Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Strategic Plan

Research Toward Development of an Effective AIDS Vaccine

Fiscal Year 2001

Table of Contents

Figures

VACCINE RESEARCH CENTER
STRATEGIC PLAN

Research Toward Development of an Effective AIDS Vaccine

Introduction

The Vaccine Research Center is dedicated to translating the latest concepts in disease pathogenesis and immunology into new vaccine strategies, providing safe and effective means to prevent and control human diseases. The primary focus of the Vaccine Research Center (VRC) will be to conduct research to develop an effective AIDS vaccine. The global epidemic of HIV infection is one of the most significant infectious disease threats to human health. Although new AIDS diagnoses and deaths have fallen significantly in many developed countries, the HIV/AIDS epidemic continues to accelerate in the developing world. There are an estimated 5.5 million new HIV infections each year, and AIDS now causes more deaths worldwide than any other infectious disease. In 1999, HIV/AIDS was the fourth overall leading cause of mortality worldwide, resulting in an estimated 2.8 million deaths. Beyond the human tragedy of HIV/AIDS, the epidemic poses a significant impediment to the economic growth and political stability of many countries. In developing countries and in segments of the U.S. population, anti-HIV therapies are frequently beyond financial reach. Accordingly, effective, low-cost tools for HIV prevention are urgently needed to bring the HIV epidemic under control. A globally effective, accessible vaccine remains the best hope for ending this pandemic.

To combat HIV, we now have at our disposal new information about the molecular and immunological basis of disease and improved tools for analysis of virus structure and measurement of immune responses. This scientific knowledge forms the basis for new ideas that may lead to novel strategies for effective vaccination. In addition, the scientific and industrial infrastructure has advanced to facilitate production and evaluation of vaccines. Nonetheless, the process of moving vaccine concepts through preclinical development and into initial clinical trials can be slow and unpredictable. Years of investment and research are required to progress through initial vaccine research, preclinical testing and development of a vaccine. In this setting, the VRC has a unique opportunity and responsibility to facilitate the transition of new concepts in microbial pathogenesis, mechanisms of immunity, and vaccine design into clinical applications.

The impact and importance of vaccines cannot be overstated. Vaccines are powerful public health tools that provide safe, cost effective and efficient means of preventing morbidity and mortality from infectious diseases. They have revolutionized the control of infectious diseases, virtually eliminating polio, smallpox and measles; however, an effective vaccine against HIV poses unique obstacles. HIV strains worldwide display tremendous genetic diversity that may limit the breadth of protective immunity elicited by

a single vaccine. Two types of HIV can be distinguished genetically and antigenically: these have been termed HIV-1 and HIV-2. HIV-2 is endemic in West Africa but is rare outside this region, whereas HIV-1 is the cause of the global pandemic. HIV-1 is subclassified into distinct genetic subtypes, or clades. For reasons that are not clear, these subtypes have distinct geographic distributions. To be effective, an HIV vaccine, or vaccines, will have to elicit immune responses against diverse strains of HIV-1. Also, because HIV attacks the primary cells of the immune system, persistent infection fails to produce effective immunity in a large percentage of the population. We are just beginning to understand how the virus evades immunologic surveillance to cause persistent infection and disease. As a result, it has been difficult to study immune mechanisms that will protect against, or control HIV-infection. These unique features of HIV infection, coupled with our lack of knowledge about protective immunity, create the foremost challenges to the development of an effective AIDS vaccine.

Historically, the process of vaccine development can be characterized as empiric, guided more by trial and error with inactivated or attenuated organisms than by rational design that builds on basic concepts in immunology and virology. While this process has been successful for numerous important infectious agents, many diseases remain for which no vaccine exists. A new science of vaccinology is now emerging that takes advantage of the latest technologies and scientific knowledge to design effective vaccine strategies. This process of rational vaccine design is closely coordinated with evaluation of vaccine candidates in animal models and human clinical trials. By embracing new discoveries and using them for the rational design of experimental vaccines, an iterative process of vaccine development, in which clinical evaluation informs basic research, will be established. Much as the great strides achieved in cancer treatment have been driven by clinical applications of new knowledge about the basic mechanisms of cancer, development of an effective AIDS vaccine will benefit from a thorough understanding of the basis of protective immunity to the virus and the mechanisms by which HIV evades immune surveillance. By having diverse components of vaccine research, development, production, and evaluation readily accessible at one site, along with a group of committed investigators with diverse skills but a common goal, the VRC has embarked on a comprehensive and systematic approach to vaccine development.

The Role of the Vaccine Research Center

The science of vaccinology is by its nature interdisciplinary, combining basic and applied research in immunology, virology, disease pathogenesis, molecular biology and structural biology with clinical trials methodology. By encompassing these activities at a single center possessing the capacity for vaccine production, the VRC hopes to advance the science of vaccine development. VRC scientists have expertise across the spectrum of the vaccine development process: basic immunology, molecular biology, preclinical testing, clinical trials and vaccine production. These areas complement each other and promote a spirit of collaboration and exchange, both within the center and with outside collaborators, which should provide a foundation for training the vaccinologists of the future.

The Vaccine Research Center has 3 major goals*:*

1. Scientific design and rational development of effective vaccine candidates.
2. Evaluation and optimization of immune responses generated by candidate vaccines.
3. Advancement of promising vaccine candidates into human trials.

These goals are based on the following hypotheses:

- A combination of broad and potent CD4- and CD8-mediated T-cell immune responses, and functional antibodies to the appropriate viral epitopes, will prevent HIV infection or control HIV disease.
- Vaccine candidates can be constructed to elicit these responses in animal models and human subjects.
- The definition of protective immune correlates will be paramount to the development of a highly effective AIDS vaccine.

Based on these hypotheses, the VRC will undertake a rational approach to vaccine design aimed at devising vaccine strategies that induce protective immune responses. The non-human primate model will be used to evaluate vaccine candidates and to elucidate the immune responses that prevent or control HIV.

There are three potential target goals for AIDS vaccines that could be addressed by immunization, each of which could have different biomarkers, or correlates of protection:

1) Sterilizing immunity, i.e., prevention of persistent HIV-infection.
2) Prevention of HIV disease and AIDS.
3) Therapeutic immunization to control HIV-infection.

Ideally, an AIDS vaccine would provide complete protection against viral infection. Studies in monkeys will evaluate immune responses that could potentially provide such protection; however, it is understood that many effective vaccines do not provide sterilizing immunity, but instead prime immune responses that allow the host to contain the invading pathogen after initial infection. In the case of HIV, vaccine-induced immunity could contribute to the effective control of HIV replication leading to protection against HIV-disease progression and potentially to a reduction of HIV transmission. Finally, vaccine candidates can also be evaluated in HIV-infected volunteers to study vaccine immunogenicity and the effect of eliciting virus-specific immune responses on HIV replication and disease course.

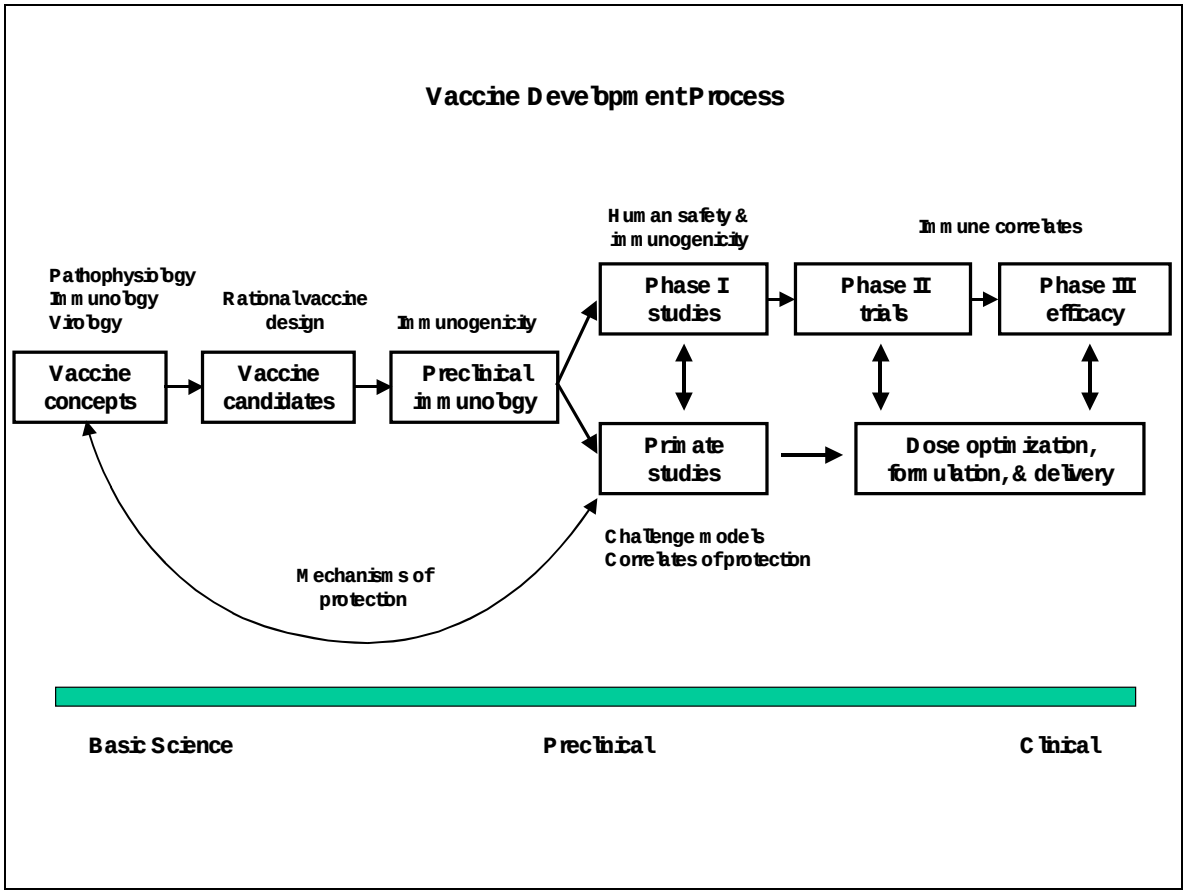

Figure 1. Integration of the VRC research activities into the vaccine development process. A systematic approach to vaccine development begins with basic research on the infectious agent, disease pathophysiology and protective immune responses. This knowledge generates hypotheses for vaccine concepts that can be tested in animal and human trials. Vaccine candidates are constructed and screened, and the most promising candidates are advanced to further testing. The non-human primate animal model is used both for evaluation of vaccine strategies and for elucidation of correlates of protective immunity. Ultimately, hypotheses of protective immunity are tested in phase III vaccine trials.

This strategic plan describes the VRC's goals and objectives, and the resources needed to achieve them. These goals are integrated with the NIAID and OAR Strategic Plans for Vaccines, which focus on rational vaccine design, development of vaccines against pathogens associated with emerging and potentially re-emerging global health problems, development of vaccines against pathogens of high public health importance that have been previously intractable to vaccine development, and vaccine discovery and development in newly identified areas of vaccine need.

MAJOR GOALS

GOAL 1. Scientific design and rational development of effective vaccine candidates.

Recent data from several animal model systems strongly suggest that both humoral and cellular immunity play key roles in protection against HIV infection and disease. Based on the hypothesis that a combination of potent CD4- and CD8-mediated immune responses, and functional antibodies to appropriate viral epitopes, will prevent HIV infection or control HIV disease, the preclinical research program will explore basic science questions relevant to vaccine design. The aim is to develop vaccine candidates that induce effective humoral and cellular immune responses. Guided by continuing research that reveals a better understanding of the basic elements of protective immunity, scientists at the VRC will apply this knowledge toward the design of vaccines. A program in virus structural biology will explore the rational design of immunogens that can induce potent virus neutralizing antibodies. Development of candidate vaccines will focus on gene-based immunization, using both DNA plasmids and viral vectors, such as MVA and adenovirus, to boost DNA-induced immune responses. Small animals, and the SIV and chimeric SIV/HIV macaque model, will be used in a systematic manner to evaluate safety, immunogenicity and degree of protection provided by candidate vaccines. Such pre-clinical animal testing will be closely integrated with the basic science programs to provide information for iterative improvements in the development of new immunogens.

Objectives

A. Utilize knowledge of HIV envelope structure to design immunogens that elicit potent virus neutralizing antibodies through a program of rational structure-based design and screening of immunogens.

Antibodies that neutralize HIV develop slowly over the course of natural infection and are difficult to elicit via immunization. In particular, HIV envelope proteins have proven to be poorly immunogenic with regard to eliciting potent antibody responses. Since passive infusion of such antibodies protects primates from chimeric SIV/HIV (SHIV) infection, the focus of this program will be the rational design of a vaccine that can elicit a broad and potent antibody response. While structure-based drug design has revolutionized drug development, as exemplified by the rapid evolution of the HIV protease inhibitors, vaccine design has traditionally placed little reliance on structural information. In part, this is because the mechanisms of antigen-immune system interactions are more complex than typical drug-target interactions. Still, structural biology has the potential to contribute an unparalleled source of information toward vaccine design.

On HIV, the primary targets of neutralizing antibodies are the envelope glycoproteins, gp120 and gp41, which reside on the outer surface of the virus. By using innovative crystallographic techniques, the structure of gp120 was determined at atomic resolution, allowing direct visualization of numerous overlapping mechanisms of immune evasion.

These include conformational change, steric occlusion, islands of variation and a carbohydrate cloak, all of which serve to disguise the gp120 surface from immune detection. This new picture of the envelope structural proteins gives us a better understanding of why it is so difficult to develop protective antibodies against HIV. The question that remains to be answered is whether knowing the precise atomic coordinates, and thus having access to the underlying chemistry of gp120, permits the precise design of a broadly effective HIV vaccine.

The availability of the atomic structure of gp120 makes possible a novel approach to vaccine development using functional constraints coupled with structural information to design modified gp120 molecules. By using functionally constrained variant HIV-1 envelope glycoproteins, we propose to create modified immunogens capable of eliciting HIV-1 neutralizing antibodies. The design of such molecules will be guided by structural information and biophysical, antigenic, and immunogenic analyses of the HIV-1 envelope glycoprotein function. The aim is to disable the numerous mechanisms of natural gp120 immune evasion and thus produce a modified gp120 that elicits a broad and potent neutralizing antibody response. Such a response should generate protective immunity against HIV.

A.1 Understand envelope-based mechanisms of immune evasion.

- Study antibody recognition of gp120, including biophysical, structural and genomic characterization. Perform structural and immunologic analysis of antigenic determinants that are required for the activity of the few known antibodies that potently neutralize HIV.
- Perform structural analysis of primary envelope gp120/CD4 complexes.
- Study structures of the gp120 glycoprotein in alternative conformational states and design altered gp120 molecules with weakened mechanisms of immune evasion using atomic information from the x-ray crystal structure and other complementary methods.

A.2 Advance immunogen development through structure-assisted vaccine design (SAVD).

- Design modified gp120/41 molecules to allow immune recognition of key cryptic epitopes that are not immunogenic under native conditions.
- Construct immunogens to mimic the natural trimeric gp120/41 structure on virions.
- Express and purify modified envelope proteins, or utilize genes encoding these proteins, to evaluate the immune response in small and large animal models.
- Perform serological, biophysical and immunological analyses to determine which molecules elicit the most broad and potent virus neutralizing antibodies.
- Perform complete structural determination and analysis of promising vaccine candidates, providing the atomic information necessary to initiate subsequent cycles of the iterative process of structure-assisted vaccine design..

- Identify potential new immunogens by mass screening of recombinant immunogen libraries using known broadly neutralizing monoclonal antibodies and/or other immunologic screening assays.

B. Develop and optimize gene-based vaccine platforms that elicit broad and potent cell-mediated and humoral immunity.

Gene-based immunization has been shown to induce cellular and humoral immune response and to confer protection in animal models of human diseases such as influenza, malaria, and Ebola virus infections. Both naked DNA and replication-defective viral vectors can be used to deliver selected viral immunogens. The effectiveness of genetic immunization is thought to result from endogenous presentation of viral proteins to host antigen presenting cells that stimulate primary immune responses. Emerging data also suggest that a combination of plasmid DNA immunization, boosted with a viral vector, elicits more potent immune responses than DNA alone. Thus, the VRC will develop both plasmid-based and viral vector vaccine products. Initial efforts will focus on these two promising delivery vectors, naked DNA-related delivery and replication-defective adenovirus. Comparisons will be made to other vectors, for example, modified vaccinia ankara (MVA) and other poxviruses, Venezuelan equine encephalitis virus (VEE), inactivated HIV, and recombinant protein approaches.

DNA immunogens have several potential advantages, including ease of gene manipulation, simplicity of manufacture, relatively low cost and ease of storage. While viral vector immunogens are more difficult to construct and produce, they appear to offer the advantage of potently boosting immune responses primed with naked DNA immunization. Several gene-based vaccine candidates have been brought into clinical trials, but their relative potency and consistency in eliciting cellular immunity are likely not optimal. For this reason, the VRC will expand the number of gene-based vaccine candidates and facilitate their evaluation in non-human primate studies and phase I human trials. The current platform for DNA immunization is also likely to require modifications to improve gene expression and gene delivery. To date, the VRC has constructed numerous immunogens by inserting HIV cDNAs into relevant plasmids. Construction of these immunogens includes modification of the nucleotide sequence for optimizing protein expression. Mutagenesis to define alternative proteins with enhanced immunogenicity will be also be explored. These approaches provide great flexibility in identifying immunogens that can induce broad and potent immune responses. Various cDNAs have been tested using plasmid-based gene delivery and candidates selected that express either Gag, Pol, various Gag-Pol fusion proteins and mutants, as well as Env and Nef.

cDNAs have also been inserted into viral vectors. These vectors include replication-defective forms of adenoviruses, poxviruses, and lentiviruses. The initial priority for the VRC will be design, production and evaluation of MVA and adenoviral vaccines. Both vectors can be grown to high titer in mammalian cell lines and both can potently boost immune responses primed by DNA immunization. Poxvirus vaccines, similar to MVA,

have been successfully manufactured in mammalian cell lines and can be readily produced for phase I trials. For adenoviral vaccines, which have the potential advantage of targeting antigen-presenting cells, development efforts will include intramural and collaborative efforts to explore efficient means for production in cell lines appropriate for advancement to human trials. Although the effort to date has focused on type V ADV vectors, alternative serotypes and viruses from different species will be evaluated as vectors candidates. In addition, it is recognized that the broad genetic diversity of HIV-1 may limit the immune response elicited by a single vaccine. Thus, inserts for gene-based vaccine approaches will include not only clade B immunogens, but also viral genes derived from other HIV-1 clades.

- Develop DNA-based immunogens of multiple HIV genes for pre-clinical and clinical immunogenicity testing, including single and multi-gene constructs and genes from different genetic clades.
- Optimize immunogenicity of gene-based immunogens through modification of the nucleotide sequence for optimal codon usage.
- Construct replication-defective forms of adenoviruses that encode HIV genes analogous to DNA plasmid vectors.
- Explore cell expression systems to safely and efficiently produce adenoviral vaccine candidates.
- Construct MVA vectors which encode HIV genes analogous to DNA and adenoviral platforms for comparative studies.
- Perform systematic evaluations of immunogenicity in small animals and non-human primates (see Goal 2) and apply these data toward iterative improvements in development of new immunogens.
- Utilize rational mutagenesis to define and evaluate alternative proteins with enhanced immunogenicity.

C. Employ state of the art methods in genomics and bioinformatics to advance vaccine development.

With a few exceptions, the expanding technologies of genomics and bioinformatics have not been applied to vaccine development. Sequencing and identification of novel gene products from infectious agents is now possible using high throughput DNA sequencing methods. Although this technology is not necessary for known viral genomes such as HIV, it is likely to prove helpful for more complicated microorganisms and parasites such as are responsible for tuberculosis and malaria. There are several ways in which this technology may be applied to the development of highly effective vaccines.

C.1 Use sequencing and related technologies to predict immune responses and develop optimized vaccine strategies.

- Utilize sequencing technology to identify open reading frames in the viral genome and to identify targets for potentially protective immune responses.

C.2 Optimize existing technologies for identifying patterns of gene expression and surrogate markers.

- Utilize microarrays to identify patterns of gene expression in specific cell types after immunization to facilitate an improved understanding of gene expression in specific immune cell types after vaccination.
- Utilize quantitative RNA expression analyses to facilitate an understanding of the patterns of gene expression associated with effective immune responses.
- Determine the ability of microarray testing, quantitative RNA expression analysis and other tests to facilitate the identification of surrogate markers that may be useful in assessing the response to vaccination.

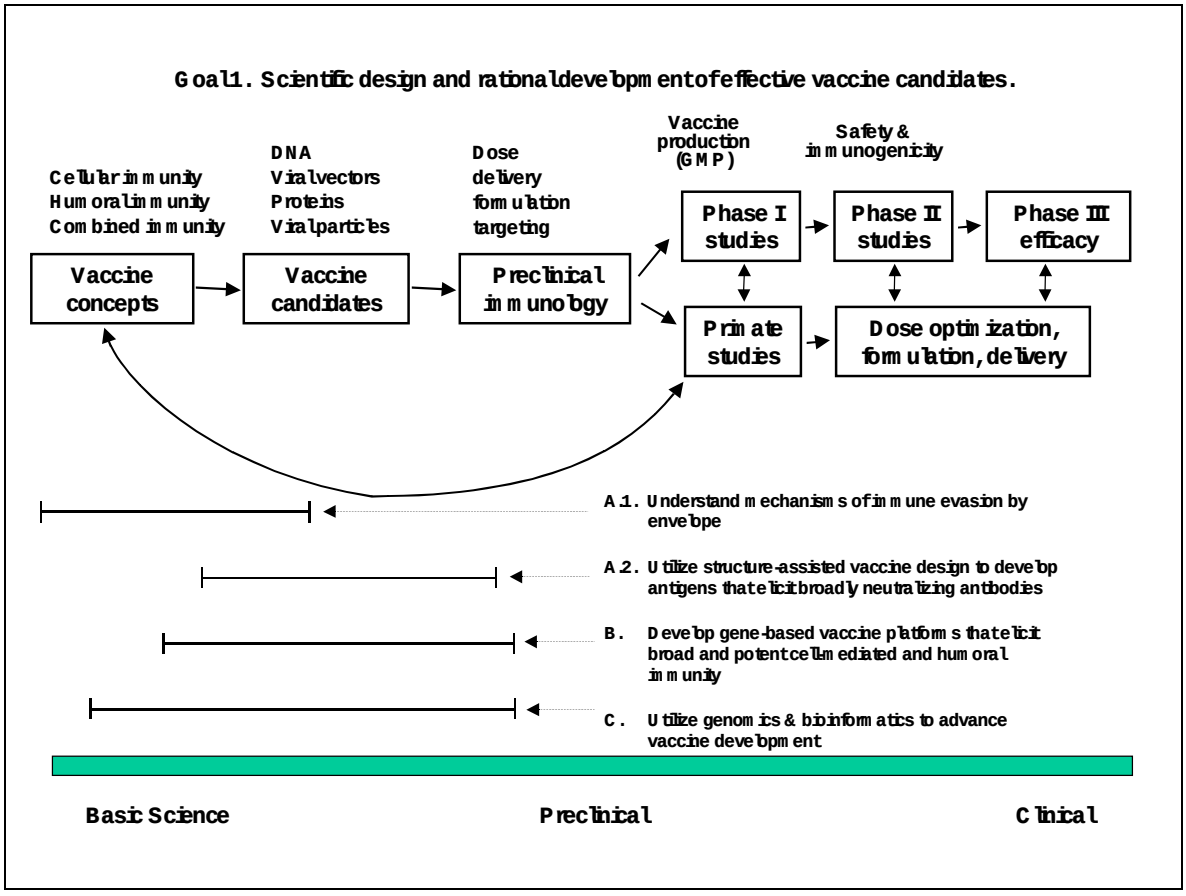

Figure 2. Summary of scientific activities related to Goal 1. Research is aimed at rational design of immunogens that will elicit a combination of broad and potent CD4 and CD8 mediated T-cell immune responses, definition of epitopes and structures that react with broadly neutralizing antibodies, and the generation of immunogens that elicit such responses to the appropriate viral epitopes.

SUMMARY: GOAL 1 RESEARCH PRIORITIES

1. Construct, express and evaluate envelope-based immunogens rationally designed to expose key neutralizing antibody epitopes.
2. Evaluate immunogenicity in small animal models and define structure-immunogenicity relationships by high throughput screening of immunogen structure and virus neutralizing antibodies.
3. Implement expression/purification systems that allow high throughput structure analysis and correlation with immunogenicity.
4. Identify new immunogens by high throughput screening of plasmid-based or viral vector recombinant immunogens, using neutralizing monoclonal antibodies.
5. Develop single and multigene DNA vaccine candidates for preclinical and clinical testing, including clade B and non-clade B immunogens.
6. Optimize DNA platform for antigen expression and delivery to antigen presenting cells in an effort to improve their immunogenicity in non-human primates and humans.
7. Construct recombinant adenoviral vectors that encode analogous HIV genes.
8. Explore cell expression systems to safely and efficiently produce adenoviral vaccine candidates and explore the use of alternative adenovirus strains as vaccine vectors.

Goal 1. Research Plan and Resources Needed

Scientific Design and Rational Development of an Effective AIDS Vaccine

A. Utilize knowledge of HIV envelope structure to design immunogens that elicit potent virus neutralizing antibodies through a program of rational structure-based design and screening of immunogens.

- Develop facilities for determining structure through X-ray crystallography.
- Develop technologies to perform biophysical, structural, genomic, and immunological characterization and analyses of envelope proteins.
- Determine atomic structure of gp120/CD4 complexes of primary HIV strains and gp120 bound by potent neutralizing antibodies.
- Develop technologies that allow redesign of envelope proteins and improved studies of structural interactions.
- Engineer, express and analyze the structure of new envelope proteins that potentially expose or stabilize key antigenic epitopes.
- Characterize functional antibody response elicited by novel protein structures.
- Evaluate novel protein expression systems to obtain sufficient protein for small animal immunization studies.
- Optimize tools for mass screening of potential immunogen libraries.

B. Develop and optimize gene-based vaccine platforms that elicit broad and potent cell-mediated and humoral immunity.

- Construct and optimize expression of numerous DNA plasmid immunogens containing various structural and non-structural HIV genes.
- Perform large-scale immunogenicity screening of plasmid-based vectors to advance the most promising candidates to larger animal studies.
- Construct replication-defective adenovirus vectors expressing HIV genes
- In collaboration with industry, evaluate cell expression systems to allow manufacture and production of adenoviral vector vaccines.
- In collaboration with industry, construct and produce MVA vectors expressing HIV genes.
- Develop and assess alternate vaccine vectors in collaboration with academic or pharmaceutical partners.

C. Employ state of the art methods in genomics and bioinformatics to advance vaccine development.

- In collaboration with existing intramural and extramural NIH programs, optimize genomic technology to identify patterns of gene expression, surrogate markers and potential targets for immune responses.
- Utilize bioinformatic resources for studies to predict the nature of immune responses to specific gene products in HIV infection.

GOAL 2. Evaluate and optimize the immune response generated by candidate vaccines.

The development of immunogens that elicit protective immunity against HIV will be guided by studies that systematically evaluate the humoral and cellular immune responses generated by vaccine candidates. Reproducible validated assays, such as those to measure CD8 T-cell function and virus neutralization will be developed and applied to animal studies and human clinical trials. Preclinical studies in small animals and primates will evaluate vaccine dose, formulation and delivery route, and will address the immunogenicity of multi-gene vectors, and vaccine combinations that prime with one immunogen and boost with a second. Concomitant studies will evaluate strategies to improve immunogenicity by targeting antigen to dendritic cells or by recruiting antigen presenting cells to the site of immunization. The accumulated knowledge from these preclinical studies will be used to develop vaccination strategies that induce optimal immune responses. The potential efficacy of vaccination strategies will be evaluated in the SIV/SHIV macaque model, where outcomes such as complete protection vs. protection against disease can be ascertained. Such pre-clinical animal testing will be closely integrated with VRC basic science and clinical programs in order to provide information on the advancement of promising candidate vaccines into human trials.

Objectives

A. Identify and develop validated, reproducible methods to quantitate vaccine-induced immune responses in humans and primates.

Measurement of vaccine immunogenicity is dependent on the availability of reliable reagents and assay methodologies, which are often developed in parallel with the vaccine itself. Assay development is comprised of basic research and development, pre-clinical validation, and translation for use in clinical trials. In all phases of assay development, issues of reproducibility, sensitivity, and interpretation limit the utility of immune-based assays. We will address these challenges of quality assurance and quality control at the levels of both basic and clinical research. Since most of these assays will be performed on-site with carefully monitored methods and technology, we will be in a position to optimize and apply standardized methodologies to measurement of vaccine responses.

One of the critical aspects of the development effort is the export of standardized reagents and assays to field sites performing clinical trials in collaboration with the VRC. Because of the limited availability of some advanced technologies, it will be important to develop standardized assays that can be generally applied. We will use VRC-developed technologies to validate and standardize reagents and assays that can be used on currently available platforms. If necessary, we will undertake the design, and manufacture of platforms that can be used to perform new assays crucial to the evaluation or implementation of vaccine field trials. These should be high-throughput assays, applicable to the heterogeneous populations and viral variants.

A.1 Develop and institute assays to measure cell-mediated immune responses to HIV in a reproducible and quantitative manner.

- Develop standardized reagents and assay methodologies to measure antigen-specific T-cell effector function based on cellular cytokine synthesis.
- Develop standardized reagents and assay methodologies to enumerate the induction of antigen-specific T-cells using epitope-specific tetramer staining
- Optimize sensitivity and reproducibility of immune assays.
- Adapt validated immune assays for high-throughput evaluation of immunogenicity.
- Apply comparable immune assays to small animal, primate and human immunogenicity studies.
- Perform comparative analysis of T-cell memory induced by a variety of vaccine strategies including various antigens, formulations, routes of delivery and adjuvants.

A.2 Develop rapid and sensitive assays to measure antibody-mediated virus neutralization.

- Develop more sensitive and reproducible assays to measure antibody-mediated neutralization of HIV.
- Develop flow cytometric high-throughput assays to measure inhibition of virus infection at the single cell level.
- Develop neutralization assays with relevant target cells including CD4 T-cells, monocytes/macrophages and dendritic cells.

A.3 Utilize innovative technologies to measure immune response.

- Optimization of high-speed flow cytometric sorting to enumerate HIV antigen-specific responses.
- Identification and characterization of potentially novel subsets of CD4 and CD8 memory T cells using extended phenotypic analysis.
- Utilization of laser capture microdissection and flow cytometic assays to probe the immune responses at mucosal surfaces.
- Development of plate-based fluorescence imaging, which provides the ability to perform high throughput assays that require constant (or repeated) measurements of individual live cells.
- Optimization of real-time PCR, DNA array analyses to evaluate immune function, HIV replication, and the impact of host genomic heterogeneity on vaccine responses.
- Development of integrated data management systems (“Bioinformatics”) to optimize data analyses and correlations.

B. Identify vaccine candidates and immunization strategies that enhance potency, antigen presentation and immunogenicity.

Multiple factors affect the breadth, potency and longevity of the immune response generated by immunization. The vaccine antigen, formulation, route of delivery, immunization schedule, adjuvant and targeting to antigen presenting cells, all play a role in ultimate immunogenicity. The VRC will conduct a systematic preclinical immunogenicity program to test various immunogens, vectors and delivery methods in order to optimize immunogenicity. This will include a focus on cytokines and chemokine immune modulators to enhance the interaction of vaccine antigen with antigen presenting cells and primary T-cells. Studies will be initiated in small animals, and advanced into non-human primates. The most promising vaccine strategies will be advanced into phase I studies.

B.1 Preclinical optimization of dose, formulation, adjuvant, delivery and antigen targeting.

- Explore improved delivery of plasmid-based immunization using nanoparticles or similar particulate adjuvants that improve immunogenicity of DNA.
- Explore modifications of gene-based immunogens that improve immunogenicity by targeting antigen to dendritic cells.
- Evaluate the effect of route of delivery on induction of specific immune responses by plasmid-based and viral vectored immunogens.
- Explore the feasibility of enhancing DNA vaccine immunogenicity using adjuvants and/or cytokines to recruit and stimulate antigen presenting cells.
- Optimize methods to influence the qualitative or quantitative nature of the immune responses to vaccines through the use of cytokines or chemokines as vaccine adjuvants.

B.2 Evaluation of multi-gene vectors and prime-boost vaccination.

- Evaluate the effect of multi-gene vaccine products (e.g., env, gag, pol) on induction of specific immune responses.
- Determine the effect of vaccine combinations such as prime-boost strategies on induction of immune responses.
- Analyze the breadth of immune responses from vaccine candidates based not only on clade B HIV, but also on analogous cDNAs from other genetic subtypes.

C. Develop rational use of the primate model to assess vaccine strategies and define immune correlates.

Animal models have proven to be of central importance in elucidating the pathogenesis of numerous viral diseases and in developing vaccine strategies to protect against transmission of a variety of viral pathogens. These models have facilitated the definition

of the molecular determinants of pathogenicity of various viruses, as well as the precise immune mechanisms that contain the spread of viruses in the infected host. Perhaps more importantly, animal models have provided a setting in which various vaccination strategies could be rapidly and efficiently assessed for their safety, immunogenicity, and the degree of protection they afford. As has been the case with other viral vaccines, the development of a safe and effective vaccine to protect against HIV will require the extensive use of experimentation in animal models. While, the ideal animal model for a viral disease is a small laboratory animal that can be infected by the virus and develops a disease that is similar to the disease in humans, none of the commonly used small laboratory animals can be readily infected with or develop an AIDS-like disease after infection with HIV. The natural reservoirs for the HIVs responsible for the worldwide AIDS epidemic have been shown to be certain African nonhuman primate species. These infections cause no disease in their natural host species. However, infection of Asian macaque species and humans with these viruses causes the disease process we know as AIDS.

The infected macaque species provide enormously powerful animal models for studying AIDS. Experimentation in these models has clarified the early immunopathologic events in an HIV/SIV infection, the central role of cytotoxic T lymphocytes in containing HIV replication, and the viral determinants of CD4+ T lymphocyte loss following HIV infection. Moreover, the SHIV-macaque models of AIDS have been used in studies demonstrating that specific neutralizing antibodies can protect against HIV. Thus, these animal models provide a powerful tool with which to assess correlates of immune protection and vaccine efficacy after challenge with pathogenic virus. These studies will provide real-time surrogate tests of protection and can be performed at the same time that vaccines are tested in humans.

Work will be done at the VRC to improve existing nonhuman primate AIDS models, by creating new challenge viruses for monkeys that more closely approximate the types of HIV isolates that are responsible for the transmission of HIV in people. New reagents will be developed for evaluating vaccine-elicited immune responses in monkeys so that studies can be done in a highly quantitative manner to assess and compare prototype vaccines. Studies will be pursued to define immune correlates of protection in monkeys against SHIV/HIV virus infections to provide guidance for vaccine strategies that could protect people against HIV infection. Finally, the immunogenicity and protective efficacy of vaccine prototypes will be evaluated in non-human primates in parallel with pilot human studies to prioritize testing in larger clinical trials. The result will be a primate animal model program that is closely coordinated with basic research and human trials, in order to systematically assess vaccine immunogenicity and efficacy.

C.1 Improve existing nonhuman primate AIDS models.

- Generate pathogenic chimeric simian/human immunodeficiency viruses that express specific HIV envelopes representative of the HIV isolates responsible for the AIDS epidemic in Africa, Southeast Asia and India.
- Create reagents that will allow for precise quantification of vaccine-elicited

immune responses in macaques, including MHC class I and II tetramers, and monoclonal antibodies specific for lymphocyte surface molecules and cytokines.

- Develop technologies to evaluate mucosal cellular and humoral immune responses in macaques, as well as viral isolates that will allow reproducible mucosal SHIV/HIV virus challenges.
- Develop a better understanding of the differences and similarities between the immune systems of people and primates, so that information generated from the primate models can be confidently translated to strategies in human.

C.2 Define immune correlates of viral containment and protection.

- Determine the nature of cellular immunity required to contain HIV replication following infection, including the diversity of CD8 T-cell epitopes that need to be recognized and the contribution of pre-existing CD4 T-lymphocyte immunity.
- Assess the contribution of neutralizing and non-neutralizing anti-viral antibodies to protection against infection.
- Evaluate the potential contribution of broadly but weakly neutralizing antibodies to vaccine protection.

C.3 Assess vaccine strategies in nonhuman primate models.

- Assess immunogenicity of various prototype HIV vaccines in macaques, carefully quantifying parameters of cellular and humoral immunity with standardized assays.
- Evaluate protection against infection and containment of viral replication following infection in vaccinated macaques.
- Determine immune competence and survival of vaccinated and challenged monkeys following infection.

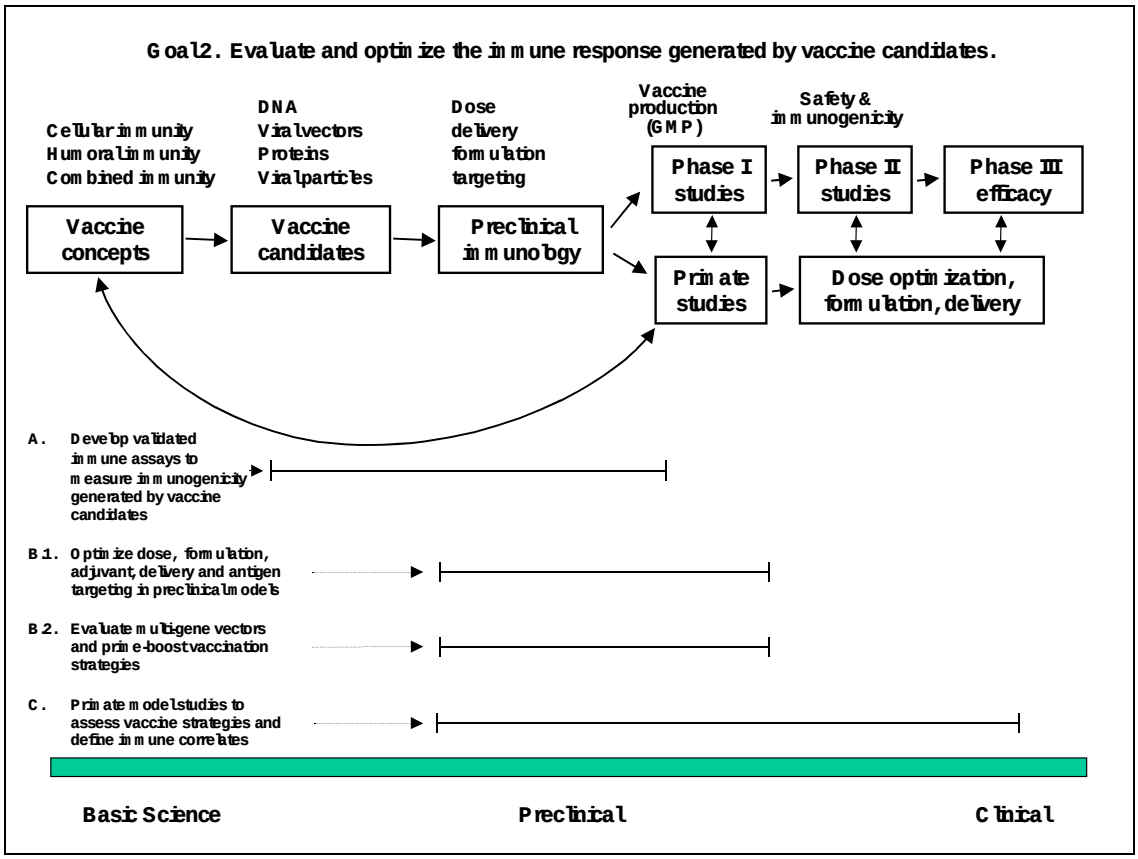

Figure 3. Summary of objectives in immune analysis and relationship to the VRC research program. Validated immune assays that can be applied to animal models and human trials will be used to systematically evaluate immune responses elicited by candidate vaccines. SIV/HIV (SHIV) animal model studies will provide data on immune correlates of protection that will inform the development and improvement of candidate vaccines.

SUMMARY: GOAL 2 RESEARCH PRIORITIES

1. Develop standardized assays of cell-mediated immune responses that can be applied to small animals, non-human primates and humans.
2. Develop rapid and sensitive assays to measure antibody-mediated neutralization.
3. Develop new reagents to improve assays of immune function in non-human primates.
4. Explore methods to enhance gene expression and delivery of gene-based immunization.
5. Explore methods to enhance immunogenicity of genetic immunization by recruiting or stimulating antigen presenting cells.
6. Evaluate cytokines, chemokines and costimulatory molecules as molecular adjuvants to direct specific types of immune responses to vaccine antigens.
7. Utilize non-human primates to assess vaccine strategies and to define protective immune correlates. Identify biomarkers from non-human primate studies for the three potential vaccine indications, sterilizing immunity, disease protection, and therapeutic immunization.
8. Perform preclinical studies of DNA immunization boosted by adenoviral vectors.
9. Perform preclinical pharmacology, toxicology, and immunogenicity studies relevant to planned human vaccine trials.

Goal 2. Research Plan and Resources Needed

Evaluate and optimize the immune response generated by candidate vaccines

A. Identify and develop validated, reproducible methods to quantitate vaccine-induced immune responses in humans and primates.

- Establish and validate quantitative intracellular cytokine assays to measure CD4 and CD8-mediated functional T-cell responses.
- Establish and validate assays to enumerate the induction of antigen-specific T-cells using epitope-specific tetramer staining.
- Develop and validate high throughput, reproducible assays to measure antibody-mediated virus neutralization of relevant primary target cells.
- Develop and apply specialized methodologies, including high speed flow cytometry, laser capture microdissection, plate-based fluorescence imaging and real time PCR to adapt validated immune assays for high throughput evaluation of immune responses elicited by candidate vaccines.
- Apply similar immune assays to small animals, non-human primates and human trials.
- Develop cells and tissue banks, and integrated data management system, to track and analyze biomarkers and correlates of protective immunity.

B. Identify vaccine candidates and immunization strategies that enhance potency, antigen presentation and immunogenicity.

- Choose and advance optimal DNA vaccine candidates for gag, pol and env genes based on small animal immunogenicity data.
- Conduct immunogenicity studies of most promising DNA vaccine candidates in non-human primate model.
- Evaluate the role of DNA formulation and delivery on DNA vaccine immunogenicity in small animal and primate models.
- Evaluate vaccine constructs and adjuvant strategies to target vaccines to antigen presenting cells.
- Construct plasmid-based and viral vectored immunogens based on non-clade B genes for comparative studies of potency and breadth of immune responses.
- Perform systematic comparative immunogenicity studies of vaccine candidates and prime-boost vaccine combinations in small animal and non-human primates.

C. Develop rational use of the primate model to assess vaccine strategies and define immune correlates.

- Procure nonhuman primates for vaccine studies, including establishment of a consistent source of primates.
- Create reagents necessary for precise quantification of vaccine-induced immune responses in macaques.

- Optimize assay methodologies and testing procedures to assess vaccines-induced immune responses in nonhuman primates, to allow comparison with human data
- Conduct primate studies to assess immunogenicity of vaccine strategies in nonhuman primates.
- Conduct primate studies to correlate the cellular and humoral immune responses induced by candidate vaccines with protection from infection or disease.

Goal 3. Advance the most promising vaccine candidates into human clinical trials.

A systematic well-coordinated process of human vaccine trials is essential to effectively develop new vaccines. While animal models are invaluable for guiding the development of vaccine approaches in general, and are indispensable for evaluating efficacy and immune correlates of protection, parallel phase I/II studies in humans are required to validate safety and immunogenicity findings. Only human phase III efficacy trials can determine vaccine efficacy. To efficiently move vaccine development forward, the VRC will marry traditional empirical vaccine development with hypothesis-driven basic and pre-clinical research. This approach will promote an iterative process in which data from clinical evaluation will inform basic research and vaccine design, and findings in animal models will help prioritize approaches to test in clinical trials. In addition to traditional phase I studies in HIV seronegative volunteers, the VRC will study the ability of vaccine candidates to augment native immunity in HIV-infected patients. Intensive evaluation of CD4 and CD8 immune responses will be correlated with control of viral replication and disease progression. In addition to the potential benefit to patients, studies of vaccine therapy will elucidate mechanisms of cellular immunity and T-cell memory that play a role in protection against HIV. Such data can then be applied to development of preventive vaccines.

The VRC will actively collaborate with intramural and extramural scientists, and facilitate the movement of ideas from the broader community into clinical trials. The VRC will maintain close ties with extramural investigators in the HVTN, where the infrastructure for conducting larger scale trials is already established, to characterize safety and comparative immunogenicity of promising vaccine approaches. This collaboration will include efforts to develop non-clade B vaccine candidates that can be evaluated at international field sites. When products emerge with real promise for licensure, the VRC will also interact with the pharmaceutical industry where there is a large capacity for, and experience in, product development and distribution. Therefore, the VRC will fill the gap between new basic concepts in immunology and initiation of clinical trials by applying state-of-the-art methods to rational vaccine design and evaluation at a single site.

Objectives

A. Develop the infrastructure to produce and test vaccine products.

The VRC is dedicated to translating basic findings into clinically relevant vaccine products. This will require the ability to manufacture candidate vaccines and to evaluate them in phase I/II clinical studies. The Center will establish the infrastructure to manage regulatory issues and to oversee the good manufacturing practice (GMP) required for vaccine production and human testing. The VRC houses a small production laboratory within the building and has plans to develop and build a GMP production facility on the NCI-Frederick campus. The planning and construction phase will begin in 2001. Individuals such as process engineers with extensive vaccine production experience will manage the VRC and Frederick facilities. They will be critical for the transfer of

technology from vaccine researchers to biologic engineers. Since licensed vaccines are ultimately produced by pharmaceutical vaccine manufacturers, the VRC will also interact with biotechnology and pharmaceutical companies to develop collaborations for the production of candidate vaccines for clinical testing. The activities of the VRC include specific scientific/product collaborations as well as efforts to stimulate the interest of industry in AIDS vaccine development.

The capability to produce vaccine candidates will expand the need for phase I clinical studies. Such vaccine evaluation presents special needs; subjects in most studies will be uninfected healthy adult volunteers. The difficulties in enrolling healthy subjects, particularly for studies related to HIV, are formidable and require a dedicated staff and ongoing recruitment plan. The motivation for most volunteers is purely altruistic, which makes it especially important that the clinical trial process is efficient, safe, and confidential. This will require special safeguards for managing volunteer records and securing databases, as well as establishing dedicated oversight systems for the safe and ethical conduct of studies.

A.1 Develop vaccine production infrastructure

- Develop the capacity for GMP production of protein, gene-based and vector-based vaccine candidates for testing in Phase I/II clinical trials.
- Develop the administrative infrastructure for filing and holding regulatory documentation for drug master files (DMF) and investigational new drug (IND) applications.
- Create a QA/QC oversight system for GMP vaccine production and post-production toxicity and potency evaluation.

A.2 Develop clinical infrastructure

- Develop the capacity to perform clinical trials which ensure the safety of volunteers and the quality control of safety and immunogenicity data.
- Establish a dedicated staff for recruitment and clinical evaluation of 200 healthy adult volunteers per year.
- Establish an integrated database and internet-accessible system for data management and analysis.
- Organize a Data and Safety Monitoring Board.
- Establish a Community Advisory Board and system for community education.
- Train physician-scientists in the science of vaccinology.

B. Conduct clinical evaluation of candidate vaccines.

Phase I clinical studies will explore the utility of current and novel vaccine strategies to identify promising vaccines and delivery methods that qualify for evaluation in higher phase studies. Various approaches such as novel proteins, vector-based, and nucleic acid-

based products will be evaluated. Studies will assess individual and combination products, delivery methods, antigen targeting and induction of mucosal immunity.

A major focus of the VRC will be the application of state-of-the-art technology to the measurement of vaccine-induced immune responses. By utilizing the same methodology across the spectrum of small animal, nonhuman primate, and clinical trials, the predictive value of the animal model systems will be enhanced over time. Both classical and novel immunologic measurements will be performed, with a major goal being to identify correlates of immunity. In addition, a significant effort will be made to develop rational and specific vaccine formulations using primary cytokines and co-stimulatory molecules rather than nonspecific adjuvant approaches that indirectly stimulate these factors. Eventually, this will allow building tailored vaccines for specific pathogens.

B.1 Perform phase I safety and immunogenicity testing of candidate vaccines to determine which strategies elicit lasting T-cell memory, and potent and broadly reactive CTL and neutralizing antibody responses.

- Perform concurrent and iterative phase I studies of candidate vaccines to efficiently determine optimal safety and immunogenicity.
- Evaluate the delivery and formulation of vaccine antigen for delivery to selected cells or immune compartments.
- Evaluate vaccine formulations containing specific immune modulators to identify components needed to build a vaccine with specific properties.
- Evaluate combined vaccine approaches and combined delivery mechanisms.
- Perform intensive monitoring of cell-mediated and antibody responses elicited by immunization.
- Analyze the secondary immune responses in vaccine recipients who suffer breakthrough infection after natural exposure to HIV in order to correlate specific immune responses with control of HIV infection.
- Define the kinetics and specificity of vaccine-induced immune responses.
- Develop new measures of mucosal immune responses.

B.2 Identify the most promising vaccines and immunization strategies for advanced clinical development

- Establish criteria for moving candidate vaccines from phase I studies to phase II and III studies.
- Establish collaborations, both nationally and internationally, for moving clinical studies from the VRC to larger multi-site clinical trial organizations.
- Collaborate with intramural and extramural researchers, biotechnology companies and the pharmaceutical industry, to identify, improve and advance promising vaccine concepts.

C. Evaluate preventive vaccine candidates in clinical protocols of therapeutic immunization.

Recent data from early initiation of antiretroviral treatment in HIV-infected patients, and from SIV primate studies, suggest that therapeutic immunization can augment native immunity and help control HIV infection. Candidate vaccines developed for prevention of HIV can be studied in HIV infected patients to evaluate immunogenicity and inform vaccine development by elucidating mechanisms of cellular immunity and T-cell memory that play a role in protection against HIV. Clinical studies of therapeutic vaccination can provide specific information on the role of HIV-specific CD4 and CD8 T cells in the control of HIV infection. This will improve our understanding of the composition of protective, or at least effective, immune responses that should be elicited by preventive vaccines. Until recently, it was difficult to measure cellular immune responses to HIV in a reproducible and quantitative manner. However, we can now characterize, precisely and quantitatively, the fundamental aspects of the HIV-specific CD4 T-cell memory response and functional CD8 T-cell responses, and correlate these with clinical and virologic parameters of HIV disease.

There is also evidence that the number and function of HIV-specific T cells may be impaired in the HIV-infected person. This impairment may result from defects in antigen presentation, T-cell proliferation or other effector functions of HIV-specific T-cells or T-cell memory (i.e., inability to maintain memory T cells through the activity of cytokines.) In addition, increased death and/or ineffective replacement of effector or memory HIV-specific T-cells, or the ability of HIV to evade recognition by an intact immune response may contribute to T-cell dysfunction. As such, it will be important to determine the degree and mechanism of this impairment, and its impact on the ability of the immune system to control HIV infection.

C.1 Conduct studies of therapeutic vaccination in HIV-infected subjects to better understand and measure effective HIV-specific T cell responses.

- Characterize, precisely and quantitatively, the HIV-specific CD4 and CD8 T-cell responses, and correlate these with clinical and virologic parameters of HIV disease.
- Determine the response complexity requirements; e.g., the breadth of epitope recognitions and T-cell receptor repertoire, required to control viral replication.
- Determine immunization strategies by which HIV-specific T-cell responses can be elicited and maintained over time.
- Study the effect of antiretroviral therapy on HIV-specific cellular immune responses, and the mechanisms underlying these effects.
- Compare the immune responses to HIV antigens in infected versus seronegative vaccinated volunteers.

C.2 Determine qualitative and quantitative aspects of T-cell functional impairment resulting from HIV infection and study mechanisms which may be responsible for the failure of the immune system to fully control HIV infection.

- Analyze the frequency of antigen specific cells and effector function following vaccination using antigen specific tetramers and intracellular cytokine staining respectively.
- Evaluate quantitative or qualitative (functional) defects in HIV-specific CD4+ and CD8+ T cells during natural infection. Determine the deficits (and mechanisms) that may be responsible for the failure of the immune system to fully control HIV infection.
- Identify and characterize potentially novel subsets of CD4+ and CD8+ memory T cells using extended phenotypic analysis.
- Perform comparative analysis of induction and kinetics of CD4 and CD8 T-cell mediated immune responses induced by a variety of vaccine formulations.
- Study the role of continuous antigen persistence and other factors such as cytokines that may be important in sustaining long-term cellular immune responses.

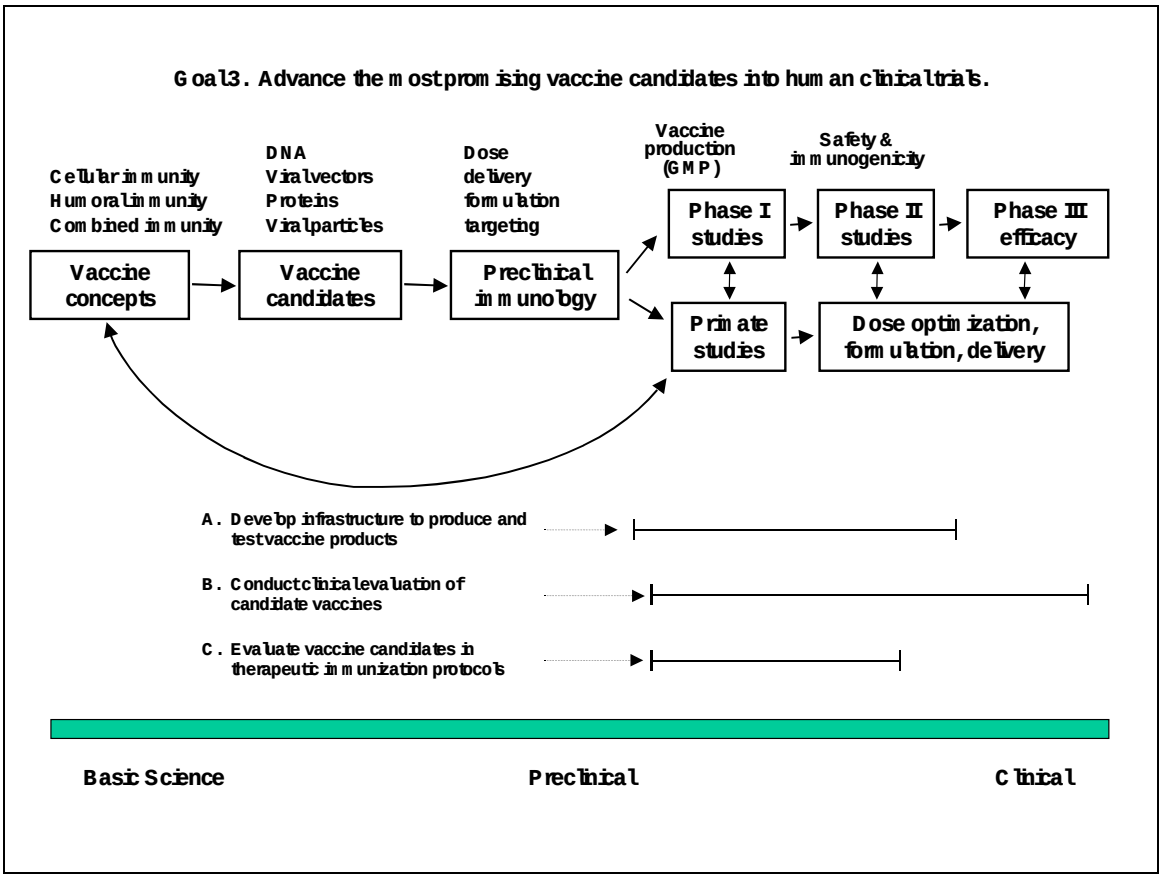

Figure 4. Integration of research activities in goal 3 into the VRC research plan. The research program is based on the hypotheses that non-human primate studies can define protective immune responses and that vaccine candidates can be constructed to elicit these immune responses in people. The infrastructure to produce and test vaccine candidates in phase I trials is critical to the vaccine development process. Systematic immune analysis of candidate vaccines in non-human primates and clinical trials will provide data on which vaccine strategies induce immune responses that correlate with protection against HIV.

SUMMARY: GOAL 3 RESEARCH PRIORITIES

1. Compare alternative vaccine concepts in humans for their ability to elicit immune responses that correlate with protective responses in non-human primate challenge models.
    - Establish, through intramural NIH facilities and extramural contracts, the capacity to produce by GMP, candidate DNA, MVA and adenoviral vectored vaccines.
    - Develop the clinical infrastructure, including recruitment of volunteers, to be able to perform several phase I vaccine trials each year.

2. Perform iterative phase I studies with DNA, adenoviral and other potential candidate vaccines, such as MVA, to systematically advance our knowledge of the safety and immunogenicity of vaccine concepts.

3. Perform phase I trials to compare immune responses elicited by prime-boost vaccine strategies.

5. Deliver the most promising vaccine candidates to the Vaccine Trials Network for evaluation in expanded trials.

6. Analyze the immune response to clades found in the developing world and initiate international collaborations to accelerate AIDS vaccine development in these countries.

7. Conduct clinical trials of therapeutic immunization using preventive vaccine candidates.

Goal 3. Research Plan and Resources needed

Advance the most promising vaccine candidates into human clinical trials

A. Develop the infrastructure to produce and test vaccine products.

- Develop a local VRC facility for small scale GMP production of vaccine products.
- Design and build a new GMP production facility for dedicated production of vaccine products.
- Institute a QA/QC oversight system and implement post-production toxicity/potency testing to ensure safe manufacturing processes.
- Develop an administrative infrastructure for obtaining and holding regulatory approvals for vaccine candidates.
- Establish the administrative and physical capacity to perform 3-5 phase I clinical trials per year.
- Establish mechanisms to recruit 200 healthy adult volunteers per year.

B. Conduct clinical evaluation of candidate vaccines.

- Advance most promising DNA vaccine candidates into phase I human testing.
- Study alternate delivery and formulations of plasmid-based vaccines.
- Study prime-boost vaccine strategies with adenoviral or MVA vectors.
- Implement mechanisms for the clinical and statistical analysis of safety and immunogenicity data generated by vaccine candidates.
- Establish systematic methods for tracking and analyzing data generated by intensive monitoring of humoral and cellular immune responses elicited during phase I vaccine trials.
- In collaboration with pharmaceutical or academic institutions, advance promising new vaccine concepts and candidates into phase I trials.

***C.* Evaluate preventive vaccine candidates in clinical protocols of therapeutic immunization.**

- Advance most promising DNA vaccine, or prime-boost strategy, into vaccine therapy trials.
- Evaluate the role of adjuvants and antigen targeting on the potency and longevity of the immune response.
- Characterize CD4 and CD8-mediated immune responses that correlate with clinical and virologic parameters of HIV disease.

SUMMARY

Goal 1. Scientific design and rational development of effective vaccine candidates.

A. Utilize knowledge of HIV envelope structure to design immunogens that elicit potent virus neutralizing antibodies through a program of rational structure-based design and screening of immunogens.
B. Develop and optimize gene-based vaccine platforms that elicit broad and potent cell-mediated and humoral immunity.
C. Utilize state of the art methods in genomics and bioinformatics to advance vaccine development.

Goal 2. Evaluate and optimize the immune response generated by candidate vaccines.

A. Identify and develop validated, reproducible methods to quantitate vaccine-induced immune responses in humans and primates.
B. Identify vaccine candidates and immunization strategies that enhance potency, antigen presentation and immunogenicity.
C. Develop rational use of the primate model to assess vaccine strategies and define immune correlates.

Goal 3. Advance the most promising candidates into human clinical trials.

A. Develop the infrastructure to produce and test vaccine products.
B. Conduct clinical evaluation of candidate vaccines.
C. Evaluate preventive vaccine candidates in clinical protocols of therapeutic immunization.

Implement programs for intramural training and collaborations, and hosting of international scholars.

A. Develop an oversight committee and review process to evaluate and fund proposals for intramural collaborative research.
B. Establish an intramural HIV Vaccine Interest Group.
C. Establish an international advisory group and create positions for visiting international scholars.