rhinoviruses. Plos one. 2014 Feb 19;9(2):e88981.

[34]. Glanville N, Mclean GR, Guy B, et al. Cross-serotype immunity induced by immunization with a conserved rhinovirus capsid protein. PLoS pathogens. 2013 Sep 26;9(9):e1003669.

[35]. Rossmann MG, Arnold E, Erickson JW, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature. 1985 Sep;317(6033), pp.145-153.

[36]. Edlmayr J, Niespodziana K, Popow-Kraupp T, et al. Antibodies induced with recombinant VP1 from human rhinovirus exhibit cross-neutralisation. European Respiratory Journal. 2011 Jan 1;37(1), pp.44-52.

[37]. Bartlett NW, Walton RP, Edwards MR, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nature medicine. 2008 Feb;14(2), pp.199-204.

[38]. Dobrikova EY, Goetz C, Walters RW, et al. Attenuation of neurovirulence, biodistribution, and

shedding of a poliovirus: rhinovirus chimera after intrathalamic inoculation in Macaca fascicularis. Journal of virology. 2012 Mar 1;86(5), pp.2750-2759.

[39]. Jahan N, Wimmer E, Mueller S. Polypyrimidine tract binding protein-1 (PTB1) is a determinant of the tissue and host tropism of a human rhinovirus/poliovirus chimera PV1 (RIPO). PLoS One. 2013 Apr 4;8(4):e60791.

[40]. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nature reviews microbiology. 2009 Jun;7(6), pp.439-450.

[41]. Zaki AM, Van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New England Journal of Medicine. 2012 Nov 8;367(19), pp.1814-1820.

[42]. Donaldson EF, Yount B, Sims AC, et al. Systematic assembly of a full-length infectious clone of human coronavirus NL63. Journal of virology. 2008 Dec 1;82(23), pp.11948-11957.

[43]. Becker MM, Graham RL, Donaldson EF, et al. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. Proceedings of the National Academy of Sciences. 2008 Dec 16;105(50), pp.19944-19949.

[44]. Eckerle LD, Lu X, Sperry SM, et al. High fidelity of murine hepatitis virus replication is decreased in nsp14 exoribonuclease mutants. Journal of virology. 2007 Nov 15;81(22), pp.12135-12144.

[45]. Smith EC, Blanc H, Vignuzzi M, et al. Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics. PLoS pathogens. 2013 Aug 15;9(8):e1003565.

[46]. Denison MR, Graham RL, Donaldson EF, et al. Coronaviruses: an RNA proofreading machine regulates replication fidelity and diversity. RNA biology. 2011 Mar 1;8(2), pp.270-279.

[47]. Eckerle LD, Becker MM, Halpin RA, et al. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS pathogens. 2010 May 6;6(5):e1000896.

[48]. Graham RL, Becker MM, Eckerle LD, et al. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. Nature medicine. 2012 Dec;18(12), pp.1820-1826.

[49]. Hotez PJ, Bottazzi ME, Tseng CT, et al. Calling for rapid development of a safe and effective MERS vaccine. Microbes and Infection. 2014 Jul 1;16(7), pp.529-531.

[50]. Jiang L, Wang N, Zuo T, et al. Potent neutralization of MERS-CoV by human neutralizing monoclonal antibodies to the viral spike glycoprotein. Science translational medicine. 2014 Apr 30;6(234):234ra59-.

[51]. Tang XC, Agnihothram SS, Jiao Y, et al. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. Proceedings of the National Academy of Sciences. 2014 May 13;111(19):E2018-26.

[52]. Ma C, Li Y, Wang L, et al. Intranasal vaccination with recombinant receptor-binding domain of MERS-CoV spike protein induces much stronger local mucosal immune responses than subcutaneous immunization: Implication for designing novel mucosal MERS vaccines. Vaccine. 2014 Apr 11;32(18), pp.2100-2108.

[53]. Ishikawa T, Yamanaka A, Konishi E. A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. Vaccine. 2014 Mar 10;32(12), pp.1326-1337.

[54]. Reed W, Carroll J. The prevention of yellow fever. Public health papers and reports. 1901;27:113.

[55]. Reed W, Carroll J, Agramonte A. The etiology of yellow fever: an additional note. 1901. Military medicine. 2001 Sep;166(9 Suppl), pp.44-53.

[56]. Reed W. Recent researches concerning the etiology, propagation, and prevention of yellow fever, by the United States Army Commission. Epidemiology & Infection. 1902 Apr;2(2), pp.101-119.

[57]. Rice CM, Grakoui A, Galler R, et al. Transcription of infectious yellow fever RNA from full-length cDNA templates produced by in vitro ligation. The New biologist. 1989 Dec 1;1(3), pp.285-296.

[58]. Bredenbeek PJ, Kooi EA, Lindenbach B, et al. A stable full-length yellow fever virus cDNA clone and the role of conserved RNA elements in flavivirus replication. Journal of general virology. 2003 May 1;84(5), pp.1261-1268.

[59]. Kapoor M, Zhang L, Mohan PM, et al. Synthesis and characterization of an infectious dengue virus type-2 RNA genome (New Guinea C strain). Gene. 1995 Sep 11;162(2), pp.175-180.

[60]. Kinney RM, Butrapet S, Chang GJ, et al. Construction of infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK-53. Virology. 1997 Apr 14;230(2), pp.300-308.

[61]. Polo S, Ketner GA, Levis RO, et al. Infectious RNA transcripts from full-length dengue virus type 2 cDNA clones made in yeast. Journal of virology. 1997 Jul;71(7), pp.5366-5374.

[62]. Puri B, Polo S, Hayes CG, et al. Construction of a full length infectious clone for dengue-1 virus Western Pacific, 74 strain. Virus genes. 2000 Feb;20(1), pp.57-63.

[63]. Blaney JE, Hanson CT, Firestone CY, et al. Genetically modified, live attenuated dengue virus type 3 vaccine candidates. The American journal of

tropical medicine and hygiene. 2004 Dec 1;71(6), pp.811-821.

[64]. Blaney JE, Hanson CT, Hanley KA, et al. Vaccine candidates derived from a novel infectious cDNA clone of an American genotype dengue virus type 2. BMC infectious diseases. 2004 Dec;4(1):1-0.

[65]. Chen W, Kawano H, Men R, et al. Construction of intertypic chimeric dengue viruses exhibiting type 3 antigenicity and neurovirulence for mice. Journal of Virology. 1995 Aug;69(8), pp.5186-5190.

[66]. Lai CJ, Zhao BT, Hori H, et al. Infectious RNA transcribed from stably cloned full-length cDNA of dengue type 4 virus. Proceedings of the National Academy of Sciences. 1991 Jun 15;88(12), pp.5139-5143.

[67]. Sumiyoshi H, Hoke CH, Trent DW. Infectious Japanese encephalitis virus RNA can be synthesized from in vitro-ligated cDNA templates. Journal of Virology. 1992 Sep;66(9), pp.5425-5431.

[68]. Khromykh AA, Westaway EG. Completion of Kunjin virus RNA sequence and recovery of an infectious RNA transcribed from stably cloned full-length cDNA. Journal of virology. 1994 Jul;68(7), pp.4580-4588.

[69]. Gritsun TS, Gould EA. Infectious transcripts of tick-borne encephalitis virus, generated in days by RT-PCR. Virology. 1995 Dec 20;214(2), pp.611-618.

[70]. Gritsun TS, Gould EA. Development and analysis of a tick-borne encephalitis virus infectious clone using a novel and rapid strategy. Journal of virological methods. 1998 Dec 1;76(1-2), pp.109-120.

[71]. Mandl CW, Ecker M, Holzmann H, et al. Infectious cDNA clones of tick-borne encephalitis virus European subtype prototypic strain Neudoerfl and high virulence strain Hypr. Journal of General Virology. 1997 May 1;78(5), pp.1049-1057.

[72]. Hurrelbrink RJ, Nestorowicz A, McMinn PC. Characterization of infectious Murray Valley encephalitis virus derived from a stably cloned genome-length cDNA. Journal of general virology. 1999 Dec 1;80(12), pp.3115-3125.

[73]. Campbell MS, Pletnev AG. Infectious cDNA clones of Langat tick-borne flavivirus that differ from their parent in peripheral neurovirulence. Virology. 2000 Mar 30;269(1), pp.225-237.

[74]. Yamshchikov VF, Wengler G, Perelygin AA, et al. An infectious clone of the West Nile flavivirus. Virology. 2001 Mar 15;281(2), pp.294-304.

[75]. Yoshii K, Igarashi M, Ito K, et al. Construction of an infectious cDNA clone for Omsk hemorrhagic fever virus, and characterization of mutations in NS2A and NS5. Virus research. 2011 Jan 1;155(1), pp.61-68.

[76]. Theiler M, Smith HH. The use of yellow fever virus modified by in vitro cultivation for human immunization. The Journal of experimental medicine. 1937 May 5;65(6):787.

[77]. Bonaldo MC, Sequeira PC, Galler R. The yellow fever 17D virus as a platform for new live attenuated vaccines. Human vaccines & immunotherapeutics. 2014 May 8;10(5), pp.1256-1265.

[78]. Lefeuvre A, Marianneau P, Deubel V. Current assessment of yellow fever and yellow fever vaccine. Current infectious disease reports. 2004 Apr;6(2), pp.96-104.

[79]. Poland JD, Calisher CH, Monath TP, et al. Persistence of neutralizing antibody 30-35 years after immunization with 17D yellow fever vaccine. Bulletin of the World Health Organization. 1981;59(6):895.

[80]. Webster LT. Japanese B encephalitis virus: its differentiation from St. Louis encephalitis virus and relationship to louping ill virus. The Journal of Experimental Medicine. 1938 Mar 3;67(4):609.

[81]. Normile D. First new dengue virus type in 50 years. Health. 2013 Oct.

[82]. Halstead SB. Immune enhancement of viral infection. Immunity and Concomitant Immunity in Infectious Diseases. 1982, pp.31:301-364.

[83]. Guirakhoo F, Arroyo J, Pugachev KV et al. Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. Journal of virology. 2001 Aug 15;75(16), pp.7290-7304.

[84]. Caufour PS, Motta MC, Yamamura AM, et al. Construction, characterization and immunogenicity of recombinant yellow fever 17D-dengue type 2 viruses. Virus Research. 2001 Nov 5;79(1-2), pp.1-4.

[85]. Guy B, Guirakhoo F, Barban V, et al. Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. Vaccine. 2010 Jan 8;28(3), pp.632-649.

[86]. Arroyo J, Miller C, Catalan J, et al. ChimeriVax-West Nile virus live-attenuated vaccine: preclinical evaluation of safety, immunogenicity, and efficacy. Journal of virology. 2004 Nov 15;78(22), pp.12497-12507.

[87]. Schnell MJ, Mebatsion T, Conzelmann KK. Infectious rabies viruses from cloned cDNA. The EMBO journal. 1994 Sep;13(18), pp.4195-4203.

[88]. Conzelmann KK, Schnell M. Rescue of synthetic genomic RNA analogs of rabies virus by plasmid-encoded proteins. Journal of virology. 1994 Feb;68(2), pp.713-719.

[89]. Radecke F, Spielhofer P, Schneider H, et al. Rescue of measles viruses from cloned DNA. The EMBO journal. 1995 Dec;14(23), pp.5773-5784.

[90]. Clarke DK, Sidhu MS, Johnson JE, et al. Rescue of mumps virus from cDNA. Journal of virology. 2000 May 15;74(10), pp.4831-4838.

[91]. Marsh GA, Virtue ER, Smith I, et al. Recombinant Hendra viruses expressing a reporter gene retain pathogenicity in ferrets. Virology journal. 2013 Dec;10(1), pp.1-7.

[92]. Yoneda M, Guillaume V, Ikeda F, et al. Establishment of a Nipah virus rescue system. Proceedings of the National Academy of Sciences. 2006 Oct 31;103(44), pp.16508-16513.

[93]. Collins PL, Murphy BR. New generation live vaccines against human respiratory syncytial virus designed by reverse genetics. Proceedings of the American Thoracic Society. 2005 Aug;2(2), pp.166-173.

[94]. Katz SL. John F. Enders and measles virus vaccine—a reminiscence. InMeasles 2009 (pp. 3-11). Springer, Berlin, Heidelberg.

[95]. Buynak EB, Hilleman MR. Live attenuated mumps virus vaccine. 1. Vaccine development. Proceedings of the Society for Experimental Biology and Medicine. 1966 Dec;123(3), pp.768-775.

[96]. Stokes J, Weibel RE, Villarejos VM, et al. Trivalent combined measles-mumps-rubella vaccine: Findings in clinical-laboratory studies. JAmA. 1971 Oct 4;218(1), pp.57-61.

[97]. Morris JA, Blount Jr RE, Savage R. Recovery of cytopathogenic agent from chimpanzees with goryza. Proceedings of the Society for Experimental Biology and Medicine. 1956 Jul;92(3), pp.544-549.

[98]. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. New England Journal of Medicine. 2009 Feb 5;360(6), pp.588-598.

[99]. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. The Lancet. 2010 May 1;375(9725), pp.1545-1455.

[100]. Glezen WP, Taber LH, Frank AL, et al. Risk of primary infection and reinfection with respiratory syncytial virus. American journal of diseases of children. 1986 Jun 1;140(6), pp.543-546.

[101]. Subramanian KS, Weisman LE, Rhodes T, et al. Safety, tolerance and pharmacokinetics of a humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. The Pediatric infectious disease journal. 1998 Feb 1;17(2), pp.110-115.

[102]. Abarca K, Jung E, Fernández P, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of motavizumab, a humanized, enhanced-potency monoclonal antibody for the prevention of respiratory syncytial virus infection in at-risk children. The Pediatric infectious disease journal. 2009 Apr 1;28(4), pp.267-272.

[103]. Kamal-Bahl S, Doshi J, Campbell J. Economic analyses of respiratory syncytial virus immunoprophylaxis in high-risk infants: a systematic review. Archives of pediatrics & adolescent medicine. 2002 Oct 1;156(10), pp.1034-1041.

[104]. Kim HW, Leikin SL, Arrobio J, et al. Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. Pediatric Research. 1976 Jan;10(1), pp.75-78.

[105]. Collins PL, Melero JA. Progress in understanding and controlling respiratory syncytial virus: still crazy after all these years. Virus research. 2011 Dec 1;162(1-2), pp.80-99.

[106]. Karron RA, Wright PF, Belshe RB, et al. Identification of a recombinant live attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants. The Journal of infectious diseases. 2005 Apr 1;191(7), pp.1093-1104.

[107]. Schickli JH, Kaur J, Tang RS. Nonclinical phenotypic and genotypic analyses of a Phase 1 pediatric respiratory syncytial virus vaccine candidate MEDI-559 (rA2cp248/404/1030ΔSH) at permissive and non-permissive temperatures. Virus research. 2012 Oct 1;169(1), pp.38-47.

[108]. Luongo C, Winter CC, Collins PL, et al. Increased genetic and phenotypic stability of a promising live-attenuated respiratory syncytial virus vaccine candidate by reverse genetics. Journal of virology. 2012 Oct 1;86(19), pp.10792-10804.

[109]. Graham BS. Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. Immunological reviews. 2011 Jan;239(1), pp.149-166.

[110]. Engelhardt OG. Many ways to make an influenza virus–review of influenza virus reverse genetics methods. Influenza and other respiratory viruses. 2013 May;7(3), pp.249-256.

[111]. Enami M, Luytjes W, Krystal M, et al. Introduction of site-specific mutations into the genome of influenza virus. Proceedings of the National Academy of Sciences. 1990 May;87(10), pp.3802-3805.

[112]. Luytjes W, Krystal M, Enami M, et al. Amplification, expression, and packaging of a foreign gene by influenza virus. Cell. 1989 Dec 22;59(6), pp.1107-1113.

[113]. Fodor E, Devenish L, Engelhardt OG, et al. Rescue of influenza A virus from recombinant DNA. Journal of virology. 1999 Nov 1;73(11), pp.9679-9682.

[114]. Neumann G, Kawaoka Y. Generation of influenza A virus from cloned cDNAs—historical perspective and outlook for the new millenium. Reviews in medical virology. 2002 Jan;12(1), pp.13-30.

[115]. Koudstaal W, Hartgroves L, Havenga M, et al. Suitability of PER. C6® cells to generate epidemic and pandemic influenza vaccine strains by reverse genetics. Vaccine. 2009 Apr 28;27(19), pp.2588-2593.

[116]. de Wit E, Spronken MI, Vervaet G, et al. A reverse-genetics system for Influenza A virus using T7 RNA polymerase. Journal of General Virology. 2007 Apr 1;88(4), pp.1281-1287.

[117]. Crescenzo-Chaigne B, Van Der Werf S. Rescue of influenza C virus from recombinant DNA. Journal of virology. 2007 Oct 15;81(20), pp.11282-11289.

[118]. Muraki Y, Murata T, Takashita E, et al. A mutation on influenza C virus M1 protein affects virion morphology by altering the membrane affinity of the protein. Journal of virology. 2007 Aug 15;81(16), pp.8766-8773.

[119]. Massin P, Rodrigues P, Marasescu M, et al. Cloning of the chicken RNA polymerase I promoter and use for reverse genetics of influenza A viruses in avian cells. Journal of virology. 2005 Nov 1;79(21), pp.13811-13816.

[120]. Murakami S, Horimoto T, Yamada S, et al. Establishment of canine RNA polymerase I-driven reverse genetics for influenza A virus: its application for H5N1 vaccine production. Journal of virology. 2008 Feb 1;82(3), pp.1605-1609.

[121]. Neumann G, Fujii K, Kino Y, Kawaoka Y. An improved reverse genetics system for influenza A virus generation and its implications for vaccine production. Proceedings of the National Academy of Sciences. 2005 Nov 15;102(46), pp.16825-16829.

[122]. Vinnemeier CD, Fischer-Herr J, Meyer S, et al. Immunogenicity and safety of an inactivated 2012/2013 trivalent influenza vaccine produced in mammalian cell culture (Optaflu®) An open label, uncontrolled study. Human Vaccines & Immunotherapeutics. 2014 Feb 1;10(2), pp.441-448.

[123]. Doroshenko A, Halperin SA. Trivalent MDCK cell culture-derived influenza vaccine Optaflu®(Novartis Vaccines). Expert review of vaccines. 2009 Jun 1;8(6), pp.679-688.

[124]. Bright RA, Carter DM, Daniluk S, et al. Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin. Vaccine. 2007 May 10;25(19), pp.3871-3878.

[125]. Pushko P, Tumpey TM, Van Hoeven N, et al. Evaluation of influenza virus-like particles and Novasome adjuvant as candidate vaccine for avian influenza. Vaccine. 2007 May 22;25(21), pp.4283-4290.

[126]. Pushko P, Tumpey TM, Bu F, et al. Influenza virus-like particles comprised of the HA, NA, and M1 proteins of H9N2 influenza virus induce protective immune responses in BALB/c mice. Vaccine. 2005 Dec 30;23(50), pp.5751-5759.

[127]. Galarza JM, Latham T, Cupo A. Virus-like particle vaccine conferred complete protection against a lethal influenza virus challenge. Viral immunology. 2005 Jun 1;18(2), pp.365-372.

[128]. Quan FS, Huang C, Compans RW, et al. Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. Journal of virology. 2007 Apr 1;81(7), pp.3514-3524.

[129]. Treanor JJ, Schiff GM, Hayden FG, et al. Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial. Jama. 2007 Apr 11;297(14), pp.1577-1582.

[130]. Traynor K. First recombinant flu vaccine approved. American Journal of Health-System Pharmacy. 2013 Mar 1;70(5), pp.382-383.

[131]. Yang LP. Recombinant trivalent influenza vaccine (Flublok®): a review of its use in the prevention of seasonal influenza in adults. Drugs. 2013 Aug;73(12), pp.1357-1366.

[132]. Dormitzer PR, Suphaphiphat P, Gibson DG, et al. Synthetic generation of influenza vaccine viruses for rapid response to pandemics. Science translational medicine. 2013 May 15;5(185):185ra68-.

[133]. Burns CC, Shaw J, Campagnoli R, et al. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. Journal of virology. 2006 Apr 1;80(7), pp.3259-3272.

[134]. Mueller S, Papamichail D, Coleman JR, et al. Reduction of the rate of poliovirus protein synthesis through large-scale codon deoptimization causes attenuation of viral virulence by lowering specific infectivity. Journal of virology. 2006 Oct 1;80(19), pp.9687-9696.

[135]. Dormitzer PR, Grandi G, Rappuoli R. Structural vaccinology starts to deliver. Nature Reviews Microbiology. 2012 Dec;10(12), pp.807-813.