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Gene Therapy of Cystic Fibrosis Using Influenza Virus

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Abstract

Cystic fibrosis is a genetic disease due to a mutation in a gene encoding the cystic fibrosis transmembrane conductance regulator chloride channel. In this proposed method, by genetically manipulating the gene structure in the influenza virus and inserting this manipulated gene into the body, we force the cell to produce a healthy version of the CFTR gene. The influenza virus belongs to the family Orthomyxoviridae and enters the body through SAA2,6Gal receptors in epithelial cells (1). If we genetically modify the related gene in the influenza virus and insert the CFTR gene, the influenza virus, which has been modified by gene therapy, can force the infected cell to express the healthy CFTR gene mRNA. Based on this method, it seems that the cause of this disease, which is the absence of CFTR proteins on the patient's cell surface, can be alleviated to some extent by inserting the healthy transcript of CFTR into the membrane phospholipids.

Keywords: Influenza virus, cystic fibrosis, CFTR, gene therapy

Introduction

Dorothy Hansine Andersen was the first who expansively described and documented cystic fibrosis (CF) in the 1930s (2). CF is an autosomal recessive genetic disorder and is the most ubiquitous fatal inherited ailment in whites, with an incidence rate of around 1 per 3,500 births (1). There is still no accepted treatment for this illness despite many efforts in genetic engineering. The cause of the disease is a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome7, encoding a transmembrane chloride channel. Roughly seventy percent of mutations are related to the F508delta mutation, which is the deletion of phenylalanine at position 508. More than 2,000 types of mutations have been documented so far. In general, heterozygotes are phenotypically healthy (3-7).

The accessibility of tools for interrogating individual genomes, as well as the human genome sequence provide an extraordinary chance to apply genetics to medicine. Mendelian conditions that are triggered by the dysfunction of a single gene, suggest great instances, which clarify how genetics can provide insight into diseases. The CF is presented here as an instance of the prevalent fatal illnesses. Current progressions in clarifying disease mechanism and reasons for phenotypic variations, and also in developing the treatments reveals that genetics still play a key role in CF investigations 25 years after finding the gene that causes the disease (8).

This protein is a chloride channel that works by hydrolyzing ATP and activating cAMP and can regulate other ion channels and it is responsible for the excretion of salt and the exchange of chlorine with bicarbonate. In the CF patients, the bicarbonate secretion is reduced, and subsequently, the pH value of the body is reduced because the bicarbonate is one of the main factors regulating the pH balance (9). The CFTR gene is expressed in lung epithelial cells and blood cells (8,10,11). The disease is not restricted to the pediatrics; currently, 40 percent of CF patients are adults, but the first symptoms of the disease appear in childhood, and only 13% of patients are over 30 years old. The CFTR gene encodes a protein that controls multiple functions, including the regulation of the sodium channel. As the ions in the lungs play a significant role in lung fluid movements, any imbalance in ion-exchange slows down the lung fluid movements. The concentration of mucus in the lungs develops complications such as persistent cough, wheezing, shortness of breath, decreased ability to exercise, recurrent lung infection, nasal congestion, and acute conditions in the CF patients; the chlorine content increases on the skin surface of such patients.

The CF leads to malnutrition, liver disease, and growth retardation through a gradual decline in lung function and pancreatic insufficiency. The illness can happen differently in different people; for example, the symptoms begin during infancy in some children. Other people may not have any symptoms until adolescence or adulthood, and may sometimes be mistaken for other conditions, such as asthma, for a long time (12). Abnormal secretion of sticky mucus can develop airway obstruction and provide a suitable environment for bacterial growth and pneumonia (5,13). Moreover, the ailment might be accompanied by other discomforts, such as issues with the digestive system. Studies show that the metabolism of essential fatty acids increases in the CF disease, which gradually reduces essential fatty acids, and subsequently the serum levels of fat-soluble vitamins and minerals such as zinc, copper, and selenium are decreased (14,15). There are diverse issues in the lumen of the gastrointestinal tract including intussusception, distal intestinal obstruction syndrome (DIOS), meconium ileus, and constipation. In this ailment, the pancreas is incapable to secrete digestive enzymes, thus decreasing the ability to absorb nutrients from the gastrointestinal tract and leading to reduced growth. Other discomforts observed in these patients are foul-smelling stool due to lack of production of digestive enzymes, abdominal pain, and loss of limbs. Findings from investigations in the United States demonstrate that CF also impacts the liver.

The involvement of liver in this disease is considerably less common than both pancreatic and pulmonary illnesses that exist in 80-90% of CF patients; liver diseases (LD) influence only one-third of CF patients, yet, due to the lessening mortality from extrahepatic causes, its recognition and treatment are significant clinical concerns. New clarifications recommend that genetic modifiers influence the clinical expression of LD in CF; their identification is a key concern as it may permit to recognize CF patients at risk for the development of LD and early institution of prophylactic approaches. Oral bile acid therapy, proposed to improve biliary secretion in terms of bile acid composition and bile viscosity, is now the only reachable therapeutic method for CF-related LD. Nevertheless, the influence of this treatment on the natural history of LD has not been well-defined and long-term influences on clinically relevant consequences need to be further examined. CF patients with progressive liver failure and/or with life-threatening consequences of portal hypertension, who as well have slight pulmonary involvement that is estimated to support long-term survival, should be provided with liver transplantation. CF patients have a one-year survival rate of 80% after transplantation with profitable influences on the quality of life, body compositions, nutritional status, and lung functions in most of the patients.

Besides, the risk of gallstones is higher in CF, although the main cause of death in these patients is lung congestion (16-18). The male reproductive system disorder is another expected concern in CF patients.

The CF is characterized by wide-ranging clinical signs, such as reproductive obstacles. Almost all males influenced by CF are infertile due to azoospermia associated with the pathology of male ducts, while CF females have diminished fertility.

Modern investigations suggest that men with congenital bilateral absence of the vas deferens (CBAVD) will possibly experience CF. The CBAVD is a rather prevalent reason for male infertility, accounting for at least six percent of obstructive azoospermia cases and 1–2% of patients with male sterility. A genetic basis for CBAVD has recently been provided by its link with CF, and today, in most cases, CBAVD is an incomplete or mild sort of CF illness. Numerous subjects with CBAVD demonstrate characteristically mild CF-compatible clinical manifestations, however, the lasting prognosis could not be as innocuous as it currently appears: further information will be reachable by long or medium-term follow-ups. When CBAVD has precisely been diagnosed and if the couple is scheduling a pregnancy by artificial reproductive technologies, it is essential to test both the affected man and his wife for CFTR mutations. This testing has several complex implications and should be carried out along with genetic counseling. Other topics are argued in this paper including CF mutations in non-CBAVD forms of male infertility, and the potentially ambiguous part of the genetic analysis of CF when employed to rule out other imaginable reasons for infertility in azoospermic men (19).

The disease not only influences children but also has been described in forty percent of adults among patients with the ailment. The first signs of the disease occur in childhood, and only thirteen percent of

patients are over thirty years old. Infected people may develop secondary kidney complications that may be the consequence of antibiotic therapy (20).

The CFTR potentiator ivacaftor was certified as an oral remedy for CF cases with the G551D-CFTR mutation (21). Some examples of the treatments currently in use include antibiotics, mucociliary clearance, and bronchodilators.

One of the available remedies is gene therapy so that the CF gene therapy intends to deliver copies of the coding sequence of normal (i.e. wild-type) CFTR DNA to target cells. This could be accomplished by utilizing non-viral or viral delivery procedures (22-25).

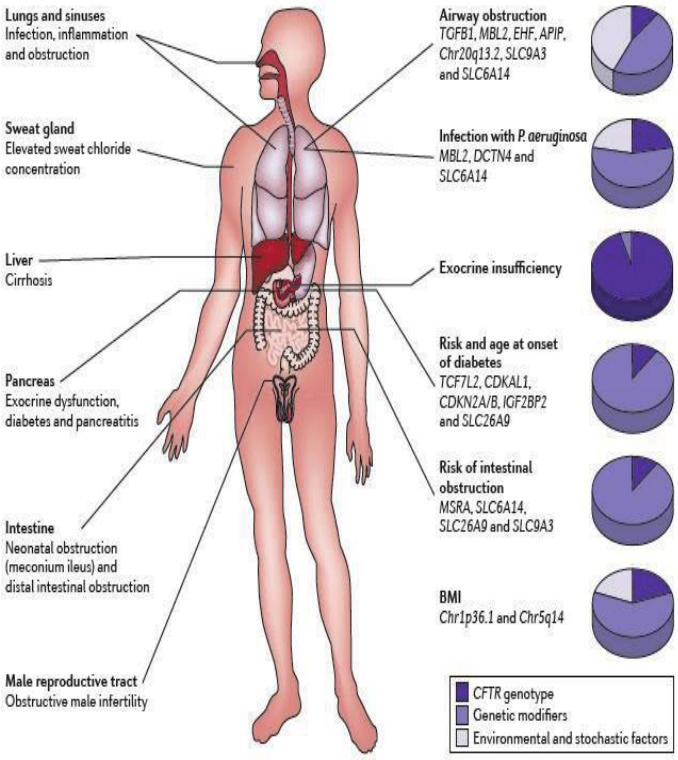
Non-viral gene therapy consists of the transfer of DNA complexes or naked DNA to cells by vectors other than viruses. Non-viral vectors under analysis are self-assembling polyethyleneimine complexes, DNA nanoparticles (DNA complexed with peptides), and cationic lipid GL67A (26-28).

The objective of this article was to employ the influenza virus for gene therapy. Influenza A virus (IAV) is a single-stranded negative-sense RNA virus (family Orthomyxoviridae), which leads to infections in birds and some mammals. The virus moves in cells through endocytosis post binding to SAα2,6Gal receptors on epithelial cells (29-31).

One of the reasons for the failure of gene therapy by a viral vector is that the response becomes stronger each time the virus enters the body. Therefore, it may not have enough time for replication due to the repeated entry of the virus into the body, and it may be destroyed by the immune response, thus losing its effectiveness over time. Interestingly, the flu virus mutates genetically every year. Two types of influenza (flu) virus, A and B, develop the flu. Type A is ordinarily the cause of the annual epidemic. These viruses are repetitively changing and generating subtypes or strains that are different from the first virus but retain some of its features. The flu-causing strains may vary from year to year, so it is possible to have a new virus available for treatment each year, but to advance this, we must produce a flu vaccine each year before gene therapy.

The unique properties of viruses make them suitable for gene transfer to eukaryotic cells. Various types of viruses, including adenoviruses and herpesviruses, have been employed for in vitro use in gene therapy programs against CF. Retroviral vectors can remain permanently in the genomes of infected cells but the cells require mitosis to produce their own genes. Adenovirus vectors can easily present genes in different cell types, but the deletion of infected cell immunity often restricts gene expression in vivo (32).

A replication-defective vector on the basis of HIV was assessed for gene delivery into the lung. The tropism of this vector has been sustained by means of incorporating the vesicular stomatitis virus G protein into its envelope. The HIV vector was proficiently transduced into the non-dividing epithelial cells of airway in vitro, while a murine-based retroviral vector did not. Investigations on a human bronchial xenograft model verified high-level gene transduction using a CFTR HIV vector into CF-derived, undifferentiated cells of the xenograft. The expression of CFTR was constant and after the graft matured, able to the functional correction of CF defect. When the HIV vector was instilled after differentiation of the epithelium, it could not successfully transduce the xenograft cells. This transduction block seems to be at the entry-level, while post-entry restrictions cannot be ignored. Further development of this system for gene therapy of CF may lead to better understand likely entry and post-entry blocks (33,34).



One of the reasons why the flu virus is better in gene therapy for CF is that the virus mutates genetically every year, and every year the FLU vaccine against the flu virus enters the market. Therefore, we need to use a virus against which the immune response is not very strong to have maximum productivity in this gene therapy. On the other hand, the virus should not be strong enough to result in acute conditions. Therefore, the influenza virus appears to be more effective than other viral vectors for gene therapy.

Materials and Methods

Cystic fibrosis is an autosomal recessive disorder that is the most prevalent illness among whites. Although it most often influences children, 40% of patients with the disease are adults and the symptoms of this disease include the airway obstruction due to thickening of the lung mucus, recurrent abdominal pain, weight loss, indigestion, intestinal obstruction, nausea and vomiting, fatty diarrhea, growth retardation, and liver involvement. The signs and symptoms of CF differ depending on the severity of the ailment, and even in a sick person, the symptoms may get worse or improve over time.

There are presently many approaches to treat CF, such as gene therapies with viral and non-viral vectors. Extensive clinical trials have resulted in the rapid development of a variety of vectors, including adenoviruses, adeno-associated viruses, and liposomes, to recover the main defects in the CF. Clinical investigations have indicated that host immune response and low vector efficiency are identified as key barriers to efficient CF gene therapy (35).

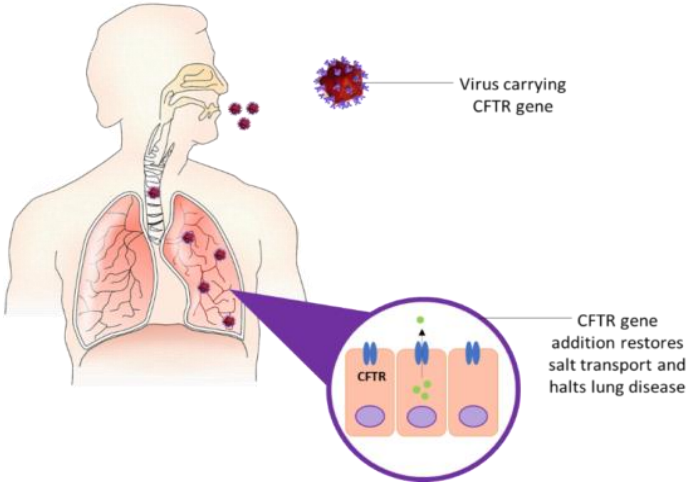
The flu virus belongs to the family Orthomyxoviridae, which includes types A and B, and selects birds and some mammals as hosts.

Influenza virus appears to have a unique feature compared to other viruses tested in gene therapy so that the virus mutates genetically every year, and the FLU vaccine enters the market each year for that virus. Therefore, the use of this virus for treatment makes it possible to recruit a new virus every year for treatment. It is significant to note that a vaccine specific to the virus must be injected before gene therapy to avoid acute conditions in the CF patients.

In previous examples of gene therapy with viral vectors, the main cause of treatment failure has been that the repeated injections of the manipulated virus have induced a strong immune response against

the injected virus, so the virus will not have much time to enter the cell and proliferate, but the flu virus, because of its annual mutation, permits the virus to proliferate in the course of treatment.

In the proposed method, we add the healthy transcript of the CFTR gene to the gene collection of the influenza virus and inject the manipulated virus. Following injection, the virus enters the cell through SAα2,6Gal receptors on lung epithelial cells, and later, the host cell starts to synthesize the viral mRNA and the cell co-synthesizes the healthy CFTR gene. Thus, this protein enters the cell membrane and can solve some of the problems of patients to some extent. Consequently, the exchange of sodium and chlorine ions is expected to be somewhat normalized and the lung fluid movement to regain the mobility, and so the lethal factor in CF is estimated to be somewhat moderated.



Discussion and Conclusion

This investigation aimed to assess the gene therapy of CF disease using the influenza virus. There are two reasons for using this virus compared to other viruses. First, the virus binds to SAα2,6Gal receptors on lung epithelial cells. Therefore, if we introduce the genetically manipulated influenza virus into the body, it will enter the lung epithelial cells directly and immediately force the cell to produce a healthy transcript of the CFTR gene, and since the main reason for death in CF is airway obstruction due to the absence of CFTR chloride channels on the cell surface, this approach can reduce partly the risk of death from the disease. The second reason for the selection of this virus over other viruses is that this virus undergoes a genetic mutation every year, and so it allows us to have a new virus every year for gene therapy. Besides, a new vaccine is introduced twice a year for the same season's flu, as the flu virus shifts rapidly (35-37). Although the effectiveness of the vaccine varies from year to year, the majority of them provide moderate to high immunity to influenza (38). Therefore, we will have a new flu virus available every year to force it into gene therapy by modifying its gene and adding the healthy transcript of the CFTR gene. As such, the response against the virus does not appear to be strong enough to cause the test to fail. On the other hand, because the vaccine against the virus has been injected before gene therapy, the virus will not be strong enough to provoke a strong response from the body, and so the manipulated virus is expected to be used only in treatment and not to cause significant pathogenicity.

Reference

1. A. Gambaryan, R. Webster & M. Matrosovich. Differences between influenza virus receptors on target cells of duck and chicken. 147, 1197–1208(2002). DOI 10.1007/s00705-002-0796-4.

2. Derichs N. Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. Eur Respir Rev. 2013;22(127):58-65. doi:10.1183/09059180.00008412.

3. Boucher R C. Status of gene therapy for cystic fibrosis lung disease. 1999 Feb 15; 103(4): 441–445. doi: 10.1172/JCI6330.

4. Castellani C, Assael BM. Cystic fibrosis: a clinical view. Cell Mol Life Sci. 2017;74(1):129-140. doi:10.1007/s00018-016-2393-9.

5. Cooney AL, McCray PB, Sinn PL. Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward. Genes. 2018 Nov; 9(11): 538. doi: 10.3390/genes9110538.

6. Donnelley M, Parsons DW. Parsons. Gene Therapy for Cystic Fibrosis Lung Disease: Overcoming the Barriers to Translation to the Clinic. Frontiers in pharmacology. 2018; 9: 1381. doi: 10.3389/fphar.2018.01381.

7. Kulkarni H, Kansra S, Karande S. Cystic fibrosis revisited. J Postgrad Med. 2019;65(4):193-196. doi:10.4103/jpgm.JPGM_263_18.

8. Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet. 2015;16(1):45-56. doi:10.1038/nrg3849.

9. ANDERSEN DH. CYSTIC FIBROSIS OF THE PANCREAS AND ITS RELATION TO CELIAC DISEASE: A CLINICAL AND PATHOLOGIC STUDY. Am J Dis Child. 1938;56(2):344–399. doi:10.1001/archpedi.1938.01980140114013.

10. Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration. 2000;67(2):117-133. doi:10.1159/000029497.

11. Kerem E, Corey M, Kerem BS, Rommens J, Markiewicz D, Levison H, Tsui LC, Durie P. The relation between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (ΔF508). New England Journal of Medicine. 1990 Nov 29;323(22):1517-22. DOI: 10.1056/NEJM199011293232203.

12. Drumm ML, Konstan MW, Schluchter MD, Handler A, Pace R, Zou F, Zariwala M, Fargo D, Xu A, Dunn JM, Darrah RJ. Genetic modifiers of lung disease in cystic fibrosis. New England Journal of Medicine. 2005 Oct 6;353(14):1443-53. DOI: 10.1056/NEJMoa051469

13. Anguiano A, Oates RD, Amos JA, Dean M, Gerrard B, Stewart C, Maher TA, White MB, Milunsky A. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. Jama. 1992 Apr 1;267(13):1794-7. doi:10.1001/jama.1992.03480130110034.

14. Strandvik B. Relation between essential fatty acid metabolism and gastrointestinal symptoms in cystic fibrosis. Acta Paediatr Scand Suppl. 1989;363:58-65. doi:10.1111/apa.1989.78.s363.58.

15. Bertolaso C, Groleau V, Schall JI, Maqbool A, Mascarenhas M, Latham N, Dougherty KA, Stallings VA. Fat soluble vitamins in cystic fibrosis and pancreatic insufficiency: efficacy of a nutrition intervention. Journal of pediatric gastroenterology and nutrition. 2014 Apr;58(4):443. doi: 10.1097/MPG.0000000000000272.

16. Kelly T, Buxbaum J. Gastrointestinal manifestations of cystic fibrosis. Digestive diseases and sciences. 2015 Jul 1;60(7):1903-13. doi:10.1007/s10620-015-3546-7.

17. Sabharwal S. Gastrointestinal Manifestations of Cystic Fibrosis. Gastroenterology & Hepatology. 2016 Jan;12(1):43-47.

18. Naehrig, S., Chao, C.-M., & Naehrlich, L. (2017). Cystic Fibrosis. Deutsches Aerzteblatt Online. 2017 Aug; 114(33-34): 564–574. doi: 10.3238/arztebl.2017.0564.

19. Quinzii C, Castellani C. The cystic fibrosis transmembrane regulator gene and male infertility. J Endocrinol Invest. 2000;23(10):684-689. doi:10.1007/BF03343794.

20. Robertson MB, Choe KA, Joseph PM. Review of the abdominal manifestations of cystic fibrosis in the adult patient. Radiographics. 2006;26(3):679-690. doi:10.1148/rg.263055101.

21. Derichs N. Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. Eur Respir Rev. 2013;22(127):58-65. doi:10.1183/09059180.00008412.

22. Yan Z, McCray Jr PB, Engelhardt JF. Advances in gene therapy for cystic fibrosis lung disease. Human molecular genetics. 2019 Oct 1;28(R1):R88-94. https://doi.org/10.1093/hmg/ddz139.

23. Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Curr Gene Ther. 2013;13(6):421-433. doi:10.2174/15665232133666131125095046.

24. SM Wold W, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Current gene therapy. 2013 Dec 1;13(6):421-33.

25. Griesenbach U, Alton EW. Progress in gene and cell therapy for cystic fibrosis lung disease. Curr Pharm Des. 2012;18(5):642-662. doi:10.2174/138161212799315993.

26. Rogan MP, Stoltz DA, Hornick DB. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. Chest. 2011;139(6):1480-1490. doi:10.1378/chest.10-2077.

27. Burney TJ, Davies JC. Gene therapy for the treatment of cystic fibrosis. The application of clinical genetics. 2012; 5: 29–36. doi: 10.2147/TACG.S8873.

28. Sumner-Jones, S. G., Gill, D. R., & Hyde, S. C. (2010). Gene therapy for cystic fibrosis lung disease. Milestones in Drug Therapy, 47–64. https://doi.org/10.1007/978-3-0346-0165-8_4.

29. Shinya K, Kawaoka Y. Influenza virus receptors in the human air way. Uirusu, 2006;56(1):85-89. doi:10.2222/jsv.56.85.

30. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. Nature. 2006;440(7083):435-436. doi:10.1038/440435a.

31. Yamaya M, Shinya K, Hatachi Y, Kubo H, Asada M, Yasuda H, Nishimura H, Nagatomi R. Clarithromycin inhibits type a seasonal influenza virus infection in human airway epithelial cells. Journal of Pharmacology and Experimental Therapeutics. 2010 Apr 1;333(1):81-90. DOI: https://doi.org/10.1124/jpet.109.162149.

32. Lundstrom, K. (2018). Viral Vectors in Gene Therapy. Diseases 6(2): 42. doi: 10.3390/diseases6020042.

33. Wagner JA, Gardner P. Toward cystic fibrosis gene therapy. Annual review of medicine. 1997 Feb;48(1):203-16. https://doi.org/10.1146/annurev.med.48.1.203.

34. Castellani S, Conese M. Lentiviral vectors and cystic fibrosis gene therapy. Viruses. 2010 Feb;2(2):395-412. doi: 10.3390/v2020395.

35. Goldman MJ, Lee PS, Yang JS, Wilson JM. Lentiviral vectors for gene therapy of cystic fibrosis. Hum Gene Ther. 1997;8(18):2261-2268. doi:10.1089/hum.1997.8.18-2261.

36. Houser K, Subbarao K. Influenza vaccines: challenges and solutions. Cell host & microbe. 2015 Mar 11; 17(3): 295–300. doi: 10.1016/j.chom.2015.02.012.

37. Mameli C, Cocchi I, Fumagalli M, Zuccotti G. Influenza vaccination: Effectiveness, Indications and Limits in the Pediatric Population. Frontiers in pediatrics. 2019; 7: 317. doi: 10.3389/fped.2019.00317.

38. Pica N, Palese P. Toward a universal influenza virus vaccine: prospects and challenges. Annual review of medicine. 2013 Jan 14;64:189-202. https://doi.org/10.1146/annurev-med-120611-145115.