A Universal Influenza Vaccine: The Strategic Plan for the National Institute of Allergy and Infectious Diseases

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Running Title: A Universal Influenza Vaccine

A priority for the National Institute of Allergy and Infectious Diseases is development of a universal influenza vaccine providing durable protection against multiple influenza strains. NIAID will use this strategic plan as a foundation for future investments in influenza research.

Abstract

A priority for the National Institute of Allergy and Infectious Diseases (NIAID) is development of an influenza vaccine providing durable protection against multiple influenza strains, including those that may cause a pandemic, i.e., a universal influenza vaccine. To invigorate research efforts, NIAID developed a strategic plan focused on knowledge gaps in three major research areas, as well as additional resources required to ensure progress towards a universal influenza vaccine. NIAID will use this plan as a foundation for future investments in influenza research and will support and coordinate a consortium of multidisciplinary scientists focused on accelerating progress towards this goal.

Keywords: Strategic Plan, Influenza, Universal Vaccine

Introduction

The National Institute of Allergy and Infectious Diseases (NIAID) has a longstanding commitment to advancing basic and translational research on influenza to inform the development of new and improved diagnostics, therapeutics and vaccines. NIAID has made one of its highest priorities the development of a universal influenza vaccine that would provide long-lasting protection against multiple strains of the virus, including strains with the potential to cause a pandemic.

There are two epidemiological forms of influenza, seasonal ("interpandemic") and pandemic [1]. Seasonal influenza epidemics, caused by influenza A and B viruses, result in 3-5 million severe cases and 300,000-500,000 deaths globally each year [2, 3]. Influenza pandemics caused by influenza A emerge at unpredictable intervals. They cause significantly increased morbidity and mortality compared to seasonal influenza. Four such pandemics have occurred in the past century, in the years 1918, 1957, 1968, and 2009 [4]. Furthermore, in the past few decades, animal influenza viruses, such as H5N1 and H7N9 avian influenza, have caused sporadic human infections and deaths [5]. These viruses, termed pre-pandemic influenza viruses, are acquired through close contact with infected animals, but do not demonstrate sustained person-to-person spread. However, there is global concern that viral mutations may allow efficient transmission among humans and lead to the next influenza pandemic.

Seasonal influenza vaccine effectiveness ranges between 10-60 percent [6]. The lowest effectiveness occurs when vaccine strains are not well matched to circulating strains. Reliance on egg passaging for vaccine production may allow for additional mutations during manufacturing and further compromise vaccine effectiveness in a given season [7]. Seasonal influenza vaccines provide virtually no protection against novel pandemic strains. The cornerstone of both seasonal and pandemic influenza prevention and control is strain-specific vaccination. Seasonal influenza viruses are subject to ongoing antigenic changes referred to as "drifts." For influenza A these drifts can be pronounced each season; they are much more gradual for influenza B.

Strains used in annual vaccines are selected twice annually following the influenza seasons in the northern and southern hemispheres [1]. Similarly, the emergence of a novel influenza virus with pandemic potential requires the development of a strain-specific vaccine to protect humans for an epidemic which might never occur. The current strategy for seasonal influenza vaccination keeps us at least one year behind this ever-evolving virus. The strategy for pandemic influenza leads to making, testing and stockpiling vaccines which may never be used.

To limit the public health consequences of both seasonal and pandemic influenza, vaccines that are more broadly and durably protective are needed. Figure 1 illustrates the steps that can guide ongoing research in this area. Advances in influenza virology, immunology, and vaccinology make the development of a "universal" influenza vaccine more feasible than a decade ago. For example, broad availability of deep gene sequencing techniques allows better and more efficient characterization of viruses and enables tracking of genetic changes in influenza viruses over time [8]. In addition, advances in structural biology allows researchers to relate how seemingly minor changes in the structure and conformation of the hemagglutinin (HA) protein, affects function, antigenicity, and immunogenicity [9, 10].

To focus research efforts, NIAID convened a workshop in June, 2017, entitled "Pathway to a Universal Influenza Vaccine," assembling scientists from academia, industry, and government to identify and develop criteria that would define a universal Influenza vaccine (Box 1), to discuss knowledge gaps in the quest for this vaccine, and to identify research strategies to address these gaps [11].

Criteria for a universal influenza vaccine.

A universal influenza vaccine should:

- Be at least 75% effective against symptomatic influenza infection;
- Protect against group I and group II influenza A viruses (influenza B would be a secondary target);
- Have durable protection that lasts at least 1 year and preferably through multiple seasons;

Building on discussions conducted during the workshop, NIAID herein proposes a strategic plan to reinvigorate pursuit of a universal influenza vaccine. This plan outlines activities in three main areas of influenza research: transmission, natural history, and pathogenesis studies utilizing prospective cohorts; influenza immunity and correlates of immune protection; and strategies in rational vaccine design to elicit broad, protective immune responses. The three research areas are not prioritized and advances in each are expected to be interdependent. The strategic plan also includes a description of research resources essential to advancing these three research areas that NIAID will develop, support and provide for the scientific community. Broad collaboration and coordination in the field is vital. NIAID intends for this strategic plan to serve as a foundation for its own research investments and envisions a transformative effort toward successful development of a universal influenza vaccine.

Research Area 1: Improve Understanding of Transmission, Natural History, and Pathogenesis of Influenza Infection

Improvements in influenza vaccines have been hindered by an incomplete understanding of influenza transmission, natural history and pathogenesis. Several cross-sectional studies have linked the results of viral surveillance with representative manifestations and characterization of host immune responses; however, there are no cohort studies that collect data from the same individuals over multiple influenza seasons with distinct vaccination and infection histories and apply modern approaches to analyzing the immune repertoire. Collection of clinical, immunologic, and virologic data from clinical cohorts along with comprehensive standardized assays will be vital in understanding the evolving immune response to influenza and how repeated exposure to influenza viruses and vaccines shapes it. Similarly, influenza transmission has historically been difficult to study and the impact of vaccination on transmission is not understood. Host and virologic factors that contribute to mild and severe disease, as well as the role of secondary infections, represent

additional underdeveloped areas of influenza research. Investment in basic research, including natural history and pathogenesis studies, will inform more effective strategies for universal vaccine design.

Objective 1.1. Expand understanding of influenza transmission and identify targets for improved disease control measures.

Our understanding of transmission of both seasonal and pandemic influenza is inadequate. Key unanswered questions include the relative contribution of aerosols, droplets, and fomites as modes of transmission and the impact of environmental factors (e.g., temperature and humidity) on each; the role of specific human sub-populations in epidemic or pandemic spread; and the level of herd immunity required to interrupt seasonal or pandemic influenza transmission. A better understanding of how influenza transmission occurs, what factors drive transmission, and what will prevent transmission are important for the development of improved vaccines. Studies under this objective will address the following:

- Identify physical and environmental factors (e.g., droplet size, temperature, humidity) that facilitate influenza transmission.
- Determine dynamics of viral spread across geographic regions.
- Identify host factors that impact transmission.
- Identify clinical features of disease that facilitate transmission.
- Develop analytic and modeling tools to define the level of immunity in the community required to prevent transmission.
- Determine the contribution of anti-HA stem antibodies in prevention of transmission.
- Determine the role of anti-neuraminidase (NA) antibodies in preventing virus budding, release, and transmission.

Objective 1.2. Identify factors associated with severity of influenza disease.

Most people with seasonal influenza have mild illness and recover in less than two weeks; however, some will have complications resulting in hospitalization and even death [13]. Those at high risk of complications from seasonal influenza include infants, the elderly, and those with medical comorbidities; these groups may respond differently to vaccination than healthy adults. An understanding of how to reduce severe influenza disease with seasonal or pandemic strains may guide novel strategies for the development of improved vaccines. Research addressing this objective will aim to:

- Identify viral factors impacting disease during human influenza infections.
- Identify host genetic and non-genetic factors (e.g., age, comorbidity) that affect susceptibility to severe influenza outcomes.
- Identify immune markers associated with reduced disease severity.
- Determine the mechanisms of immune dysregulation that may contribute to severe disease.
- Determine the role of bacterial or viral co-infections in the severity of influenza disease.

Objective 1.3. Precise characterization of circulating influenza viruses.

Knowing the antigenic diversity of influenza viruses that circulate in animals and humans is vital to guiding the development of universal influenza vaccines. Improving viral surveillance in animal reservoirs proximate to humans and characterizing the risk posed to humans may identify novel antigenic targets. Accurately predicting how circulating influenza viruses will evolve is critical to improving vaccine efficacy. Research addressing this objective will aim to:

- Develop the capability to rapidly characterize circulating influenza viruses from humans and animal reservoirs to assess the breadth of protection required from vaccines.
- Develop and test models predicting the influence of pre-existing immunity on virus evolution to anticipate the next emerging dominant seasonal influenza strain.

- Improve genotypic and phenotypic characterization of circulating viruses associated with adverse clinical outcomes, host immunity, and vaccine failures.
- Improve understanding of antigenic drift and immunodominance of various influenza antigens.

Research Area 2: Precise Characterization of Influenza Immunity and Correlates of Immune Protection

In contrast to strategies for the development of seasonal influenza vaccines, the goal of most universal influenza vaccine strategies is to induce broadly protective immunity. The ability of influenza viruses to undergo antigenic drift and evade antibody-mediated immunity complicates the design of broadly-protective vaccines. The role of T cell-mediated immunity in influenza infection and disease has historically received little research attention; however, it may offer another pathway to achieve broad protection. While current seasonal influenza vaccine approaches do not induce strong CD8+ T cell responses, universal influenza vaccine strategies may need to incorporate this component of the immune response to be fully successful.

The immune correlate of protection used historically as a surrogate of influenza vaccine efficacy, the hemagglutination inhibition (HAI or HI) assay, measures antibody-mediated inhibition of viral attachment, and as such, limits comprehensive assessment of vaccine-elicited immunity. “Functional” antibodies are measured by microneutralization (MN) assays, which measure the effect of antibodies on viral attachment and infection of mammalian cells. It will be essential to identify immune correlates beyond HAI and neutralizing activity in both humans and animal models, as well as standardized assays to measure potential correlates of protection, in order to accelerate design, development, and testing of a universal influenza vaccine.

Comprehensive profiling of human immune responses coupled with high-throughput viral sequencing and bioinformatics can determine the critical immune components required for induction of long-term, broadly-protective immunity against influenza. By applying these techniques across populations, biomarkers and

correlates of effective responses can be elucidated [14]. The knowledge gained from Research Area 2 will help to elucidate protective immune mechanisms triggered by both natural influenza infection and vaccination.

Objective 2.1. Improve understanding of how and when exposure to influenza antigens shapes the host response to influenza infection and vaccination.

Humans encounter numerous influenza strains and vaccinations throughout their lifetime with immune responses determined by the genetics of the virus as well as intrinsic host factors such as genetics, age, health and immune status. Recent data provide strong evidence that infection with influenza strains circulating during one's childhood elicits a lifelong immunologic imprint that impacts subsequent responses to vaccinations and to novel strains and helps protect against unfamiliar HA subtypes from the same phylogenetic group as the original infecting virus [15]. This phenomenon is termed "immunologic imprinting." While induction of protective immunological memory can be a positive outcome of such prior exposures, some evidence suggests that pre-existing immunity from infection or vaccination may limit the generation of protective responses to novel influenza strains or vaccines [16-18]. The emergence of transformative new technologies such as high-throughput sequencing and single-cell sorting provides the opportunity to understand in a fundamental manner viral evolution and human immune repertoires. Further research addressing the following aims will enable our understanding of the mechanisms that underlie the role of immunologic imprinting and the effect of serial influenza exposure and vaccination on vaccine efficacy. The following studies will address this objective:

- Explore how immunity develops and evolves over time in different age cohorts.
- Characterize differences between immunity resulting from vaccination and from influenza infection.
- Define the influenza-specific B cell repertoire by birth year during times of changing viral subtype dominance across several decades.
- Characterize immune responses in those with a limited HAI response to infection or to vaccination.

- Define the balance of antibody responses to HA and NA and level of protection afforded.

Objective 2.2 Delineate the innate and adaptive immune responses to both natural infection and influenza vaccination.

Development of a universal influenza vaccine requires a deeper understanding of innate and adaptive immune responses that are necessary for protection, both systemically and at local tissue sites. Innate immune mechanisms may contribute to the protection afforded by HA antibodies. Growing evidence suggests that tissue resident innate and adaptive immune cells play a dominant role in protection, and that development of tissue-specific immunity is influenced by the route or location of initial antigen exposure, vaccine formulation, and vaccine modality [19, 20]. Once influenza infection is established, protection from clinical symptoms requires both B cell/antibody and T cell responses [21]. Research efforts under this objective aim to:

- Determine the contribution of innate immune cells in guiding adaptive immune responses to influenza infection and vaccination.
- Define the mechanisms of broadly protective humoral immunity against influenza, including processes that affect immunodominance based on antigen specificity, avidity, accessibility, or precursor frequency.
- Evaluate the roles of CD4+ and CD8+ T cells in protective immunity to influenza and immune-mediated pathogenesis and immune dysregulation including the use of vaccine prototypes in the human challenge model.
- Define mechanisms regulating the induction, development, regulation, trafficking, and maintenance of tissue resident B and T cell immunity, and the impact on protection from influenza infection.

Objective 2.3. Identify alternative mechanisms of protection beyond HAI-mediating antibodies.

The commonest way to measure immunity against influenza is to determine HA antibody levels that prevent viral attachment. However, recent evidence indicates that some broadly cross-reactive antibodies protect by mechanisms other than virus neutralization, for example, by antibody-dependent cellular cytotoxicity (ADCC). Standard neutralization assays do not assess these alternative mechanisms of protection. Therefore, novel assays are needed to test these mechanisms as additional correlates of human immunity relevant to vaccine efficacy. Research efforts under this objective aim to:

- Identify additional immune correlates of protection in the context of influenza vaccination.
- Develop methodologies to measure cytokines, antibody/B cells and T cell responses in relevant tissue sites.
- Improve the epitope specificity of immunological measurements.
- Define how the mechanisms of protective immunity differ for induction of sterilizing immunity versus protection from symptomatic disease.

Objective 2.4. Standardize/harmonize non-HAI based assays.

To facilitate the characterization of the immune responses, new assays and reagents must be developed, standardized and harmonized, particularly as new vaccine platforms and antigenic targets evolve. The following research efforts will address this objective:

- Measure specific HA stem antibody responses, including standardized protocols and reagents for assay development and validation.
- Develop standard *in vitro* high-throughput surrogate assays to measure influenza-specific ADCC, antibody-dependent cellular phagocytosis or complement dependent cytotoxicity [22-24].
- Improve high-throughput assays for measuring influenza neutralization against multiple strains.

- Determine NA content in vaccine and correlate levels of NA antibody commensurate with protection.
- Assess T cell-mediated immune responses (CMI) including tissue-resident responses.

Research Area 3: Support Rational Design of Universal Influenza Vaccines

Annual influenza vaccines do not provide robust protection against antigenically drifted variants, different influenza viral subtypes, or durable protection extending beyond the next influenza season. The next-generation influenza vaccine must capitalize on knowledge gained by assessment of the protective immune correlates elicited by natural infection and seasonal vaccination, and by experimental vaccine formulations (including adjuvants) and prime-boost regimens to rationally advance vaccine designs that maximize antibody- and cell-mediated immune responses. One path forward would utilize a coordinated, iterative approach in which identified correlates of protection in trials conducted through NIAID's clinical trial network informed the design of next generation vaccines. Targeted, incremental advances in vaccine design (e.g., inclusion of additional antigens or adjuvants) may help improve seasonal influenza vaccine effectiveness, inform efforts to achieve a universal influenza vaccine that confers broad, durable protection against multiple influenza viruses and reduce the need for annual vaccinations. An alternative path forward would utilize the combination of new insights and knowledge of the humoral and T cell responses defined by this strategy to design wholly new vaccine candidates for comparison to seasonal influenza vaccines in terms of superior efficacy, breadth, and durability.

The new era of vaccinology is driven by advances in structural biology and genomics, multiparameter single-cell sorting technology to facilitate B cell and T cell repertoire interrogation, rapid isolation of broadly neutralizing antibodies, and novel computational methods for protein design. In addition, new/improved T cell epitope predictive algorithms are providing insights into the effector/regulatory functionality of HLA-restricted, epitope-specific T cell subsets. These advances support a multidisciplinary and rationally-informed approach towards the design of vaccines for viruses whose major antigenic determinants are too variable to

use conventional vaccine approaches. These emerging strategies and approaches, coupled with a multitude of new vaccine expression and delivery platforms, make the next generation of improved influenza vaccines within reach. Research Area 3 aims to demonstrate the utility of new antigen design strategies, platforms and adjuvants in eliciting protective immune responses against influenza. Resulting vaccine strategies will be evaluated using an iterative approach to deliver clinical evidence supporting advancement of influenza vaccine(s) that provide broader protection against multiple influenza types and subtypes. NIAID has a history of pioneering structure-based and rational vaccine design through its intramural Vaccine Research Center as well as through its extramural grantees. Coordination of these resources will be promoted in the NIAID strategic plan towards development of a universal influenza vaccine.

Objective 3.1. Design new immunogens that elicit a wider breadth of protection.

Current seasonal influenza vaccines are focused on eliciting immunity to the globular head domain of the major viral glycoprotein, HA. However, this domain exhibits high plasticity and evolves continuously. Hence it does not represent an ideal target for broad protection. In contrast, conserved parts of the virus, such as the membrane-proximal stem domain of HA, the viral NA, the ectodomain of the ion channel M2 (M2e), and internal viral proteins, may be better targets. In addition to neutralizing activity, the contributions of other antibody effector functions and T cells must be considered when designing immunogens to achieve robust broad and durable protection against influenza. Research efforts under this objective aim to:

- Advance new vaccine approaches into clinical trials that exploit emerging antigen design strategies, novel technologies, and/or platforms.
- Refine and optimize vaccine products based on correlates of protection data obtained from cohorts, preclinical studies, and clinical trials, including data specific to age and risk subgroups.
- Identify vaccine candidate(s) that provide broad protection, superior to the seasonal influenza vaccine, and advance candidates to next phase of testing.

- Define mechanisms of vaccine-induced protection.
- Explore trial designs testing efficacy of vaccines that do not have traditional immunological endpoints of HAI or neutralization.

Objective 3.2. Test adjuvants and alternative delivery methods to enhance breadth and durability of immunity.

Adjuvants not only enhance the immune response against an immunogen and provide dose-sparing benefits, they can also be used to enhance specific components of the immune response which can impact durability and breadth of protection. To date, a limited number of influenza vaccines licensed in the United States include an adjuvant. Durable protection from influenza may require new vaccine designs that apply advances in the discovery and development of adjuvants to optimize vaccine responses. In addition, vaccine trial designs involving heterologous prime-boost strategies and/or alternative vaccination routes may offer broader and more effective tissue-resident protection compared to classical intramuscular administration. Research efforts under this objective include:

- Identify new antigen/adjuvant combinations that induce broad and long-term protection.
- Evaluate alternate routes of vaccine delivery (e.g., intranasal, oral, topical) in preclinical and clinical studies.
- Perform studies to examine heterologous prime-boost approaches to maximize protective immune responses.
- Evaluate use of currently available vaccines delivered by alternative methods or in different sequences and combinations.

Objective 3.3. Test promising vaccine platforms/candidates in 'iterative Phase I/II' clinical trials.

Early preclinical assessment will aid in down-selection of novel vaccine platforms, adjuvants and delivery systems. Clinical trials currently underway testing next generation vaccine candidates will reveal whether

specific immune strategies are feasible, safe and elicit broad, durable cross-reactive responses. Together, data on immunological assessments of correlates of protection generated from longitudinal cohort studies and ongoing and future Phase I/II novel vaccine trials will inform clinical refinement of influenza vaccine platforms through this iterative process. Research efforts under this objective aim to:

- Conduct Phase I clinical evaluation in healthy volunteers (including children) of new influenza vaccine candidates that have demonstrated broad protection in preclinical models.
- Conduct Phase I/II trials of vaccine candidates proven safe, and with promising immunogenicity profiles, in targeted age groups or at-risk populations.
- Evaluate the results of Phase II trials to identify next generation vaccine candidate(s) that elicit profiles predictive of broad against multiple influenza types and subtypes and protection more durable than one year.

Research Resources and Cross-Cutting Tools to Improve Influenza Vaccines

A coordinated effort of guided discovery, facilitated product development, and managed progress through iterative testing in clinical trials will be critical to achieving the goal of a universal influenza vaccine. NIAID will support gaps as outlined in Table 1, and also coordinate the efforts of a consortium of scientists to ensure movement towards the short-, medium, and long-term goals outlined in Table 2: Supplementary Material. In addition, NIAID will expand its support of the following research resources to enable progress in Research Areas 1-3 outlined in this strategic plan.

Animal Models to Advance Vaccine Development.

No single animal model completely recapitulates human disease. Currently, mice and ferrets are extensively used in basic research and translational studies, with recent advances enabling researchers to interrogate many aspects of influenza pathogenicity, transmission, and host immunity. However, there are still gaps and limitations in these models, including lack of immunological tools for species other than mice, differences in

host immune responses across species, inability to recapitulate in animals the effects of the complexities of human preexisting immunity, and a lack of standardized assays across different laboratories. The following will address the gaps in existing animal models:

- Determine the optimal utilization of current animal models for studying efficacy, natural history/pathogenesis, transmission, and mechanisms of immunity.
- Develop appropriate tools and reagents to maximize utility of current and future animal models.
- Develop new models to mimic the human immune response and to replicate the human experience of influenza immunity building over time.

Establish longitudinal cohorts for influenza research.

Humans encounter numerous influenza strains and vaccinations throughout their lifetimes and responses are determined by viral genetics, host factors, and prior exposure experiences. Expanded studies of influenza in humans will help answer questions related to transmission, pathogenesis, and immunity. Recent data provide strong implications regarding “immunologic imprinting” and the impact of serial vaccination that requires further investigation using long-term human cohorts, including cohorts of infants naïve to influenza infection or vaccination [15, 16]. Data from natural history studies in prospective cohorts are essential to understanding the immune response to influenza infection and vaccination. The following will address current knowledge gaps:

- Determine how various factors (e.g., genetics, age, immune status) impact influenza immunity and pathogenesis.
- Identify and utilize existing community-based, prospective, longitudinal cohort studies of influenza or other respiratory infections that may allow assessment of influenza immunity or vaccine effectiveness.

- Improve understanding of the heterogeneity of virus shedding among individuals and within the same individuals over time.

Increase capacity and capability for conducting human challenge studies.

There are many limitations of current animal models and clinical studies of natural infection in humans. Disease and susceptibility patterns in typical animal models such as ferrets and mice do not represent the spectrum of disease observed in humans. Natural history studies of humans are limited by the inability to pinpoint exposure and timing of illness, the lack of understanding of preexisting immunity, and the inability to predict what viruses are circulating yearly. Influenza challenge trials in healthy human volunteers may overcome certain of these limitations and offer a unique opportunity to ask focused questions regarding influenza pathogenesis and vaccine efficacy in a controlled manner. The following will address these gaps:

- Increase the number of sites capable of performing human challenge studies.
- Optimize and standardize available influenza challenge strains, disease models, and infection methods.
- Use the human challenge model to address questions related to influenza pathogenesis, immunity, and correlates of protection.
- Evaluate next generation universal influenza vaccine candidates in healthy volunteers for down-selection by comparison to best available conventional vaccines.
- Compare data from human challenge studies with data from studies of natural infection.

Develop systems biology approaches for influenza.

Systems biology is an interdisciplinary field that seeks to integrate diverse data sets and computational and mathematical analysis to build models of biological processes. The complex process by which individuals respond to an infection or vaccine, and the diversity of responses that individuals generate to the same

infection or vaccination, are well suited to systems biology analysis [14, 25]. The application of these approaches to analyze samples from infected persons or vaccinees may prove critical in revealing molecular and cellular signatures and biological processes that reflect effective, broad and long-term protective immunity against influenza. To address the knowledge gaps outlined above, specific goals include:

- Develop analytical tools to integrate and analyze diverse and multi-scale influenza infection and vaccination data sets from clinical samples to uncover correlates of protection and signatures of effective responses.
- Identify pathogen and host factors that correlate with and/or contribute to heterogeneous responses to influenza infection and vaccination, including protection from disease.
- Identify novel molecular signatures associated with vaccine/adjuvant candidates.
- Promote open sharing of data sets from clinical cohorts and animal models to enable computational identification of correlates of interest across multiple studies.
- Develop computational models that can predict protective responses to infection or vaccination.

CONCLUSION

Developing an influenza vaccine that improves the breadth and durability of protection against seasonal influenza and provides protection from pandemic strains is a high scientific priority for NIAID. NIAID will accelerate its efforts for developing a universal influenza vaccine by supporting a consortium of scientists focused on addressing obstacles that have limited progress toward this goal. NIAID will also support the expansion of research resources by establishing longitudinal cohorts, supporting improved animal models of influenza infection, and expanding capacity for conducting human challenge studies. In collaboration with scientists, industry, international partners, the World Health Organization, and regulatory agencies, NIAID is committed to helping achieve this important public health goal.

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Footnote Section:

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References

1. Paules C, Subbarao K. Influenza. Lancet **2017**; 390:697-708.
2. World Health Organization. Influenza. Available at: http://www.who.int/immunization/topics/influenza/en/. Accessed July 26, 2017.
3. Centers for Disease Control and Prevention. Disease Burden of Influenza. Available at: https://www.cdc.gov/flu/about/disease/burden.htm. Accessed September 18, 2017.
4. Centers for Disease Control and Prevention. Pandemic Influenza - Past Pandemics. Available at: https://www.cdc.gov/flu/pandemic-resources/basics/past-pandemics.html. Accessed July 28, 2017.
5. World Health Organization. Influenza - Avian and Other Zoonotic Influenza. Available at: http://www.who.int/influenza/human_animal_interface/en/. Accessed September 18, 2017.
6. Centers for Disease Control and Prevention. Seasonal Influenza Vaccine Effectiveness, 2005-2017. Available at: https://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm. Accessed January 31, 2018.
7. Paules CI, Sullivan SG, Subbarao K, Fauci AS. Chasing Seasonal Influenza - The Need for a Universal Influenza Vaccine. N Engl J Med **2017**.
8. Dinis JM, Florek NW, Fatola OO, et al. Deep Sequencing Reveals Potential Antigenic Variants at Low Frequencies in Influenza A Virus-Infected Humans. J Virol **2016**; 90:3355-65.
9. Joyce MG, Wheatley AK, Thomas PV, et al. Vaccine-Induced Antibodies that Neutralize Group 1 and Group 2 Influenza A Viruses. Cell **2016**; 166:609-23.

10. Wu NC, Wilson IA. A Perspective on the Structural and Functional Constraints for Immune Evasion: Insights from Influenza Virus. J Mol Biol **2017**; 429:2694-709.
11. Paules CI, Marston HD, Eisinger RW, Baltimore D, Fauci AS. The Pathway to a Universal Influenza Vaccine. Immunity **2017**; 47:599-603.
12. Ekiert DC, Kashyap AK, Steel J, et al. Cross-neutralization of influenza A viruses mediated by a single antibody loop. Nature **2012**; 489:526-32.
13. Centers for Disease Control and Prevention. People at High Risk of Developing Flu–Related Complications. Available at: https://www.cdc.gov/flu/about/disease/high_risk.htm. Accessed September 18, 2017.
14. Tsang JS. Utilizing population variation, vaccination, and systems biology to study human immunology. Trends Immunol **2015**; 36:479-93.
15. Gostic KM, Ambrose M, Worobey M, Lloyd-Smith JO. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. Science **2016**; 354:722-6.
16. McLean HQ, Thompson MG, Sundaram ME, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis **2014**; 59:1375-85.
17. Andrews SF, Huang Y, Kaur K, et al. Immune history profoundly affects broadly protective B cell responses to influenza. Sci Transl Med **2015**; 7:316ra192.
18. Cobey S, Hensley SE. Immune history and influenza virus susceptibility. Curr Opin Virol **2017**; 22:105-11.
19. Rosato PC, Beura LK, Masopust D. Tissue resident memory T cells and viral immunity. Curr Opin Virol **2017**; 22:44-50.
20. Kumar BV, Ma W, Miron M, et al. Human Tissue-Resident Memory T Cells Are Defined by Core Transcriptional and Functional Signatures in Lymphoid and Mucosal Sites. Cell Rep **2017**; 20:2921-34.
21. McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. N Engl J Med **1983**; 309:13-7.
22. Wines BD, Vanderven HA, Esparon SE, Kristensen AB, Kent SJ, Hogarth PM. Dimeric FcgammaR Ectodomains as Probes of the Fc Receptor Function of Anti-Influenza Virus IgG. J Immunol **2016**; 197:1507-16.
23. Mullarkey CE, Bailey MJ, Golubeva DA, et al. Broadly Neutralizing Hemagglutinin Stalk-Specific Antibodies Induce Potent Phagocytosis of Immune Complexes by Neutrophils in an Fc-Dependent Manner. MBio **2016**; 7.
24. Brown EP, Dowell KG, Boesch AW, et al. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods **2017**; 443:33-44.
25. Pulendran B. Systems vaccinology: probing humanity's diverse immune systems with vaccines. Proc Natl Acad Sci U S A **2014**; 111:12300-6.

Table 1: Filling the research gaps leading to a universal influenza vaccine

RESEARCH GAPS	ACTIONS
Research Area 1: Improve Understanding of Transmission, Natural History, and Pathogenesis of Influenza Infection	
Understanding influenza transmission	• **Expand existing programs** • **Increase support for investigator-initiated applications** • **Launch targeted funding opportunities**
Identify factors that impact disease severity	
Expand characterization of circulating influenza virus	
Research Area 2: Precise Characterization of Influenza Immunity and Correlates of Immune Protection	
Improve understanding of host response to infection/ vaccination	• **Expand existing programs** • **Increase support for investigator-initiated applications** • **Launch targeted funding opportunities** • **Initiate study of infant immunity imprinting in response to vaccination and infection**
Delineate innate and adaptive immune response to natural infection and vaccination	
Identify mechanisms of protection beyond HAI	
Standardize/harmonize non HAI assays	
Research Area 3: Support Rational Design of Universal Influenza Vaccines	
Design new immunogens to widen breadth of protection	• **Expand existing programs** • **Increase support for investigator-initiated applications** • **Launch targeted funding opportunities** • **Utilize NIAID clinical trial sites**
Test adjuvants and delivery methods	
Test candidates in iterative Phase I/II trials	
Research Resources and Crosscutting Tools	
Develop/improve animal models and reagents to advance vaccine development	• **Expand existing programs** • **Increase support for investigator-initiated applications** • **Launch targeted funding opportunities** • **Initiate study of infant immunity imprinting in response to vaccination and infection** • **Utilize NIAID clinical trial sites**
Establish longitudinal cohorts	
Expand human challenge study capability and capacity	
Develop systems biology approaches for influenza	
NIAID to launch a multidisciplinary consortium and coordinate activities to advance all research areas	

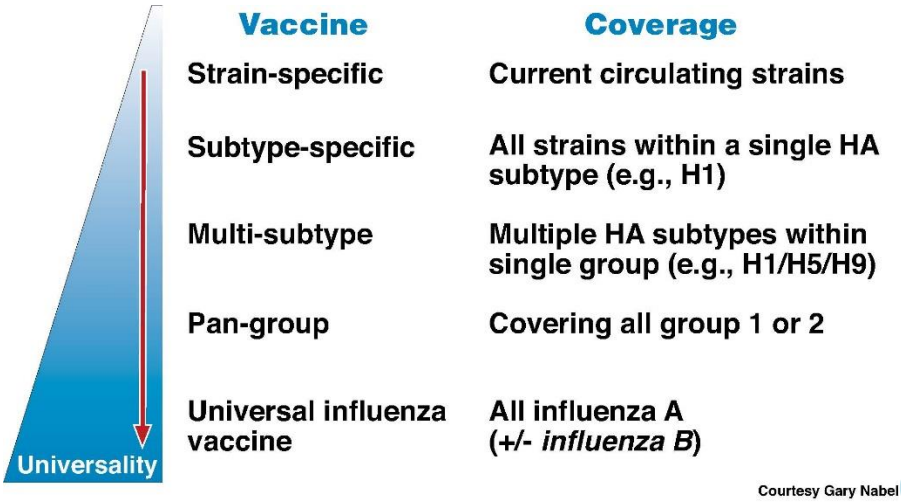

Figure 1: Steps Toward a Universal Influenza Vaccine. Breadth or coverage of vaccine protection against influenza viruses ranging from strain-specific protection (least breadth) to pan-group protection [11], then universal influenza vaccine (most breadth) [12].