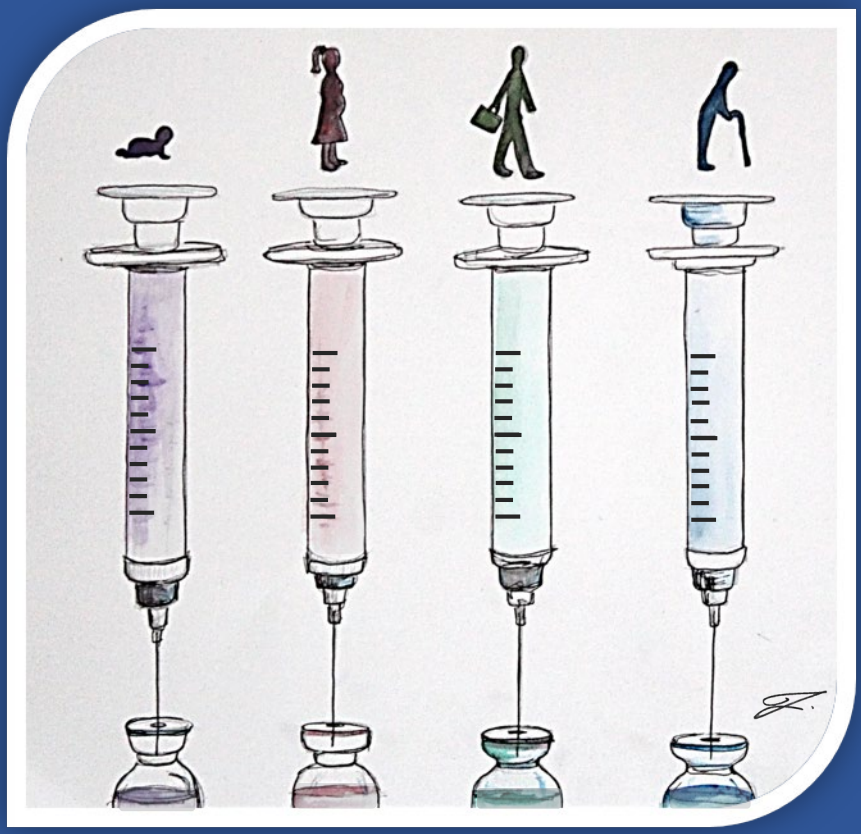
NIAID

National Institute of Allergy and Infectious Diseases

2018 NIAID STRATEGIC PLAN FOR RESEARCH ON VACCINE ADJUVANTS



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1. INTRODUCTION

1.1. BRIEF OVERVIEW OF ADJUVANTS IN LICENSED VACCINES

The use of adjuvants began in 1920 when the French veterinarian Gaston Ramon discovered that co-administration of inactivated diphtheria toxoid with starch, breadcrumbs, or other substances led to an increase in antitoxin responses to diphtheria. In 1926, this finding was followed by Alexander Glenny's discovery of the adjuvanticity of aluminum salts and their clinical utility in diphtheria immunization.

Until 2009, aluminum-based adjuvants, collectively termed "alum," were the only adjuvants in vaccines licensed for use in the United States. That year, the U.S. Food and Drug Administration (FDA) licensed the first of five vaccines containing novel adjuvants, which are described below and summarized in Table 1:

- Cervarix®, a human papillomavirus (HPV) vaccine containing AS04, a combination of alum and 3-O-desacyl-4'-monophosphoryl lipid A (MPL), an immune-stimulating lipid developed by GlaxoSmithKline (GSK). In the United States, Cervarix® is approved for use in females 10 through 25 years of age to prevent infection from HPV types 16 and 18, which cause about 70% of cervical cancer cases.
- Q-Pan H5N1, a pandemic H5N1 influenza vaccine containing AS03, an oil-in-water emulsion combination adjuvant developed by GSK. This vaccine is part of the U.S. vaccine stockpile but is not commercially available. It is intended for use in people 18 years of age and older.
- FLUAD®, an influenza vaccine containing MF59®, a squalene-based oil-in-water emulsion adjuvant developed by Novartis, for use in people 65 years of age and older. FLUAD® was first licensed in Italy in 1997 and is now used as a pediatric and adult influenza vaccine in more than 30 countries.
- Shingrix®, a shingles (herpes zoster) vaccine containing AS01, a liposome-based combination adjuvant containing MPL and the saponin QS-21, developed by GSK. Shingrix® is for use in adults 50 years of age and older.
- Heplisav-B®, a hepatitis B vaccine containing CpG-oligodeoxynucleotide (ODN), a short, single-stranded DNA molecule that mimics unmethylated bacterial DNA and triggers innate immune responses through activation of Toll-like receptor 9 (TLR9), developed by Dynavax. This vaccine is for use in people 18 years of age and older.

Table 1. FDA-approved vaccines containing novel adjuvants (2009-2018)

Year	Adjuvant	Vaccine Name
2009	AS04	Cervarix®
2013	AS03	Q-Pan H5N1
2015	MF59®	FLUAD®
2017	AS01B	Shingrix®
2017	CpG-oligodeoxynucleotide	Heplisav-B®

1.2. THE NEED FOR NEW VACCINE ADJUVANTS

Live-attenuated vaccines and some vaccines based on inactivated or killed agents do not require exogenous adjuvants, because the vaccines contain their own natural/endogenous adjuvants that can stimulate both the innate and adaptive arms of the immune system. However, subunit vaccines, which contain specific purified molecular entities, generally lack immune stimulators found in whole organism–based vaccines, leading to a need for exogenous adjuvants to induce protective immunity and desirable vaccine efficacy.

Unfortunately, not all adjuvanted vaccines induce the types of immune responses required for durable protective immunity. New or improved adjuvants may enhance the safety and efficacy of current vaccines, and they also are needed to improve vaccine efficacy in at-risk populations such as neonates, young children, pregnant women, the immunocompromised, and the elderly. Finally, vaccines do not exist for many known and newly emerging infectious agents, and the availability of new adjuvants, along with an understanding of their mode of action, may facilitate development of such vaccines.

1.3. NIAID'S MISSION AND EXTRAMURAL FOCUS ON VACCINE ADJUVANT RESEARCH

The overall mission of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), is to improve human health by supporting research to understand, treat, and prevent infectious and immune-mediated diseases. Critical components of NIAID's research agenda in support of this mission are the discovery and development of adjuvants, traditionally defined as vaccine components that augment and target immune responses.

NIAID's three extramural divisions—the Division of Allergy, Immunology, and Transplantation (DAIT); the Division of Microbiology and Infectious Diseases (DMID); and the Division of AIDS (DAIDS)—all support aspects of adjuvant research that are described in detail in Sections 2, 3, and 4. Briefly, the mission of each division and its adjuvant research focus is described below.

DAIT supports basic and clinical research to understand immune system development; define the mechanisms governing immune system function across the lifespan, providing knowledge that can be applied to develop and improve treatment and prevention strategies for inducing immunity against pathogenic infections; and develop better diagnostic, treatment, and prevention strategies for immune-mediated diseases and the rejection of transplanted organs. DAIT adjuvant research focuses on fundamental immunology, adjuvant discovery, and early-stage development. The latter includes compound screening, determination of adjuvant mechanisms of action, lead compound optimization and formulation, efficacy testing in animals, and investigational new drug (IND)–enabling studies. Adjuvant science programs supported by DAIT began in 2004 and include the Adjuvant Discovery Program, the Adjuvant Development Program, and the Molecular Mechanisms of Combination Adjuvants Program. Through these programs, more than 2 million compounds have been screened, and numerous adjuvant candidates, encompassing eight different adjuvant types

or classes, are being further developed. Other programs invested in characterizing immune responses to adjuvants include the NIAID Systems Approach to Immunity and Inflammation Program and the Human Immunology Project Consortium (HIPC).

DMID supports basic and applied research to control and prevent diseases caused by virtually all human infectious agents except HIV. The DMID portfolio includes a broad range of research from early discovery through product development for vaccines, therapeutics, and diagnostics, including novel vaccines (adjuvanted and unadjuvanted) and platform technologies. Currently, DMID is testing more than 100 different adjuvant candidates/formulations encompassing all stages of vaccine development. DMID also offers a comprehensive suite of preclinical services for the development and evaluation of adjuvanted and unadjuvanted vaccines.

DAIDS supports a comprehensive research portfolio to advance biological knowledge of HIV/AIDS and its related coinfections and comorbidities. The ultimate goal is to halt the spread of HIV through the development and implementation of an effective vaccine and biomedical prevention strategies that are safe and desirable. The adjuvants used in HIV vaccine candidates, as of April 2018, are listed in Appendix D.

1.4. NIAID'S STRATEGIC PLAN FOR RESEARCH ON VACCINE ADJUVANTS

As technologic and scientific advances increased our understanding of the pathways regulating innate and adaptive immune responses, NIAID recognized opportunities to develop novel adjuvants or modify existing adjuvants to safely trigger long-term protective immunity and durable vaccine efficacy. In 2010, NIAID prepared its first Strategic Plan for Research on Vaccine Adjuvants and convened an Adjuvant Blue Ribbon Panel (BRP) to review the plan.

The 2010 BRP made the following recommendations:

- Support additional research to understand how new and existing adjuvants and combinations of adjuvants enhance vaccine-specific immune protection
- Foster discovery and advancement of promising adjuvant candidates through optimization and preclinical testing stages
- Develop/improve and standardize animal models to evaluate adjuvant safety and efficacy, including development/use of animal models that more faithfully reflect immune responses of neonates/young children and the elderly
- Expand/standardize reagents for adjuvant discovery and development, including pathogen-specific reagents, and immunologic reagents for animals beyond traditional mouse models
- Identify more accurate predictors of adjuvant efficacy and/or reactogenicity
- Determine the effects of formulation on adjuvant mechanisms of action and safety and effectiveness of adjuvanted vaccines

Between 2010 and 2018, NIAID's extramural adjuvant discovery and development research programs have implemented these recommendations. Because of significant

advances in adjuvant science, many of which were driven by NIAID-supported programs, NIAID has updated its 2010 adjuvant research agenda, culminating in the 2018 Strategic Plan, which focuses on three areas:

- Fundamental immunology and adjuvant discovery
- Adjuvant development and preclinical evaluation
- Clinical evaluation of adjuvanted vaccines

NIAID convened a second BRP on April 23-24, 2018, to assess this Plan and provide insights and recommendations, which are included in appropriate sections of the Plan and summarized in Table 2.

Table 2. 2018 Blue Ribbon Panel Recommendations

Fundamental Immunology and Adjuvant Discovery

Fundamental Immunology
• Expand fundamental mechanistic studies to:
  o Identify innate correlates of adaptive immunity, including correlates of both protection and persistent immunity, for healthy and at-risk populations
  o Determine how antigen selection, including antigen processing/presentation mechanisms, impacts adjuvant functionality
 	
Adjuvant Discovery
• Expand discovery efforts beyond TLRs, to include adjuvants that trigger inflammasome components, target specific immune cells, or mimic immunologic outcomes of natural infections that induce long-term protective immunity
• Foster precision adjuvant discovery by first determining clinical/immunologic needs, followed by designing a discovery process to identify compounds that meet those needs
• Support development of functional screening methods that better mimic *in vivo* responses, taking into account human variability and at-risk populations
• Compare/optimize adjuvants for specific cell targeting and delivery routes
• Encourage development of standard operating procedures and best practices that maximize screening efforts and data sharing for cross-study analyses and benchmarking to licensed products
• Support central core services to further improve program integration: formulation assistance, production of standardized reference antigens, and animal testing (multiple models) for head-to-head adjuvant comparisons
• Emphasize importance of considering formulation at the early stages of adjuvant discovery

Adjuvant Development and Preclinical Evaluation

- Explore iterative testing of candidate adjuvants with a broad panel of antigens to identify the most promising combinations for further development
- Establish a public database/portal that describes the characteristics, functionality, and safety profiles of novel compounds developed in NIAID's adjuvant programs, including links to publications and clinical trials information, and procedures for requesting access to these adjuvants
- Support studies that develop methods to extrapolate adjuvant outcomes seen with one antigen to additional antigens
- Expand support of current Good Manufacturing Practice (cGMP) capabilities to support small-scale cGMP production of promising adjuvants for clinical trials

Clinical Evaluation of Adjuvanted Vaccines

- Support clinical trials of novel adjuvants and expand clinical comparisons of different adjuvants formulated with the same antigen
- Support clinical trials of adjuvanted vaccines in at-risk populations (e.g., elderly, children)
- Support comprehensive immunological analyses of samples from clinical trials to generate detailed immune profiles of adjuvant functionality
- Increase use of human challenge models, where applicable, to better assess the efficacy of adjuvanted vaccines
- Ensure at least a 12-month post-vaccination assessment for safety and immune durability

2. FUNDAMENTAL IMMUNOLOGY AND ADJUVANT DISCOVERY

2.1. RECENT PROGRESS IN NIAID-SUPPORTED ACTIVITIES

Fundamental Immunology

Fundamental immunology research supported by NIAID's portfolio of investigator-initiated grants and solicited research programs has identified many novel innate immune molecules and increased our understanding of innate and adaptive immunity. For example, investigator-initiated studies have led to the discovery of pathogen-associated molecular patterns (PAMPs), which trigger innate immune responses and are the basis of many adjuvants (e.g., they mimic PAMP-induced immune function). More recently, NIAID-supported investigators have determined that live/viable bacteria contain a unique class of PAMPs, termed vita-PAMPs, which are bacterial messenger RNAs (mRNAs) and cyclic di-adenosine monophosphate (c-di-AMP) that serve as signatures of viability and trigger specific innate immune responses. Similarly, investigators supported by one of NIAID's solicited research programs, Systems Approach to Immunity and Inflammation, have:

- Identified novel functions for more than 260 genes, many of which provide insights into signaling pathways triggered by innate immune receptors and will aid in mechanism-of-action studies of novel candidate adjuvants
- Developed a suite of ENU-mutant mice (https://mutagenetix.utsouthwestern.edu/) and Collaborative Cross (CC) mice (http://csbio.unc.edu/CCstatus/index.py) that can be used to confirm or determine an adjuvant's mechanism of action. Mice from both resources can be used in various ways to identify immune receptors or signaling pathways responsible for adjuvant function.

Novel animal models also have been developed through investigator-initiated research projects. For example, researchers at the University of Minnesota have developed the "dirty mouse" model by determining that the microbiome of pet store mice dramatically impacts immune system function. Dirty mice exhibit immune functionality more reflective of adult human responses, while immune function in laboratory mice is more similar to that of human infants. Co-housing the pet store mice with laboratory mice increases the microbiome diversity of the laboratory mice and changes their immune functionality to that of the pet store mice (e.g., more similar to adult human responses).

Adjuvant Discovery

NIAID began its first solicited program in adjuvant science in 2003 with the Adjuvant Discovery Program. This contract program supported the identification and characterization of novel, effective, and safe vaccine adjuvants. The scientific scope of this program included:

- Use of high-throughput screening methods for adjuvant identification

- Validation of adjuvant activity in human cells
- Optimization of lead adjuvant candidates through formulation and medicinal chemistry guided by structure–activity relationship (SAR) studies
- Mechanism of action studies
- Verification of adjuvanticity in animal models

The program was renewed in 2009. Building on knowledge gained from the 2003 and 2009 programs and to address recommendations made by the 2010 Adjuvant BRP, the 2014 program renewal emphasized discovery of adjuvants (1) for use in at-risk populations and (2) that induced mucosal immune responses. Recognizing the limitations of a program that renews only every five years, and following the recommendations of the 2010 Adjuvant Blue Ribbon Panel to increase the investment in adjuvant research, in 2017, NIAID began using the annual Small Business Innovation Research (SBIR) contract solicitation to support additional adjuvant discovery activities. This program, while new, already has expanded the investigator pool and increased the diversity of adjuvant candidates for the development pipeline.

Since its inception, researchers funded by NIAID's Adjuvant Discovery Program have screened more than 2 million compounds *in vitro* and more than 10^{24} *in silico* and identified a wide variety of promising adjuvant candidates, including:

- Synthetic TLR2, 4, 5, 7, 8, 7/8, and 9 agonists; nanoemulsions (for mucosal delivery)
- C-type lectin receptor (CLR) agonists
- CD1d ligands (natural killer T [NKT] cell inducers)
- Synthetic RIG-I agonists
- Carbohydrate-based adjuvants

Figure 1 shows representative immune receptors and pathways targeted by compounds and formulations that have resulted from the NIAID Adjuvant Discovery Program.

Fig. 1

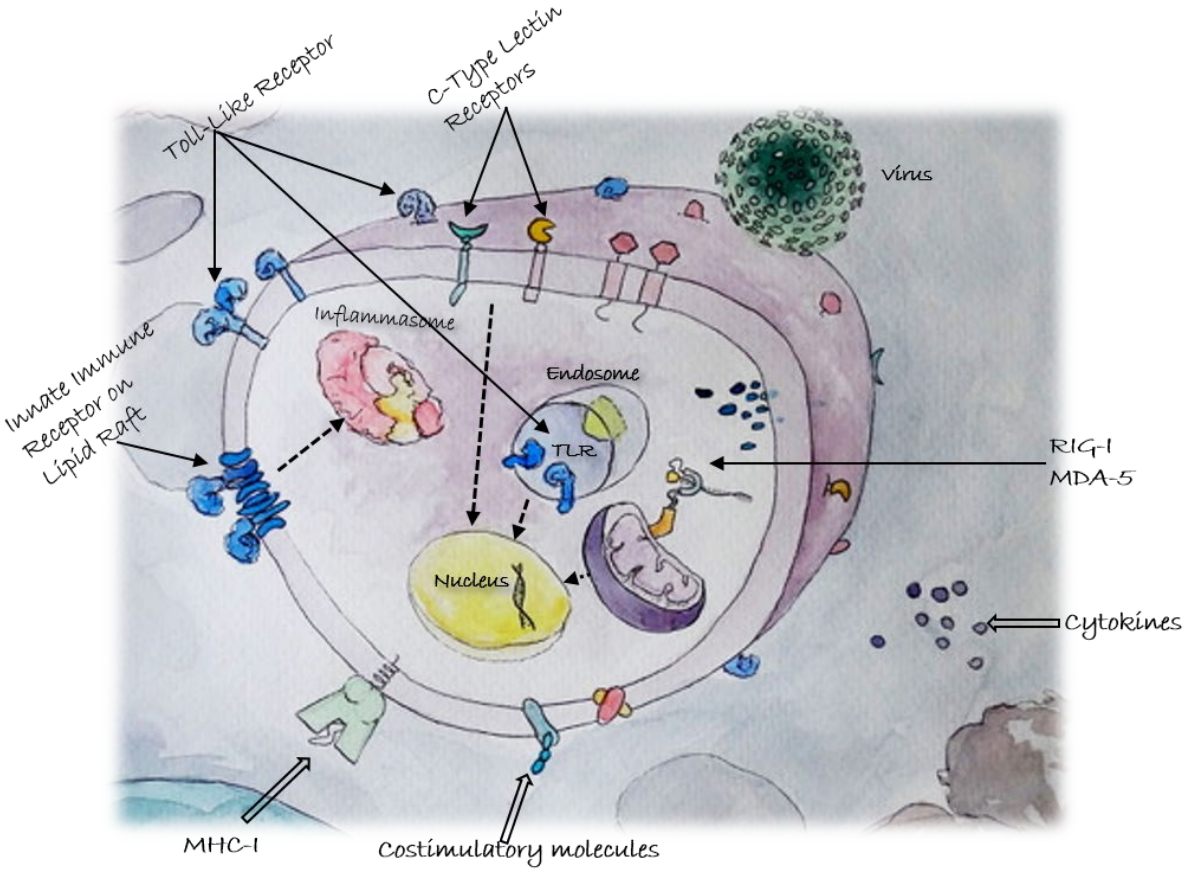

Figure 1: Vaccine adjuvants from NIAID's adjuvant discovery and adjuvant development programs target a variety of cellular receptors and signaling pathways. Some of these adjuvants (solid arrows) engage cell-surface and intracellular RIG-I-like receptors and C-type lectin receptors. In addition, adjuvants have been developed that directly act on signaling molecules of these receptors, act by rearranging lipid rafts and associated receptors, or provide adjuvanticity through yet undefined mechanisms (dashed-line arrows).

Specific accomplishments of these programs include identification of the following molecules with adjuvant activity, including:

- Novel CpG-ODNs that exhibit higher adjuvanticity than previously published CpG-ODN and are not species specific (e.g., able to trigger both mouse and human TLR9). One of these novel CpG-ODNs, originally identified through *in silico* screening, is now part of an inulin-CpG combination adjuvant that has been used in two clinical trials of influenza vaccines.
- Co-adjuvants in the form of small molecules that enhance the activity of an adjuvant but do not exhibit intrinsic adjuvant activity themselves. Such co-adjuvants have been shown to extend NF-κB signaling in antigen-presenting cells (APCs) and thus enhance lymphocyte activation.
- Novel oxoadenine-based TLR7/8 agonists with high potency and low reactogenicity

- Amphotericin B as a novel TLR2/TLR4 agonist with adjuvant activity and low reactogenicity
- Adjuvant formulations that target adjuvant activity to specific APCs or lymph nodes, reducing reactogenicity of potent imidazoquinoline (IMDQ)-based TLR7 agonists, such as:
  - Lysophospholipid conjugates of IMDQ
  - IMDQ-ligated nanogel
  - Hyaluronic-acid conjugated IMDQ
- First small-molecule TLR4 agonist, which is currently being developed as a combination adjuvant with TLR7 agonists for influenza vaccines
- Novel RIG-I agonists, as follows:
  - A small molecule compound called KIN, currently being developed for vaccines against emerging RNA viruses, including pandemic influenza and Zika
  - Synthetic, Sendai-virus-derived double-strand RNA (dsRNA) adjuvant for intranasal immunization against influenza
- Nanoemulsions (NEs) for intranasal or intramuscular injection, which act as delivery platform and adjuvant. NEs inactivate viruses and bacteria and can be used to produce killed, whole organism adjuvanted vaccines. Intranasal formulations can elicit strong Th1/Th17 responses upon boost with NE-adjuvanted vaccine, overcoming preexisting antigen-specific Th2 dominance. This approach is being developed for a recombinant influenza vaccine and a *B. pertussis* vaccine.
- Selective TLR8-agonist (methylpyrimidine-diamine) without TLR7 activity and ultra-low inflammatory profile
- Dendrimeric IMDQ construct with pure TLR7 activity, which has low reactogenicity and enhanced adjuvanticity (compared to monomeric IMDQ) and promotes antibody affinity maturation and epitope spreading
- First chemokine receptor CCR1 agonist adjuvant (bis-quinoline)
- NOD-1 agonist adjuvant (glutamyldiaminopimelic acid-derivative)
- Novel water-soluble TLR2 agonist
- Synthetic CRX compounds (MPL-derivatives), which are TLR4 agonists with high safety profiles. Several of these compounds were developed as adjuvants for intramuscular delivery and sublingual formulation (combination adjuvant with methylglycol chitosan) for influenza vaccines. They also are being developed as combination adjuvants with novel TLR7/8 agonists.
- Protein cage nanoparticles (PCN) for mucosal delivery to induce tertiary lymphatic tissue

The Adjuvant Discovery Program has also identified molecules with valuable, non-adjuvant properties that include:

- Novel antimicrobials
  - Antivirals against Zika and dengue viruses
  - Antibiotics such as a modified TLR7/8 agonist without TLR activity, which acts as a potent inhibitor of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA)
  - Pure TLR7 agonists currently being evaluated as HIV latency-reversal agents

- Large libraries of TLR7, TLR8, and TLR7/8 agonists that modulate immune responses and can be used as tools to study TLR7 and TLR8 signaling pathways

2.2. PROGRAM GOALS AND RESEARCH CHALLENGES

Goal 1: Strengthen Immunology Research

The most significant advances in NIAID's Adjuvant Discovery Programs have come from increased knowledge of innate immune receptors and their mechanisms of action (Figure 1).

The first innate immune receptor agonists developed as adjuvants targeted TLR4 and TLR9. NIAID-supported researchers have expanded screening to include molecules that bind to NOD-like receptors and C-type lectin receptors, as well as molecules involved in signal transduction from innate immune receptors, such as various kinases and interferon regulatory factors. Furthermore, an increased understanding of how innate and adaptive immune cell subsets contribute to vaccine-induced immunity is leading to the discovery of adjuvants that target cells other than macrophages and dendritic cells. These cells include B cells, mast cells, and NKT cells.

While considerable progress has been made in discovery of innate immune receptors and their mechanisms of action, several challenges remain. These include:

- **Limitations of existing methods to evaluate potential adjuvant function *in vivo*.** Most screening approaches are based on a limited number of parameters, such as NF-κB activation or the induction of select cytokines from either cell lines or primary cells. *In vitro* induction of those factors may not translate into adjuvanticity *in vivo*. Better methods to evaluate adjuvant function, based on increased knowledge of immunologic mechanisms, will ensure that promising candidates are not being overlooked.

- **Selection of appropriate animal models for adjuvant screening.** Host species differences in immune receptor specificity and cellular distribution, immunogenetics, and pathogen susceptibility can complicate the discovery of adjuvants for human use. One example is TLR8, which was thought to be nonfunctional in mice until the recent discovery of the mouse TLR8 ligand and the realization that it differs significantly from the human TLR8 ligand. The selection of appropriate animal models for adjuvant screening is critically important.

- **Adjuvants for at-risk populations, especially the very young and frail elderly.** The recent successes of the AS01-adjuvanted Shingrix® vaccine and MF59®-adjuvanted influenza vaccine in the elderly and the AS03- and MF59®-adjuvanted influenza vaccines licensed outside the U.S. for young children clearly demonstrate that adjuvants can overcome age-associated immune deficits. However, additional adjuvants are still needed for use in the very young and the frail elderly.

Goal 2: Support Technology Development for Adjuvant Discovery

Adjuvant discovery methods have improved in recent years for two main reasons:

- Technologic advances have allowed for more efficient *in vitro* screening of compounds with progressively higher throughput rates.
- Advances in medicinal chemistry have enabled rapid and systematic modifications of molecules to alter their immune stimulatory properties.

In addition, the focus of adjuvant discovery has shifted from natural compounds and/or derivatives of known innate immune receptor agonists to small molecules that bear no structural similarities to natural compounds. These molecules may bind to sites on an immune molecule that may or may not overlap with the binding site for the natural ligand. These types of adjuvants are identified by high-throughput screening of chemical libraries.

Recently, *in silico* approaches have been employed (1) to pre-screen compound libraries to reduce the number of compounds that need to be screened *in vitro* or (2) to explore the potential utility of computer-generated structures before synthesis and *in vitro* testing. While *in silico* screening has the potential to revolutionize adjuvant science, this approach still faces challenges, which include the needs to:

- **Determine structure–activity relationship (SAR) to improve the accuracy of *in silico* screening methods.** SAR analysis defines the relationship between a compound's chemical or three-dimensional structure and its biological activity. Insights gained through SAR analysis can be used to rationally alter structure and hence the potency or functionality of a compound. Application of SAR to *in silico* screening of adjuvants requires knowledge of the structure of the immune receptor being targeted.

- **Define binding site requirements.** Recent identification of small-molecule compounds that can bind to innate immune receptors but lack structural similarity to the natural receptor ligands highlights gaps in our understanding of the requirements for receptor activation. In addition, ligand binding is not necessarily associated with receptor activation. Thus, *in silico* modeling alone can be misleading and needs to be coupled with laboratory validation to improve overall accuracy.

- **Provide access to supercomputing facilities.** The most frequently used algorithms for *in silico* screening simulate static docking of putative ligands to their receptors, rather than the dynamic interactions that occur naturally between receptor and ligands. Modeling such dynamic interactions requires supercomputing capacity that is not readily available to many researchers.

2.3. NIAID'S STRATEGY TO ADVANCE ADJUVANT DISCOVERY

Based on NIAID's goals and recommendations from the BRP, NIAID's 2018 Strategic Plan for Research on Vaccine Adjuvants focuses on two major areas: strengthen

immunology research and support technology development.

- Strengthen immunology research, to specifically improve evaluation of adjuvant function, provide new animal models for adjuvant screening, and facilitate the discovery of adjuvants for use in at-risk populations. To meet this goal, NIAID will continue to support and expand current programs, and develop new programs, as follows:
  - Current programs
    - The Systems Approach to Immunity and Inflammation Program, which is identifying novel immune response genes through development and analysis of novel mouse models. The data and novel mouse models generated through this program will enhance adjuvant discovery efforts.
    - The Immunity in Neonates and Infants program and the Immunity in the Elderly program, which are characterizing immune functionality and facilitating identification of possible adjuvant targets in these at-risk populations.
    - The Adjuvant Discovery Program, which continues screening, preclinical testing, and early-stage development of new adjuvant candidates and will pursue discovery of immune modulating or tolerogenic adjuvants.
    - The Molecular Mechanisms of Combination Adjuvants (MMCA) program, which is providing novel insights into the mechanisms of combination adjuvant activity and the basis for synergistic adjuvanticity.
  - FY 2019 new NIAID initiatives
    - Maintaining Immunity After Immunization, which will increase understanding of how durable immunity is induced and maintained in response to infection or vaccination and will identify novel targets for adjuvant discovery
    - Collaborative Cross (CC) Mouse Model Generation and Discovery of Immunoregulatory Mechanisms, which will support the use of CC mouse lines to increase understanding of the host genetics involved in immune regulation and function
  - NIAID will continue to use the SBIR contract mechanism to fund additional adjuvant discovery by small business entrepreneurs and the development of immunologic reagents for underrepresented animal models that may be better models of human immune responses (e.g., ferrets for influenza research).
  - In addition to its solicited adjuvant portfolio, NIAID will maintain and expand its portfolio of unsolicited adjuvant grants using a variety of mechanisms, such as SBIR, R01, R03, R21, and T grants.

- Support technology development for adjuvant discovery. For example:

  - NIAID will expand support of structural immunology studies to enhance SAR analyses and improve the accuracy of *in silico* adjuvant discovery methods. Currently, most of the structural immunology research supported by NIAID is through investigator-initiated grants. While these studies have provided valuable insights into the structure–function relationships of many important immune molecules, technologic advances in this area would increase the number of

available structures for use by the adjuvant discovery community. NIAID will consider using the SBIR contract funding mechanism and other approaches to grow this important research area.

- NIAID's Office of Cyber Infrastructure and Computational Biology (OCICB) provides sophisticated biocomputing resources to NIAID's intramural researchers. NIAID extramural staff will work with OCICB staff to make these supercomputing capabilities available to adjuvant researchers in the extramural community.

3. ADJUVANT DEVELOPMENT AND PRECLINICAL EVALUATION

3.1. RECENT PROGRESS IN NIAID-SUPPORTED ACTIVITIES

Adjuvant Development Program

NIAID expanded its solicited program in adjuvant science in 2008 with the Adjuvant Development Program. This contract program supports:

- Preclinical development of novel adjuvants coupled with specific vaccines, including mechanistic analyses of the novel adjuvant/antigen combinations
- Formulation and dosing studies
- Optimization of immunization regimens and delivery routes
- cGMP production

This program was recompeted in 2013 and 2018 with the goal of ensuring further development of promising adjuvant candidates through preclinical testing and IND-enabling studies, thus expanding the number of adjuvants available for clinical evaluation. Several of the compounds evaluated through the Adjuvant Development Program originated from NIAID's Adjuvant Discovery Program, and some have been or are currently being tested in more than 20 clinical trials. In 2016, NIAID began using the annual SBIR contract solicitation to support additional adjuvant development activities.

The adjuvant/antigen or adjuvant/vaccine combinations assessed through the Adjuvant Development program and SBIR contract program include:

- Microcrystalline inulin with seasonal, pandemic, and universal influenza vaccine candidates
- Nanoemulsions with a plant-derived, recombinant H5 influenza vaccine or HIV envelope virus-like particle (VLP) vaccine
- Fully synthetic TLR4 agonists plus plant-derived saponin (QS-21), coupled with a recombinant E protein from West Nile virus
- A small-molecule RIG-I agonist combined with split H5N1 influenza virus, UV-inactivated West Nile virus, or a VLP-based vaccine for Zika virus
- The protein-based mucosal double-mutant heat-labile toxin (dmLT) adjuvant, with BCG and the recombinant fusion protein, 5fu, from *Mycobacterium tuberculosis* and a recombinant IpaB-IpaD fusion protein from *Shigella sonnei*
- A novel semi-synthetic analog of QS-21 (TiterQuil-1055™) with Fluzone®, Fluarix®, and Flublok® influenza vaccines

Adjuvants in HIV Preclinical Vaccine Development

NIAID also supports adjuvant development within vaccine-focused research programs. NIAID's HIV preclinical vaccine development activities focus predominately on variations of HIV Env immunogens in combination with an array of adjuvants and/or delivery

systems to enhance potency and durability. These studies are conducted in both small animals and nonhuman primates. An extensive variety of adjuvants and adjuvant combinations have been tested, with the most common adjuvants being Alhydrogel® and other aluminum salts formulations, MF59®, AS0 series, Adjuplex™, ISCOMATRIX®, pIL-12, and multiple TLR agonists alone or in combination (3M-052, CpG, poly ICLC, R848, GLA-SE).

Adjuvants in Non-HIV Preclinical Vaccine Development

NIAID's vaccine research and development activities for non-HIV infectious diseases include three different types of vaccines: live attenuated vaccines; inactivated vaccines; and subunit vaccines composed of purified protein, recombinant protein, or carbohydrate. These preclinical research and development efforts focus on early discovery of adjuvanted vaccine targets and IND-enabling activities, including providing biological resources, animal testing, process development, Good Laboratory Practice (GLP) toxicity assessment, and cGMP production of vaccines or vaccine components. Early-stage research on adjuvanted vaccines includes pairing of antigens with adjuvants, formulation studies, and the design and construction of novel vaccine candidates in which an adjuvant is built into the vaccine construct. Examples of this latter approach include co-display of the adjuvant and antigen on the surface of nonpathogenic engineered microbes and nanoparticle molding technologies for co-delivery of adjuvant and antigen. NIAID also supports development of animal models that are relevant for or mimic natural infection or diseases of public health importance to evaluate vaccine formulation. These models are especially important for vaccine research for biodefense or emerging infectious diseases, since the data generated may be utilized for vaccine emergency use authorization, accelerated approval, or pivotal efficacy studies under the Animal Rule.

NIAID also supports the development of adjuvanted vaccines in some high-priority research areas through investigator-initiated research grants and various program initiatives, such as SBIR contracts, Centers of Excellence for Translational Research, Advanced Development of Vaccine Candidates for Biodefense and Emerging Infectious Diseases, and Research to Advance Vaccine Safety. Selected examples of current activities include evaluation of:

- Candidate tuberculosis (TB) vaccines containing traditional and novel adjuvants or adjuvant systems, as follows:
  - The cyclic dinucleotide (CDN) class of molecules (a vita-PAMP), which includes c-di-AMP, as regulators of *M. tuberculosis* physiology and the host immune response to infection and vaccination. Research is underway to determine the adjuvant potential of c-di-AMP in a preventative tuberculosis vaccine.
  - The cationic liposomal adjuvant CAF09, which induces both CD4+ and CD8+ T cells
  - An adjuvant platform to produce micelles and vesicles incorporating mycolic acid, CpG, and MPL for intranasal immunization
  - A wide range of adjuvants, including alum, GLA-SE, GLA-AF, QS-21, CpG-SE, and liposomes, which are being evaluated independently and in combination with

either anionic, neutral, or PEG liposomes to identify the optimal adjuvant pairing with the *M. tuberculosis* antigen ID93

- Candidate malaria vaccines containing:
  - The novel glycolipid adjuvant 7DW8-5 combined with a live attenuated sporozoite vaccine
  - Traditional adjuvants such as Alhydrogel®, MPL/Alhydrogel®, ISA 51 VG, ISA 720 VG, GLA-SE, GLA-LSQ, AddaVax™, and CpG 7909, in malaria subunit-based vaccines
  - Nano/microparticle technology platforms for a malaria transmission-blocking vaccine

Programs in Support of Adjuvanted Vaccine Development

Several of NIAID's basic immunology programs facilitate selection of appropriate antigens and evaluation of antigen-specific immunity generated by candidate adjuvanted vaccines, including:

- The Immune Epitope Database and Analysis Resource (IEDB, www.iedb.org), which provides public access to B and T cell immune epitope information curated from the scientific literature or through direct submission by the research community, and sophisticated epitope prediction and analysis bioinformatics tools
- The B-Cell Epitope Discovery and Mechanisms of Antibody Protection program and the Large-Scale T-Cell Epitope Discovery program
- The NIH Tetramer Core Facility (http://tetramer.yerkes.emory.edu/)
- The Human Immunology Project Consortium (HIPC) program, which uses novel approaches, including metabolomics, transcriptomics, and proteomics, to conduct comprehensive systems immunologic analyses of human immune responses to infections, adjuvants, and/or vaccines
- The Cooperative Centers on Human Immunology (CCHI) program, which is developing novel technologies and bioinformatics tools to conduct mechanistic analyses of human immune responses to infections and vaccines (adjuvanted and unadjuvanted)
- The Modeling Immunity for Biodefense program, which includes the development of computational tools that analyze antibody-antigen interactions and facilitate design of more immunogenic or cross-protective antigens that can be paired with adjuvants

Preclinical Development Services and Support

NIAID also provides a comprehensive suite of preclinical development services that fill particular knowledge and experimental gaps critical to move adjuvanted vaccine candidates along the product development pathway. These resources include:

- BEI Resources, a central repository that supplies organisms and reagents, including those relevant for vaccine or adjuvant research to the research community
- The Preclinical Models of Infectious Diseases program, which provides services to researchers to study the full range of pathogens, including bacteria, viruses, parasites,

fungi, and other agents such as toxins and prion proteins. The program's main focus is to help researchers who have developed a product such as a vaccine that needs to be tested in an animal model, but who lack either the resources or expertise needed to perform that testing, and to support the development and refinement of animal models for infectious diseases of importance to humans.

- NIAID's Vaccine Development Services, which are intended for use in the investigation, control, prevention, and treatment of a wide range of infectious agents and to support the development of vaccines; vaccine components, including adjuvants; vaccine delivery systems; and challenge material. Vaccine testing services include assay development, immunogenicity and efficacy studies, clinical and nonclinical sample testing, and safety and toxicity testing. Vaccine manufacturing services include writing product development plans, product optimization, non-cGMP and cGMP manufacturing of antigens and adjuvants, assay development, and regulatory activities, including audits.

NIAID will continue to support new program initiatives to address the development and evaluation of important vaccines and vaccine technologies, including adjuvants. Examples of some existing programs are:

- Centers of Excellence for Translational Research (for emerging/reemerging vaccine technologies)
- Novel Vaccine Technologies and Strategies to Promote Sustained Vaccine Efficacy (for malaria and pertussis)
- Advanced Development of Vaccine Candidates for Biodefense and Emerging Infectious Disease (for vaccines, including influenza and TB; and technologies, including adjuvants)
- Research to Advance Vaccine Safety

These services and programs support a wide range of preclinical development needs for vaccines, including adjuvanted vaccine construction; *in vitro* and *in vivo* assessment of candidate products; generation of data regarding optimization, synthesis, and formulation of candidates; manufacturing using cGMP standards; and safety assessments such as GLP toxicity studies.

3.2. PROGRAM GOALS AND RESEARCH CHALLENGES

Goal 1: Expand Availability of Novel Adjuvants for Preclinical Vaccine Development and Clinical Evaluation

Vaccine developers tend to look for adjuvants with well-established safety records, preferably in humans, when selecting an adjuvant for their candidate vaccines. NIAID's adjuvant development activities include mechanism-of-action studies to gain insights into immune pathways triggered by adjuvants and ensure that off-target effects are identified and mitigated early in the development process. These efforts will be crucial in assembling a pool of well-characterized, potent adjuvants with proven safety records that can be used to accelerate development of effective vaccines.

The major challenge associated with early stage development and preclinical assessment of novel adjuvants and adjuvant vaccines is:

- **The ability to correlate safety and efficacy observed in animal models to human responses.** Animal models, while valuable, are not able to emulate many of the factors affecting responses in humans. These factors include:

    - Pre-existing conditions and medications; recent infection history and chronic infection status (e.g., cytomegalovirus [CMV], Epstein-Barr virus [EBV])
    - Genetics/epigenetics (e.g., species-specific TLR expression patterns and ligand binding)
    - Microbiome composition
    - Behavior, including exercise and recreational drug use
    - Nutrition and diet
    - Environmental exposures

In addition, current animal models may not identify the most effective vaccine regimens or predict adjuvant-vaccine reactogenicity in humans. Although certain cytokines, other soluble molecules, and transcriptional networks have been associated with reactogenicity, well-defined signatures that reliably predict reactogenicity have not been identified. Concerns about adverse events have slowed the development and licensure of adjuvanted vaccines, especially for at-risk populations such as the very young, pregnant women, and the elderly.

Goal 2: Optimize Formulation, Antigen/Adjuvant Pairing, and Support cGMP Production

Development of novel adjuvanted vaccines involves activities that require specialized, multidisciplinary teams with strong formulation expertise. NIAID's adjuvant and vaccine development programs support formulation studies, including the iterative process required to optimize antigen/adjuvant pairing, and cGMP production of lead adjuvant and adjuvant-antigen formulations for further downstream preclinical development and clinical evaluation. Formulation studies are crucial during adjuvant development and vaccine construction. However, they also must be considered during the early stages of adjuvant discovery and adjuvant-antigen pairing, since inadequate or suboptimal formulation may prevent promising novel adjuvants or adjuvanted vaccines from moving forward in development.

The major challenges associated with these activities include:

- **Formulation considerations.** Subtle differences in formulations can significantly impact immunogenicity, stability, and safety of adjuvanted vaccines. In addition, due to significant advances in antigen discovery and development of protein expression platforms, most vaccines currently in development are subunit vaccines. Pairing the correct protein antigens with the appropriate adjuvants during the early development stage is critical for future success of a safe and efficacious vaccine. Currently, given the

lack of correlates or surrogates of protection for most pathogens, head-to-head comparisons of multiple adjuvants with specific vaccine antigens may be the most efficient process for identifying the optimal adjuvants for a given antigen.

Despite the vital importance of formulation, including co-formulation of adjuvant/antigen pairing, adjuvant development efforts frequently lack adequate consideration of formulation, which can impact:

- Proper adjuvant selection and retention of antigenicity/immunogenicity
- Targeting of the adjuvant to the correct immune cells and lymphatic tissues
- Co-localization of antigen and adjuvant, or proper antigen conformation
- Timed release of the adjuvant to prevent induction of excessive innate immune responses and the potential for reactogenicity
- Selection of the optimal delivery system and vaccination strategy

- **cGMP manufacturing.** Bottlenecks for vaccine developers in academia and small businesses are (1) limited access to proprietary adjuvants; (2) development of scalable, robust manufacturing processes for promising novel adjuvants; and (3) lack of sufficient quantities of cGMP material for well-characterized adjuvants/adjuvanted vaccines required for early phase clinical trials.

3.3. NIAID'S APPROACH TO ADVANCE ADJUVANT DEVELOPMENT AND PRECLINICAL EVALUATION

To address the issues raised above and to continue the development of promising adjuvanted vaccine candidates, NIAID will support the following activities:

- Expand availability of novel adjuvants for preclinical vaccine development and clinical evaluation by improving the ability to correlate safety and efficacy profiles generated in animal models to human responses.

  To accomplish these efforts, NIAID will:

  - Continue NIAID's Preclinical Models of Infectious Disease program, which provides a central resource for development and refinement of animal models, including traditional small laboratory animals, nonhuman primates, and nontraditional animals such as swine. This program also offers *in vivo* screening and efficacy testing in appropriate animal models.
  - Continue NIAID's SBIR contract program for the development of immunologic reagents for underrepresented mammalian models, including ferrets, guinea pigs, and swine, to expand the types of animal models that can be used for detailed immunologic analyses of adjuvant function
  - Maintain and expand development of novel animal models that more accurately reflect human immune responses to adjuvanted vaccines across all age groups/populations through NIAID's Systems Approach to Immunity and Inflammation program and through a new funding opportunity, starting in 2019, to

support the use of CC and CC-RIX mouse lines that more faithfully reproduce human immune responses
    - Increase support to HIPC and CCHI investigators to:
        - Accelerate further development and validation of fully human artificial "immune systems" and immune tissue explants
        - Conduct additional systems immunology analyses of head-to-head comparisons of novel adjuvant/antigen combinations

- Optimize formulation and antigen/adjuvant pairing activities.

  To accomplish these efforts, NIAID will:

    - Renew the Adjuvant Development Program in 2018, which includes a stronger emphasis on formulation and head-to-head comparisons of adjuvant/antigen combinations. NIAID also will continue to award SBIR contracts to increase adjuvant development. Community access to adjuvants developed under these programs will be expanded through targeted NIAID supplements.
    - Expand development of novel adjuvanting technologies, including conjugation technologies or self-adjuvanting vaccine platforms, through the SBIR adjuvant development contracts and other funding mechanisms
    - Expand development of co-adjuvants to include checkpoint inhibitors that enhance APC or T-cell responses by extending immune activation or by blocking negative regulators
    - Continue to support adjuvant process development to enable cGMP production capabilities for adjuvant production through NIAID's Adjuvant Development program
    - Encourage and continue to support preclinical development of adjuvanted vaccines in the areas of product optimization, process development, formulation studies, cGMP production, and GLP safety assessment through NIAID's Vaccine Development Services, Broad Agency Announcements (BAAs), partnership programs, and SBIR contract solicitations to accelerate the transition of adjuvanted vaccine candidates into clinical evaluation

4. CLINICAL EVALUATION OF ADJUVANTED VACCINES

4.1. RECENT PROGRESS IN NIAID-SUPPORTED ACTIVITIES

Adjuvanted Vaccines for Non-HIV Infectious Diseases

Since 1998, NIAID has supported 133 vaccine clinical trials for non-HIV infectious diseases, primarily through the Vaccine and Treatment Evaluation Units (VTEUs). Forty-seven of these clinical trials have included adjuvanted vaccines, with adjuvants such as Alum (used in 13 of these trials), MF59®, AS03, glucopyranosyl lipid adjuvant (GLA, a synthetic TLR4 agonist), and GLA with QS-21 in liposomal formulation. Recently completed and ongoing trials of adjuvanted vaccines for non-HIV infectious diseases are summarized in Appendix C and described below:

- Phase I and II clinical trials of inactivated influenza vaccine candidates with established adjuvants in multiple combinations, as part of its influenza pandemic preparedness program
- Exploration of novel vaccination strategies, including heterologous prime-boost interval studies using H7 influenza vaccines
- In collaboration with GSK, the direct comparison of two adjuvants, AS03 and MF59®, with seasonal influenza vaccines. The trial has been completed and results will be released to the public once data analyses are completed.
- A first-in-human Phase I clinical trial with a purified formalin-inactivated Zika virus vaccine candidates with alum adjuvant, in a rapid response to this emerging viral disease threat
- Two clinical trials exploring vaccines with novel adjuvants
  - TLR7/8 agonist, Imiquimod, to enhance the efficacy of a pre-pandemic H5 influenza vaccine
  - Recombinant double-mutant heat-labile toxin (dmLT), which acts as both antigen and adjuvant in a vaccine against enterotoxigenic *E. coli* (ETEC). The dmLT adjuvant can induce strong mucosal immune responses after parenteral delivery.
- Evaluation of tuberculosis (TB) vaccine candidates in Phase I clinical trials using new combination adjuvants, GLA-LSQ and GLA-SE
- Evaluation of new combination adjuvants such as GLA-LSQ for a malaria vaccine or Alhydrogel® plus GLA in Aqueous Formulation (GLA-AF) for schistosomiasis vaccines
- Use of the human challenge models to evaluate, compare, or narrow the field of choices of different vaccine formulations for hookworm vaccines

Adjuvanted Vaccines for HIV

NIAID's HIV vaccine program has been exploring multiple vaccine platforms to achieve protective immunity against HIV that include HIV DNA vaccines or viral vectors boosted or combined with adjuvanted recombinant Env protein(s). Two complementary strategies are being used: an empirical approach that builds on partial clinical success to expeditiously move vaccine candidates into human testing and a theoretical approach that

selects vaccine candidates for further development based on an understanding of the immune response to HIV infection. Completed and ongoing trials of HIV adjuvanted vaccines are described below and summarized in Appendix D.

The Empirical Approach

- This approach relies mostly on the landmark RV144 study in Thailand, the first and only (as of April 2018) large clinical trial to demonstrate modest efficacy for an investigational HIV vaccine. RV144 evaluated the safety and estimated the efficacy of a prime-boost combination of two vaccine components given sequentially: ALVAC-HIV, which uses a canarypox virus as a vector—or carrier—to deliver HIV genes; and the recombinant AIDSVAX B/E, HIV Env protein formulated with alum hydroxide gel (Rehydragel™). At the end of the 3.5-year study period, investigators observed a 31% reduction in risk of HIV infection among vaccine recipients compared to those who received a placebo.

  Analysis of the immune responses induced by the vaccine suggests that a lower risk of infection is associated with:

  - Plasma immunoglobulin G (IgG) antibodies targeting the V1V2 region of HIV Env
  - Low plasma levels of HIV-Env-specific IgA (which compete with protective IgG)
  - Polyfunctional CD4+ T cells (CD154, interleukin-2 [IL-2], IL-4, interferon-gamma [IFN-γ], and tumor necrosis factor alpha [TNF-α])
  - IgG antibodies harboring Fc effector functions needed for antibody-dependent cell-mediated cytotoxicity (ADCC), but only in vaccinees with low-plasma, HIV-Env-specific IgA

  To follow up on this encouraging outcome, the P5 partnership (Pox-Protein-Public-Private-Partnership, http://www.vaccineenterprise.org/content/P5Partnership) was established in 2010 to substantiate and improve upon RV144 using a modified pox-protein HIV vaccine. Phase I studies include:

  - Side-by-side comparison of adjuvant formulations
    - Boost of HIV Env formulated with Rehydragel™ vs. MF59® after ALVAC prime (HVTN 107)
    - Boost of HIV Env formulated with MF59® vs. AS01B after DNA prime (HVTN 108)
    - Boost of HIV Env formulated with MF59® vs. AS01B after ALVAC prime (HVTN 120)

- Prior to completion of the RV144 trial, many adjuvants were tested in Phase I clinical trials in combination with HIV immunogens: Rehydragel™, Alhydrogel®, Adju-Phos®, MF59®, AS01B, and GLA-SE. Two Phase IIb studies used similar recombinant Env subunit proteins only (without the ALVAC prime) as part of the regimen (VAX003, VAX004) that were formulated with Rehydragel™, but these studies did not show efficacy.

The Theoretical Approach

- This approach targets the induction of broadly neutralizing antibodies (bNAbs) against HIV. Based on HIV natural infection studies and an exquisite characterization of the structure of the HIV Env spike, there is a better understanding of the requirements to induce bNAbs. The immunogens must first engage the B cell harboring the germline lineage of desired antibodies, and then, through multiple cycles of affinity maturation, the gene encoding immunoglobulins may acquire the mutations needed to provide broad and potent HIV neutralization activity. T follicular helper (Tfh) cells, the drivers of affinity maturation in the lymph node, also are sought to generate better bNAbs. In addition to high levels of somatic hypermutation, other unusual structural features of bNAbs include a long heavy chain complementarity-determining region 3 (HCDR3), capable of reaching through the glycan shield of the virus, and autoreactivity.
- Two additional clinical trials involving direct comparisons of adjuvants are under development. The first is supported by the HIV Vaccine Trials Network (HVTN) and will use a stabilized trimer BG505 SOSIP.664 immunogen, adjuvanted with 3M052+Alum, CpG 1018, or GLA-LSQ. The second trial, a partnership between NIAID and the U.S. Military HIV Research Program, will test a DNA prime, gp145 HIV Env protein boost, adjuvanted with several Army Liposome Formulations (ALFs), Alhydrogel®, and dmLT.

4.2. PROGRAM GOALS AND RESEARCH CHALLENGES

Goal 1: Increase the Availability of Well-Characterized Novel Adjuvants for Use in Vaccine Clinical Trials

One of NIAID's major goals is to develop vaccines against pathogens and their toxins. This goal includes clinical evaluation in both domestic and international populations, which often have distinct pathogen exposure histories that can impact host immunity. Therefore, adjuvants capable of inducing Th1/Th17, Tfh, CD8+ T cell, or mucosal immune responses or that are effective in at-risk populations are especially needed.

Challenges associated with these activities include:

- **Difficulty in reconciling human clinical data and animal preclinical data to improve the design and further evaluation of adjuvanted vaccine candidates.** The primary endpoints of early-phase clinical trials of adjuvanted vaccines are safety and immunogenicity. However, sample sizes are generally small and lack sufficient statistical power to dissect safety or identify correlates of protection, both of which are critical parameters in determining whether a vaccine candidate moves into later-phase clinical trials or needs further refinement. Preclinical animal efficacy studies may provide some insights regarding safety and efficacy, but species differences in immunity, pharmacogenetics, and reactogenicity need to be considered.
- **Limited number of adjuvants available from commercial sources.** Although NIAID has supported many clinical trials of vaccine candidates against infectious diseases,

those requiring adjuvants continue to rely on a limited number of sources. Access to novel adjuvants already in clinical development is hampered by intellectual property (IP) restrictions. In addition, adjuvant IP holders may either oppose or not be interested in direct comparisons of their formulation with those they do not own. Bringing more potent adjuvants into the clinic represents a significant financial and administrative burden for any adjuvant developer. Many of these developers reside at academic institutions or own small businesses and have neither experience with the process nor the funding needed to bring products into the clinic.

Goal 2: Maximize Evaluation of Data from Clinical Trials

Although many well-designed clinical trials generate valuable datasets and contain a wealth of information, the lack of correlates of protection for most pathogens complicates the rational selection of an adjuvant for the clinical evaluation of a novel vaccine. Other issues related to evaluation of data from clinical trials of adjuvanted vaccines include:

- Formulations identified in Phase I clinical trials as safe and immunogenic that may fail in advanced clinical development or efficacy field trials
- Immunological analyses of clinical trial samples commonly focus on certain parameters known or assumed to be associated with protection against a specific disease. However, such assumptions limit the breadth of immunologic information produced and our ability to identify and validate immune correlates of protection associated with adjuvant functionality.
- The lack of standardized assays to measure the immunogenicity of an adjuvant combined with different antigens. This data would establish immunological profiles of adjuvants and determine how much those profiles are affected by the antigen component of the vaccine.

Sample-sparing techniques, such as those supported by NIAID's Sample Sparing Program, have improved significantly in recent years allowing more parameters to be determined with small numbers of cells or volumes of whole blood or serum. Simultaneously, the cost of multiplex assays has decreased making the routine generation of high-density datasets from clinical trials samples highly feasible.

The major challenge associated with this goal is:

- **Access to data from adjuvant studies and adjuvanted vaccine and human challenge clinical trials.** Many investigators conducting adjuvant research or clinical trials do not deposit their datasets into publicly accessible databases such as ImmPort, because they either are unaware of relevant databases or lack incentives or the expertise to comply with data submission requirements. These requirements may include the collection of specific meta-data parameters, data types, and use of controlled vocabularies.

4.3. NIAID'S APPROACH TO ADVANCE THE CLINICAL EVALUATION OF ADJUVANTED VACCINES

To address the challenges raised above and to continue the evaluation of novel adjuvants in clinical trials, NIAID plans to foster/support the following activities:

- Generation, collection, curation, and public availability of clinical data on the immunological activity of vaccine adjuvants to inform their use in subsequent trials
- Expansion of NIAID-sponsored research using human challenge models to evaluate the efficacy of candidate adjuvanted vaccine formulations
- Support of clinical trials to compare and down-select novel adjuvanted vaccine candidates
- Continued/expanded investment in the development of computational models that can reconcile data from human clinical trials and preclinical animal studies to improve the design and efficacy of adjuvanted vaccines
- Continued investment in the development of sample sparing assays to enable evaluation of more immunological parameters in small clinical samples
- Use of NIAID-sponsored programs such as HIPC, CCHI, and Systems Biology programs to:
  - Conduct research to identify biomarkers and correlates/surrogates of immune protection or safety/reactogenicity
  - Assist in the design of immunological analyses of clinical trials samples
  - Enable collaborations between existing NIAID networks and clinical trials sites through targeted administrative supplements
- Expansion of NIAID's Bioinformatics resources, including ImmPort (www.immport.org), to:
  - Develop a public database of reference adjuvants and their immunologic activity
  - Provide access to immunologic and other datasets generated by NIAID-supported clinical trials

5. SUMMARY

The goal of NIAID's adjuvant discovery and development programs is to generate a diverse panel of well-defined, safe, and effective adjuvants that can be paired with candidate vaccines to induce the desired protective immune profiles in all relevant populations.

To achieve this goal, NIAID will continue to:

- Support and expand its fundamental immunology research portfolio to identify the requirements for inducing effective immune responses and long-lasting protection in all individuals
- Advance adjuvant discovery and early stage development, specifically targeting the critical immunologic parameters described in the relevant sections above
- Support the discovery of adjuvanted vaccines; animal testing and screening, including GLP safety assessment of adjuvanted vaccine formulations; process development; and production of clinical grade materials of vaccines and vaccine adjuvants
- Explore the feasibility of a NIAID-sponsored cGMP vaccine/adjuvant manufacturing facility for small-scale clinical trials
- Explore creative approaches to promote and accelerate clinical testing of promising vaccine candidates formulated with novel adjuvants

6. APPENDICES

6.1. APPENDIX A: ABBREVIATIONS

ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
ALF	Army Liposome Formulation
APC	Antigen-Presenting Cell
BAA	Broad Agency Announcement
bNAb	Broadly Neutralizing Antibody
BRP	Blue Ribbon Panel
CC	Collaborative Cross
CCHI	Cooperative Centers for Human Immunology
c-di-AMP	Cyclic Di-Adenosine Monophosphate
CDN	Cyclic Dinucleotide
cGMP	Current Good Manufacturing Practice
CLR	C-Type Lectin Receptor
CMV	Cytomegalovirus
DAIDS	Division of AIDS
DAIT	Division of Allergy, Immunology, and Transplantation
DMID	Division of Microbiology and Infectious Diseases
dmLT	Double-Mutant Heat-Labile *E. coli* Toxin
dsRNA	double-strand RNA
EBV	Epstein-Barr virus
ETEC	Enterotoxigenic *E. coli*
FDA	Food and Drug Administration
GLA	Glucopyranosyl Lipid Adjuvant
GLP	Good Laboratory Practice
GSK	GlaxoSmithKline
HCDR3	Heavy Chain Complementarity-Determining Region 3
HIPC	Human Immunology Project Consortium
HPV	Human Papillomavirus
HVTN	HIV Vaccine Trials Network
IEDB	Immune Epitope Database and Analysis Resource
IgG	immunoglobulin G
IL-2	interleukin-2
IFN-γ	interferon-gamma
MMCA	Molecular Mechanisms of Combination Adjuvants
MPL	Monophosphoryl Lipid
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NE	Nanoemulsion
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NKT	Natural Killer T
OCICB	Office of Cyber Infrastructure and Computational Biology
ODN	Oligodeoxynucleotide
PAMP	Pathogen-Associated Molecular Patterns

PCN	Protein Cage Nanoparticles
SAR	Structure–Activity Relationship
SBIR	Small Business Innovation Research
TB	Tuberculosis
Tfh	T Follicular Helper
TLR	Toll-Like Receptor
TNF-α	tumor necrosis factor alpha
VLP	Virus-Like Particle
VTEU	Vaccine and Treatment Evaluation Units

6.2. APPENDIX B: BLUE RIBBON PANEL MEMBERS

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6.3. APPENDIX C: NIAID-SPONSORED CLINICAL TRIALS FOR NON-HIV ADJUVANTED VACCINES, RECENTLY COMPLETED OR ONGOING (AS OF APRIL 2018)

Adjuvant	**Pathogen**	**Pathogen Vaccine Antigen/Approach**	**Phase ClinicalTrials.gov Identifier**
dmLT	Enterotoxigenic *E. coli*	dmLT antigen	Phase I NCT02531685
AS03 vs. MF59®	Influenza A/H5N8	A/gyrfalcon/Washington/41088-6/2014	Phase I NCT02624219
AS03 vs. MF59®	Influenza A/H5N8	A/gyrfalcon/Washington/41088-6/2014	Phase I NCT03014310
AS03	Influenza A/H7N9	Monovalent A/Hong Kong/125/2017	Phase II NCT03312231
AS03	Influenza A/H7N9	Monovalent A/Hong Kong/125/2017, with seasonal IIV4	Phase II NCT03318315
AS03	Influenza A/H7N9	Monovalent A/H7N9 A/Shanghai/2/2013	Phase II NCT02921997
MF59®	Influenza A/H7N9	Monovalent A/H7N9 A/Shanghai/2/2013, with live attenuated H7N9	Phase I NCT02251288
GLA-SE vs. AP10-602	*M. tuberculosis*	ID93 antigen	Phase I NCT02508376
Alhydrogel® +/− GLA-AF	*N. americanus*	Co-administered Na-GST-1 and Na-APR-1 antigens	Phase Ib NCT02476773
Alhydrogel® +/− GLA-AF or CpG 10104	*N. americanus*	Na-GST-1 antigen	Phase II NCT03172975
Alhydrogel® +/− GLA-AF (AP 10-701)	*Schistosoma spp.*	Sm-TSP-2 antigen	Phase Ia NCT02337855
Alhydrogel® +/− GLA-AF (AP 10-701)	*Schistosoma spp.*	Sm-TSP-2 antigen	Phase Ib NCT03110757
Alum	Zika	Zika virus purified inactivated vaccine	Phase I NCT02963909

6.4. APPENDIX D: NIAID-SPONSORED CLINICAL TRIALS FOR HIV ADJUVANTED VACCINES, COMPLETED OR ONGOING (AS OF APRIL 2018)

Adjuvant	HIV Vaccine Antigen/Approach	Phase ClinicalTrials.gov Identifier
Adju-Phos®	Ad26.Mos.HIV or Ad26.Mos4.HIV boosted by gp140 Clade C protein	Phase I NCT02788045
Adju-Phos®	Ad26.Mos4.HIV boosted by gp140 Clade C protein	Phase I NCT03060629
Alhydrogel®	gp145 Clade C Env protein	Phase I NCT03382418
GLA-SE	EnvSeq1: CH505 TF gp120 series	Phase I NCT03220724
GLA-SE	Polyvalent DNA boosted by gp120 (A, B, C, AE)	Phase I NCT03409276
pIL-12	PENNVAX®-GP DNA	Phase I NCT02431767
MF59®	ALVAC (vCP2438) boosted by bivalent subtype C gp120	Phase I/II NCT02404311
MF59®	ALVAC (vCP2438) boosted by bivalent subtype C gp120	Phase IIb/III NCT02968849
MF59®	DNA-HIV-PT123 boosted by bivalent subtype C gp120	Phase I NCT02997969
MF59®	gp140 Clade C boost to HVTN073	Phase I NCT01423825
MF59®	gp140 Clade C	Phase I NCT01376726
MF59®	MVA-C boosted by gp140, DNA-C2 boosted by MVA-C, or DNA-C2 boosted by MVA-C and gp140	Phase I NCT01418235
Rehydragel™	DNA-HIV-PT123 or NYVAC-HIV-PT1/PT4 prime with AIDSVAX B/E boost	Phase I NCT01799954
Rehydragel™	ALVAC (vCP1521) boosted by AIDSVAX gp120 B/E	Phase I NCT02109354
Rehydragel™	DNA-HIV-PT123 boosted by AIDSVAX gp120 B/E	Phase I NCT02207920

Adjuvant	HIV Vaccine Antigen/Approach	Phase ClinicalTrials.gov Identifier
Rehydragel™	ALVAC (vCP1521) boosted by AIDSVAX gp120 B/E	Phase III NCT00223080
pIL-12 and pIL-15	PENNVAX-B DNA	Phase I NCT00528489
MF59® vs. AS01B	DNA-HIV-PT123 boosted by bivalent subtype C gp120	Phase I NCT02915016
MF59® vs. AS01B	ALVAC (vCP2438) boosted by bivalent subtype C gp120	Phase I/IIa NCT03122223
MF59® vs. Rehydragel™	ALVAC (vCP2438) boosted by bivalent subtype C gp120	Phase I NCT03284710