2006–2008 IN REVIEW
Table of Contents

Introduction .................................................. 1
   Letter from Tachi Yamada .................................. 1
   Overview .................................................. 3

Section 1: HIV Vaccine Landscape .............. 6
   A Brief History of HIV Vaccine R&D Efforts ........... 7
   The First Wave ........................................ 7
   The Second Wave ....................................... 7
   The Third Wave ........................................ 9
   Current Prospects ...................................... 11

Section 2: Overview of the Model ............... 12
   The CAVD Model ....................................... 13
   Grants Management and Alliance Management .......... 15
   CAVD Legal Agreements ................................ 17
   Evaluation of the CAVD ................................ 19

Section 3: Scientific Updates ...................... 22
   Antibody Approaches .................................. 23
      • Rational Antibody Vaccine ....................... 24
      • Targeting Broadly Conserved Regions of HIV-1 .. 26
      • Novel Immunogens ................................ 27
      • Stabilized Transition State Immunogens ........ 28
      • V3 as a Potential Epitope ....................... 29
   T-cell Approaches ..................................... 29
      • New and Improved Viral Vectors ................. 30
      • Optimizing T-cell Vaccine Inserts .............. 33
      • T-cell Vaccine Delivery ......................... 33
      • STEP Trial: Lessons Learned .................... 34
   New Approaches ........................................ 35
      • Mucosal Immunity ................................ 35
      • Innate Immunity .................................. 35
      • Adaptive Immunity ................................ 36
      • Dendritic Cells .................................. 37
      • Clues from HEPS and Long-Term Controllers .... 38
      • Allogeneic Vaccines ............................... 39
   Central Service Facilities: Improving the Infrastructure for Evaluating Vaccines .......... 39
      • The Comprehensive Antibody Vaccine Immune Monitoring Consortium ........ 40
      • The Comprehensive T-Cell Vaccine Immune Monitoring Consortium .......... 41
      • Mouse Immunology Laboratory .................. 42
      • Vaccine Immunology Statistical Center .......... 43
      • HIV Specimen Cryorepository ................... 44
   Conclusion ............................................... 45

Section 4: The Challenge Ahead ................. 46

Appendices .................................................. 48
   1. Principal Investigators ............................... 49
   2. Collaborating Institutions ........................... 50
   3. Site Map ............................................ 52
   4. List of Publications ................................ 54
   5. List of Abbreviations ............................... 57
When Bill and Melinda started their foundation, they knew they would be tackling some of the most complicated biomedical challenges facing the world today. The development of an effective HIV vaccine is one such challenge. A vaccine is essential to ending the worst epidemic in recent history. The foundation is dedicated to this pursuit for the long haul.

To date, the foundation has committed nearly $10 billion in global health grants to organizations worldwide. We focus our funding on two areas:

1. Accelerating access to existing health interventions and technologies

2. Supporting basic and clinical research to develop new vaccines, drugs, and other health tools to fight diseases that cause the greatest illness and death in developing countries

We have committed more than $2 billion to HIV/AIDS, which is one of our top priorities.

That is why I am so excited about the Collaboration for AIDS Vaccine Discovery (CAVD). It’s a unique approach that we believe is accelerating HIV vaccine research and development through intense and novel collaborations.

The CAVD supports the goals of the Scientific Strategic Plan of the Global HIV Vaccine Enterprise, which calls for complementing investigator-led efforts with large-scale, well-funded, and collaborative efforts across institutions and disciplines. As Dr. José Esparza and I wrote in a commentary in the Journal of Experimental Medicine: “...the ability of science to address and solve some of the major global health problems will require both the creativity of individual investigators and the infrastructure, systems, and resources needed to efficiently harness scientific knowledge to develop practical solutions” (April 16, 2007, JEM). In both approaches, innovation is imperative.

I am excited about the ongoing work of our CAVD partners in the third year of the collaboration. Each of the CAVD collaborators has an important role in ensuring that the scientific and operational obstacles to an effective HIV vaccine are dealt with expeditiously and expertly. The kind of information-sharing and real-time collaboration pursued in the CAVD is groundbreaking and will be looked to as a model of success for future efforts.

To you the CAVD collaborators: Thank you for bringing your time and talent to this important cause. The collaborations that have evolved over the last two and a half years are a testament to the fact that business as usual can be improved upon. Your work within the CAVD will bring about change, and save lives.

Tachi Yamada

President, Global Health Program, Bill & Melinda Gates Foundation
Seattle, USA, January 2009
The Bill & Melinda Gates Foundation launched the Collaboration for AIDS Vaccine Discovery (CAVD) in July 2006 with grants to 16 institutions to create an international network of highly collaborative research consortia focused on accelerating the pace of HIV vaccine development. The areas selected for initial funding were aligned with the Scientific Strategic Plan of the Global HIV Vaccine Enterprise published in PLoS-Medicine in February 2005.

The foundation initiated a Request for Proposals (RFP) process in early 2005 with three separate RFPs addressing two of the six priorities identified in the Enterprise Plan: The first one solicited projects focused on the rational design of immunogens capable of inducing broadly reactive neutralizing antibodies; the second solicited projects focused on the rational design of immunogens capable of inducing persistent high levels of T-cell immunity; and the third one focused on the creation of vaccine immune monitoring consortia as part of a robust global infrastructure to develop, expand and ensure broad access to laboratory assays and technologies, allowing valid comparisons of data across pre-clinical and clinical trials worldwide. During the process, we identified the need to provide the vaccine discovery consortia with access to two crucial areas of expertise (statistical analysis and mouse modeling), and to an essential infrastructure for preserving and sharing the materials generated under these grants.

Since 2006, the CAVD has been expanded to include 19 grantees and each of those grantees is working with a number of collaborating institutions. In total the CAVD currently comprises 95 collaborating institutions in 21 countries involving over 400 investigators, with funding totaling more than US$327 million over five years. Two grantees funded through the foundation's Grand Challenges in Global Health (GCGH) program are also collaborating within the framework of the CAVD.

This is the first report on the cumulative progress of the CAVD since the first grants were awarded in July 2006, and is meant to provide CAVD members and the broader community of HIV vaccine stakeholders with an overview of the scientific and operational progress made over the last two and a half years. This report highlights the major activities and progress to date of the consortia.

In the first section we endeavor to set the stage for the creation of the CAVD by providing a brief history of the HIV vaccine research-and-development effort that led to the need for more highly collaborative research efforts and the creation of the Global HIV Vaccine Enterprise, which provided the framework for the development of the CAVD. Next, we describe the structure of the CAVD, which currently consists of fourteen consortia and two GCGH groups focused on vaccine discovery, applying new scientific knowledge and cutting-edge research techniques to the creation and evaluation of novel vaccine candidates. These consortia are linked to five central service facilities that enable investigators to openly share data and compare results, which in turn allow the most promising vaccine approaches to be prioritized for further development. We also describe the alliance management activities and legal agreements that are used to help align CAVD consortia and facilitate a collaborative research approach.
The majority of this report focuses on the scientific advances made over the last two years. The CAVD vaccine discovery consortia have made considerable progress toward the goal of developing an effective HIV vaccine and the five central service facilities have contributed not only resources and expertise but also original research.

Toward the goal of antibody-based vaccines, CAVD researchers are discovering new naturally occurring anti-HIV-1 antibodies in humans and immunized animals which can inform the rational design of vaccine candidates capable of eliciting antibodies that neutralize a broad range of HIV-1 strains. Several teams are creating novel immunogens that closely mimic HIV-1 surface proteins in functional conformations. These and other novel immunogens are being screened for the ability to induce anti-HIV-1 antibodies.

Progress in the rational design of vaccine candidates to induce T-cell immunity has also been swift and promising. CAVD investigators are evaluating new viral vectors and engineering existing vectors to reduce anti-vector immune responses. They are testing new selections and arrangements of HIV-1 and other genes to potently and persistently stimulate T-cell responses. They are experimenting with novel vector delivery systems.

In addition to antibody-based and T-cell-based vaccine concepts, CAVD investigators are exploring other novel strategies. They are designing vaccines that are active at mucosal surfaces, which are often the entry points for HIV-1. They are studying adjuvants to stimulate innate and adaptive immunity. And they are developing HIV vaccines that target dendritic cells.

The vaccine discovery consortia and the central service facilities are collaborating to rapidly evaluate vaccine candidates in vitro and in vivo. The central service facilities are providing statistical expertise, creating and refining assays for screening vaccine candidates, making substantial progress on the creation of mouse models of T-cell and B-cell immunity, and improving methods for storing samples at a central repository.

The CAVD represents the major portion of the foundation’s support to HIV vaccine research efforts. CAVD investigators are funded to pursue research that lies within the critical path of the development of an HIV vaccine. As the foundation’s funding strategy is focused on a mission-oriented research approach to lead to the development of a vaccine that saves millions of lives, the research-and-development process pursued by the investigators is paramount. While the creation of new knowledge is important, research funded within the CAVD must link any knowledge gained to a translational and clinical development pathway which, in some cases, may include additional discovery research deemed essential for the development process. As the field advances and shifts priorities or areas of focus, so too will the funding of research projects within the CAVD.

While the CAVD structure is an experiment in itself, we have been very encouraged by the willingness of researchers to work in new ways—in a more open, collaborative spirit, sharing data and materials
early in an effort to advance research. We thank all of the CAVD investigators, program managers, lab technicians, legal counsel, administrators, and others for the passion and commitment they have shown to the pursuit of an HIV vaccine to end one of the worst epidemics in history.

We look forward to the ongoing commitment and scientific excellence of CAVD investigators as they continue to work hard to accelerate the development of an HIV vaccine.

Sincerely,

José Esparza, Nina Russell, Francine McCutchan, and Siobhan Malone

Global Health Program, HIV Vaccines
Bill & Melinda Gates Foundation
Overlapping Waves of Vaccine Attempts

The first wave focused on the idea that antibodies would be sufficient to confer HIV immunity, while the second wave was based on the concept that cellular mediated immunity might vanquish HIV. The third wave is rooted in the realization that more basic research is needed, and that success against HIV depends on a collaborative research effort that builds on lessons learned from past failures. (Esparza and Osmanov, reference on page 11).
A Brief History of HIV Vaccine R&D Efforts

Twenty-six years ago the identification of HIV had researchers optimistic that a preventive vaccine was just around the corner. Vaccines had been created for many other infectious diseases so it was logical to predict that a concerted effort to develop an HIV vaccine would yield results within a few years.

As all of us know, the task has proven more complicated than anyone imagined. HIV is a wiler foe than first anticipated, and today we still do not have an HIV vaccine. The three vaccine candidates tested to date in Phase IIb or III clinical trials have proven ineffective. In the following pages we briefly look at the history of the HIV vaccine endeavor and how this history has shaped the philosophy and research portfolio of the CAVD.

The First Wave

Soon after HIV was discovered in 1983, some researchers were confident that they could use recombinant DNA techniques to make a safe and effective vaccine. Two companies, Genentech and Chiron, raced to create a vaccine based on a recombinant HIV envelope glycoprotein—gp120. Shortly before the gp120-based candidate vaccines were to be tested in a large clinical trial, however, new data came to light indicating that the antibodies induced by that type of vaccine could not neutralize in vitro clinical isolates of HIV (R5 viruses).

The National Institutes of Health (NIH) dropped support for the trial in 1994, but former Genentech researchers formed a private company called VaxGen and in 1998 began two efficacy trials, one in North America and the other in Thailand, to the skepticism of many in the HIV/AIDS research community.

This skepticism surrounding the gp120 vaccine continued to pervade the field of HIV vaccine research. After witnessing the futile efforts of Chiron and Genentech, few pharmaceutical companies wanted to tackle the problem. Conflicts erupted among HIV vaccine researchers as to how best to move forward. How much of a role should industry play? What was the best way to increase the number of vaccine ideas in the development pipeline?

In the years following the NIH’s decision, a number of new research initiatives sought to realign the vaccine effort, including the formation of a public-private partnership called the International AIDS Vaccine Initiative (IAVI), an NIH-funded clinical research network, the HIV Vaccine Trials Network (HVTN), and a watchdog group, the AIDS Vaccine Advocacy Coalition (AVAC). President Clinton established an HIV/AIDS advisory committee and in 1997 set a goal of developing a vaccine within the next decade.

The Second Wave

The mid-90s served as a turning point not only for HIV vaccine advocacy but also for HIV vaccine science. Since the virus is highly mutable and able to evade the immune system, some researchers despaired of creating antibodies capable of neutralizing a broad array of HIV strains. Researchers did not know of a single case of a person developing protective immunity following HIV infection. (There is still no known case of protective immunity after 60 million infections to date.)
Despite the absence of protective immunity, researchers noted that some people were frequently exposed and yet resisted becoming infected with HIV. In Nairobi, for example, some sex-workers remained seronegative despite frequent sexual contact with infected partners. Other individuals became HIV infected yet managed to resist progressing to AIDS for many years. In other words, a small number of people appeared able to stave off HIV’s destruction of the immune system.

Scientists studying these individuals came to realize that cytotoxic T-cells were at least partially responsible for keeping the virus in check. These cells, part of “cell-mediated immunity” (CMI), became a target for HIV vaccine development. Could a vaccine that boosted cellular immunity protect people from HIV?

Although a vaccine that harnessed the power of T-cell responses was a relatively new concept, researchers thought it a promising one. After immunization, killer T-cells would theoretically find and kill any HIV-infected cells. The approach might keep HIV from establishing itself altogether, or boost an infected person’s ability to resist progressing from HIV infection to AIDS.

In the late 1990s and early 2000s, a number of researchers and a handful of pharmaceutical companies began to work on T-cell concepts. The vaccines involve the injection of HIV genes into the body where they are taken up into immune cells. These cells then display surface proteins marking them as “infected.” This display alerts the killer T-cell population to expand and kill infected cells.

To ferry the HIV genes into the body, researchers attached them to viruses used as vectors, including adenovirus, canarypox, and vaccinia (MVA). Still other vaccine candidates based on “naked” DNA used no vector at all.

Preliminary trials of the cell-mediated approach were already underway when the disappointing VaxGen results were announced in 2003. The negative results cast a pall over the HIV vaccine community. The virus appeared to have many ways of hiding its cell-surface proteins from antibodies, from coating them with sugars to inducing conformational changes. The challenge of developing an HIV vaccine was more difficult than anyone had realized.

To many researchers, the VaxGen trial illustrated the danger of investing too much hope in one approach. Yet history seemed to be repeating itself with cell-mediated immunity. By 2003, there were already too many “me-too” products in clinical trials, according to an analysis by AVAC. Half of the trials were testing DNA vaccines, 30% were testing a variation of the canarypox vaccine, and only 20% tested other approaches.

Some researchers questioned whether business-as-usual vaccine development was adequate to meet the challenge of HIV. The worry was that if investigators didn’t alter course, HIV vaccine research would be beset by misdirection of resources and more high-cost vaccine trial failures, and lead to the delay of a vaccine at a cost of millions of lives each year.
The Third Wave

These thoughts and feelings led a group of people in 2003 to propose a new way of doing HIV vaccine research. In a paper published in the journal *Science*, researchers called for a new concept, the Global HIV Vaccine Enterprise (the “Enterprise”), to promote a multidisciplinary and collaborative approach to the challenge of generating and testing new vaccine candidates. The Enterprise was initially proposed by HIV researchers and policymakers from the Bill & Melinda Gates Foundation, the World Health Organization-Joint United Nations Programme on HIV/AIDS (WHO-UNAIDS), the National Institute of Allergy and Infectious Diseases (NIAID), the HIV Vaccine Trials Network (HVTN), the International AIDS Vaccine Initiative (IAVI), the European Commission (EC), and other organizations around the globe.

The Enterprise called for a “big science” approach akin to the Human Genome Project. Traditionally scientific research has been characterized by individual endeavors and competitiveness between groups of researchers. Under this traditional paradigm, scientists often didn’t communicate their findings until publication, and when a research idea failed,
neither the scientist nor the publishing world had incentive to publicize the reasons for failure.

A collaborative model would harness the creative power of individual research while emphasizing the sharing of information. The Enterprise’s Scientific Strategic Plan, published in PLoS Medicine in February 2005, called for a network of consortia and research centers that would do HIV vaccine research according to a comprehensive systematic approach. The centers would share information, reagents, and procedures so that data could be compared and, if needed, merged. All goals, plans, progress, and obstacles would be shared to avoid duplication of effort. Researchers would be open to incorporating new scientific findings and new technologies into their existing efforts. The plan also called for innovation in product development and manufacturing, regulatory considerations, intellectual property issues, establishment of clinical trial sites, and community outreach in developing countries. Perhaps most importantly, the scientific strategic plan identified the key scientific challenges and areas of missing knowledge that were blocking researchers from developing an HIV vaccine.

**Current Prospects**

The Collaboration for AIDS Vaccine Discovery (CAVD) was established in 2006 with the intent
of fulfilling the Enterprise philosophy and tackling certain priority areas addressed in the scientific strategic plan. The CAVD brought together investigators in a collaborative effort that enhances individual research efforts.

The strategic plan of the Enterprise served as the basis for identifying and prioritizing the research projects funded by the CAVD. The original 16 projects were selected because each addresses one or more of the scientific roadblocks identified in the plan. The CAVD research portfolio is diversified across antibody-based and cellular immune approaches and contains a spectrum of vaccine ideas that range from “safer” to “riskier.” The CAVD’s diversified approach is based on the hope that one of these approaches will eventually lead to an HIV vaccine. Not every grant will succeed in identifying a way to develop an HIV vaccine, but failures have important lessons to teach.

The failure in September 2007 of the adenovirus-based vaccine in the STEP trial is just such an example, although it was not part of the CAVD. The vaccine failed to induce any protection against HIV, and may have made some participants more vulnerable to HIV infection. Many had hoped that the trial would confirm that the CMI approach was viable, but that still remains an open question.

While a setback, the STEP trial provides value because it reaffirms the impetus to maintain a high level of basic and applied HIV research. The CAVD has already begun funding projects to enhance our understanding of humoral and cell-mediated immunity and search for new ways to induce broadly neutralizing antibodies. Other CAVD projects involve developing improved vaccine vectors, defining immune correlates of protection, and exploring mucosal and innate immunity.

In addition to these questions, CAVD researchers are addressing the gap between basic research and vaccine development. The lack of understanding of protective immune responses to HIV has made the pharmaceutical industry reluctant to join the search for an HIV vaccine. Once the underlying science is known, industry will come forward to apply its expertise to developing and scaling up a vaccine. Until then, CAVD grants are designed to harness existing knowledge or obtain new knowledge with the clear target of designing a vaccine that can be tested in animals and humans and provide proof that an HIV vaccine can be protective.

The strength of the CAVD is that it brings scientists together to make the global vaccine search more effective. This “Big Science” collaborative approach is a great experiment and requires a change in the culture of scientists. The CAVD grantees include many of the world’s most prominent researchers in HIV vaccine science. It is a first-class team, but the team’s success depends on how the players work together.

Sources:
With large-scale scientific collaborations there is a delicate balance between maintaining the scientific independence and research flexibility of a small organization with the collaborative power of pooling resources and ideas across a large consortium. In particular, the core immune-monitoring facilities provide an opportunity to conduct and compare assays in a more thorough and standardized manner.” —David Ho
The CAVD Model

The concept of the CAVD emerged from the vision behind the creation of the Global HIV Vaccine Enterprise and the belief that a “Big Science” approach was needed to complement the efforts of independent researchers. As the Enterprise’s Scientific Strategic Plan states, investigator-led research efforts… “should not be replaced with big science. Both approaches must be undertaken. Creation of research environments that support the creativity both of individual investigators and of larger, collaborative efforts will accelerate the scientific breakthroughs needed to successfully develop a safe and effective HIV vaccine.”

In that vein, the CAVD was established by the Bill & Melinda Gates Foundation in an effort to bring together a critical mass of HIV vaccine researchers initially focused on different aspects of HIV vaccine discovery and laboratory standardization to work in new ways—use standardized reagents, share ideas and data early, and support comparative evaluation of those data to allow for an iterative process of knowledge building and problem solving. The CAVD, as a Big Science model, would not only form a new, large collaborative research effort, but it would ensure a targeted research approach to address an urgent global health need by harnessing scientific knowledge for the purpose of developing an HIV vaccine. The CAVD thus creates a collaborative environment that breeds scientific innovation by combining the independent pursuits and complementary approaches of research groups through a common targeted research agenda.

While the CAVD was launched with 16 primary grantees, it now includes 19 primary grantees and each has a number of collaborating partner institutions. In total the CAVD currently comprises 95 collaborating institutions in 21 countries involving several hundred investigators, with the collection of grants totaling more than US$327 million over five years. In the past year, the CAVD has also incorporated two research groups funded through the foundation’s Grand Challenges in Global Health (GCGH) program. As a dynamic effort going forward, the CAVD will evolve as science evolves, with some collaborators concluding their contribution and other collaborators added in the future.

The 14 Vaccine Discovery Consortia (VDC) of the CAVD and the two GCGH groups are pursuing a range of innovative strategies to design an effective HIV vaccine focusing on candidates capable of eliciting antibodies against HIV and inducing strong and lasting protective cellular immune responses against HIV. The five Central Service Facilities (CSFs) support the VDC, enabling investigators to openly share data and materials and compare results using standardized assays. The CSFs include three laboratory networks for evaluating the immune responses elicited by vaccine candidates (T-cell, antibody, and mouse immunology laboratories), a research specimen cryorepository, and a data and statistical management center. With 14 discovery consortia and five central service facilities, the CAVD is a unique structure that requires centripetal forces to keep it functioning as a whole system with a targeted research approach rather than a number of disparate groups working independently. The services provided by the five CSFs are a critical component of the system and the core of the model. In the same way a hub and spokes function on a wheel, the CSFs provide the common meeting point...
Section 2 | Overview of the Model

Collaboration for AIDS Vaccine Discovery

Vaccine Discovery Centers
- Cell Mediated Vaccine Approaches
- Humoral Vaccine Approaches
- Cell Mediated & Humoral Vaccine Approaches

Central Service Facilities
- Antibody Vaccine Immune Monitoring Consortium
- T Cell Vaccine Immune Monitoring Consortium
- Mouse Immunology Laboratory
- Vaccine Immunology Statistical Center
- HIV Specimen Cryorepository

Data & Materials

Funding

Alliance Management

Legal Agreements
for the VDC to come together for evaluation and comparison of their research results. There are three other important sets of activities and agreements used in the CAVD to help align the consortia and facilitate a collaborative research approach—grants management, alliance management, and legal agreements.

**Grants Management and Alliance Management**

The individual grant agreements between the foundation and each of the 19 primary institutions of the CAVD provide the first layer of glue for the collaboration by defining the terms and conditions of the awards, including the stated objectives of the research projects and the expected project milestones and outcomes as they relate to the overall goal of the CAVD. The ongoing management of the grants focuses on the relationship between the foundation and the grantees, and the advancement of the funded projects individually over time. Interim and annual reports, site visits, and frequent but less formal meetings and calls between the grantees and foundation staff ensure that the grant agreement is executed effectively but with flexibility, that the project milestones and timelines in the grant agreements are met or updated as needed, and that the projects are budgeted and funded appropriately over the course of the agreement. The set of activities required to manage the CAVD as an alliance of researchers, on the other hand, focuses on the collection of grantees that make up the CAVD and what is needed to make the whole greater than the sum of the parts.

What is referred to as *Alliance Management* within the context of the CAVD encompasses activities in the areas of communication, evaluation, relationship building, scientific coordination, and the creation of tools for collaboration. Together, activities in each of these areas aim to facilitate data and materials sharing, ensure cross-consortia communications and research fertilization, facilitate the use of the CSFs by the VDC, and decrease the overall transactional costs of participating in a network. Critical to the effort, the alliance management activities also create incentives for participation in the CAVD and identify new opportunities for collaboration and coordination between the research consortia.

A cornerstone of the alliance management effort to facilitate communications, data-sharing, and CSF use is the CAVD Web portal that was launched shortly after the first grants were awarded in 2006. The portal (at [www.cavd.org](http://www.cavd.org)) is made up of three main areas—a public side, a password-protected side, and a series of intra-consortium team sites. The public site describes the CAVD effort and provides an overview of the projects for other researchers, academics, and the public in general. A password-protected level provides access for the more than 700 CAVD investigators, administrators, lab managers, project managers, legal representatives, and others to CAVD announcements, study data, document libraries, study registration tools, CSF services information, and additional tools and information to facilitate inter-consortia collaborations. The third and most confidential area of the portal, the team sites, provides a space for intra-consortium collaboration. The project manager of each consortium controls the users of their team site, thus allowing the consortium a confidential virtual workspace.
To promote collaboration and enhance the effectiveness of the CAVD, three important communities of practice were established from the beginning—a Council of Principal Investigators (PIs), a Project Managers team, and a group of legal and technology transfer representatives from the 19 current CAVD grantee institutions and the two GCGH grantee institutions. The Council of PIs, made up of the 21 principal investigators (listed in Appendix 1), is convened periodically to provide a forum for communication and collaboration where issues relevant to the CAVD’s overall scientific effort and operations are discussed and decided. The Council of PIs forms an important advisory body in a model that does not have a single leader, but rather a collection of important scientific leaders. The Project Managers from each of the grants are brought together regularly via conference call, Web conferencing, or in-person meetings, to discuss and resolve operational issues and to coordinate scientific management issues across the CAVD consortia. This group is an integral part of the smooth functioning of the inter- and intra-consortia collaborations, especially related to the use of the CSFs and the sharing of materials and reagents. The legal and technology transfer institutional representatives meet annually to discuss issues as they relate to the implementation and evolution of the terms and principles of the data- and materials-sharing agreement described on the next page.

Additional communities of practice are established as needed to tackle specific issues as they arise in an effort to enhance collaboration and improve the CAVD. Similarly, an annual meeting is held every year to bring together over 200 CAVD investigators to discuss the scientific progress of their consortia and provide an opportunity for inter-consortia collaboration. A meeting in December 2006 brought together CAVD members for the first time to begin developing a common vision for the network. The second meeting in December 2007 focused
on in-depth descriptions of the projects and early results obtained, and the December 2008 meeting focused on the scientific progress made over the previous year and began to identify the path forward.

CAVD Legal Agreements

An essential feature of the CAVD is the set of legal agreements that lay the foundation for a common framework and understanding among the collaborators around data sharing and materials sharing with the intent of facilitating scientific collaboration in support of the foundation’s Global Access objectives. The foundation is committed to the notion that efficient data and material exchange within and among the CAVD consortia, and prompt dissemination of CAVD findings and materials to the broader scientific community, will greatly advance the development of a safe and effective HIV vaccine. The foundation also believes that projects funded by it should be conducted and managed in a manner that enables accessibility to CAVD discoveries for the benefit of people in developing countries most in need of them. This twofold commitment is referred to as the Global Access Policy—an integral aspect of the foundation’s grantmaking activities and philosophy to ensure that projects are funded for the purpose of ultimately developing an intended health solution for the benefit of those who need it most, and not only to advance science.

Towards this end, the CAVD developed an inter-consortia Data and Material Sharing Agreement (DMSA) that describes the principles under which the CAVD members will share data and materials between the various consortia. The CAVD also prepared language for use in institutional Material Transfer Agreements (MTAs) that provides terms and conditions for sharing materials among and between consortia in a manner that takes into account the objectives of the DMSA. These principles of the DMSA (and the related MTA language) further the Global Access objectives by establishing guidelines that facilitate the prompt and widespread sharing of data and other vaccine-related scientific information across the CAVD and more broadly with the scientific community.

The process to establish a common data- and materials-sharing agreement was an intense and collaborative effort that resulted in one of the most important features of the CAVD model—agreements that encourage the sharing of data and materials with all of the collaborating institutions of the CAVD. The concept was first presented in the request for proposals that the foundation posted in early 2005. More concrete discussions around the creation of these legal agreements began in early 2006 and were presented to the grantee Principal Investigators shortly after the initial set of grants were awarded in July 2006. By October
of that year, the foundation’s legal counsel had drafted the initial agreements through a collaborative effort with the PIs, technology transfer representatives, and legal counsel of the grantee institutions. A concerted effort was made to balance scientific, business, and legal concerns in order to create agreements that would serve the purposes of all three for the institutions involved. Within two months, the initial sixteen grantee institutions approved and signed a common data- and materials-sharing agreement and only five months later all of their collaborating institutions were signed on. This herculean effort to get nearly 100 public and private institutions from around the world signed up to a common set of principles is a testament to the dedicated involvement and passion of the scientists, administrators, and their institutions’ technology transfer and legal representatives to work in new ways to accelerate the development of a safe and effective HIV vaccine.

The DMSA provides the guiding principles for the collaborative work of the CAVD consortia and includes five annexes. (The DMSA and its annexes can be found on the CAVD Web site at www.cavd.org.) Annex A is the list of record of the CAVD collaborating institutions and can be found on the CAVD Web site as well as in Appendix 2 of this report. Annex B contains a set of defined terms used within the
agreement. The Guiding Principles for data- and materials-sharing are presented in Annex C and cover such topics as the management, treatment, and dissemination of standardized and non-standardized data, as well as inventions and publication rights. The Master CAVD Confidential Disclosure Agreement, Annex D, sets forth the terms for the exchange of confidential information between CAVD consortia. The Material Transfer Agreement (MTA), Annex E of the DMSA, is intended to streamline the process of transferring materials from and between CAVD consortia by providing a common agreed-upon template so that transferring institutions can focus on negotiating the details of the transfer itself rather than on the form used to facilitate the exchange.

With a total of 95 institutions currently signed on to the DMSA and that number continually evolving, implementation of the agreement and ongoing coordination among the investigators, the project managers, and the technology transfer and legal representatives at all of the institutions involved is critical to ensuring the success of the CAVD. As the amount of data and materials produced and shared within and outside of the CAVD’s collaborating institutions expands, the DMSA will require ongoing oversight, input, and evaluation by all collaborators.

“The CAVD Data & Materials Sharing Agreement established the principles for collaborative work, facilitated the negotiations with our research partners, and allowed us to reach agreement in a timely manner.”—Julie McElrath

Evaluation of the CAVD

While the ultimate goal of the CAVD is to develop an effective vaccine, intermediate measures of success are necessary in order to review progress at the individual grant level and to review incremental progress for the collaboration as a whole. As most of the CAVD projects are three- to five-year grants focused on pre-clinical research, progress is primarily measured through achievement of the project milestones agreed upon with the foundation in advance. However, in order to measure scientific progress within the framework of the CAVD short of the development of a vaccine and beyond individual grant milestone achievement, a formal evaluation program was initiated in November 2007.

The CAVD model is built upon the importance of collaboration, especially around the sharing of data and materials, to accelerate the pace of HIV vaccine research. To be successful, therefore, the CAVD must encourage its members to maintain a positive perception of collaboration as a means to support efficient
Section 2 | Overview of the Model

scientific discovery. The initial evaluation focused on establishing a preliminary baseline assessment of CAVD members’ definition of success and effective collaboration and documenting their perceptions of success. The baseline evaluation provided an analysis of CAVD participants’ perceptions of collaborative scientific pursuit, definitions of success for participants at all levels, suggestions for success metrics, and feedback on the CAVD structure and management. When asked about success markers for the CAVD structure, respondents suggested the following seven:

1. People working together
2. Information shared quickly
3. Efficient testing and comparing of ideas
4. Coordination among collaborators
5. Effective use of financial resources
6. Clearly demonstrated added value
7. A shift towards collective, evidence-based decision making

This feedback was used to help develop a method to track forward movement within the CAVD based upon proxy metrics of success such as the amount of data shared or material transferred among CAVD consortia. Both evaluation tools will enable the foundation staff and the CAVD members to track progress and course correct as needed to continually improve the scientific collaboration at the core of the CAVD in an effort to accelerate the development of an effective HIV vaccine.

While the perceptions survey will be repeated annually, the metric check will be done semiannually to track data such as: the number of completed studies with data sets posted on the CAVD internal portal; the number of materials transferred between consortia or with third parties; the number of inter- and intra-consortia publications and meeting abstracts; the number of patents in submission or filed; the number of statistical consultations; the number and type of study requested of the central service facilities; the number of new inter-consortia collaborations; and other data.

“The capacity to collaborate and share ideas, reagents, and data with other groups within our consortium and throughout the CAVD network has proven invaluable.” —Norman Letvin

An early review of the progress shows that inter- and intra-consortia collaborations are increasing as determined by the number of new collaborations formed, staff scientist exchanges, the amount of material transferred between consortia, and the use of the CSFs by the VDC. To date, 95 institutions have signed the CAVD Data and Materials Sharing Agreement and 45 unique new collaborations have been made between consortia within the CAVD since the grants were awarded. Five CAVD consortia have engaged in 16 separate staff scientist exchanges in another group’s lab that range, on average, between 1 and 20 days. A total of 78 MTAs have been executed—20 of which are third-party MTAs. A number of materials have been deposited in the cryorepository (antibodies, antiserum, cell lines, clinical materials, highly infected cells, HIV isolates, hybridoma cell lines, and pseudotypes) and projects are increasingly using the CAVD’s two Vaccine Immunology Monitoring Consortia (VIMC) to perform
standardized and exploratory lab assays and the Vaccine Immunology Statistical Center (VISC) for statistical consultations.

Increased research activity is reflected in the number of studies registered with the Mouse Immunology Laboratory (MIL) and Antibody and T-cell VIMCs—a total of 35 studies thus far. Sixteen of those studies have been completed with the resulting data posted on the members’ side of the CAVD portal for all CAVD researchers to review. Research generated by the CAVD is also being shared with the HIV vaccine community through papers and presentations. Twelve of the CAVD consortia submitted 44 abstracts to the AIDS Vaccine 2008 conference and a total of 85 abstracts have been submitted to international meetings. In the past two and half years, 47 original articles have been published in peer reviewed journals. (A list of those publications can be found in Appendix 4 and online at www.cavd.org.)

While these metrics help to define forward movement in the CAVD based on activities seen as important to the health of the collaboration, the scientific progress described in the next section is at the heart of the CAVD.
The CAVD provides funding to a select cadre of researchers who are pursuing some of the most promising avenues of research. Yet these scientists do not work alone. Each CAVD principal investigator is part of a larger effort of scientists worldwide who are united against the common challenge of developing a vaccine against HIV.
The scientific challenges HIV vaccine researchers face are formidable and include determining which immune responses need to be induced by a preventative vaccine, how to design a vaccine capable of coping with HIV’s genetic variability and tendency to mutate, and how to achieve lasting protection against the virus.

The CAVD is funding multiple and diverse strategies toward achieving these goals. The CAVD’s Vaccine Discovery Consortia (VDC) are exploring in parallel strategies that stimulate the production of antibodies against HIV-1, tactics that induce T-cell responses to HIV-1, methods that promote the body’s innate immune responses, and new approaches that stimulate immunity at the mucosal surfaces where HIV-1 usually initiates infection. It is likely that a combination of approaches will be necessary for the creation of an effective preventative vaccine.

Crucial to the progress of this research is the improvement of methods for evaluating immunogenicity of vaccine candidates. The CAVD’s central service facilities (CSF) are working to streamline laboratory evaluations of immune responses, provide data on the cross-comparisons of studies conducted in different laboratories, and innovate new mouse models, assays, and statistical methods for evaluating vaccine candidates.

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CAVD investigators are often associated with other HIV/AIDS vaccine research initiatives, including the Center for HIV/AIDS Vaccine Immunology (CHAVI), the International AIDS Vaccine Initiative (IAVI), EUROPRIZE, the NIH’s Vaccine Research Center, EuroVacc, and the HIV Vaccine Trials Network (HVTN). The rapid exchange of information among CAVD members, as well as between the CAVD and other vaccine research efforts, is an essential piece of the overall strategy to bring HIV-1 vaccine candidates forward for evaluation as quickly and as efficiently as possible.

This section presents an overview of the research initiatives and accomplishments of CAVD scientists. It begins with a discussion of CAVD efforts to improve antibody-based vaccines, then describes research aimed at developing effective T-cell-based vaccines, and finally recounts CAVD initiatives aimed at a variety of novel approaches, such as ways to stimulate the innate immune system and target mucosal surfaces.

Antibody Approaches

Despite early failures of a vaccine designed to elicit antibodies to the viral envelope protein gp120 to protect individuals from acquiring HIV-1 in large-scale clinical trials, several studies indicate that antibody approaches can work. In a monkey model, for example, passive administration of neutralizing antibodies followed by challenge with an HIV/SIV hybrid virus called SHIV either prevented infection or caused long-term reductions in the levels of virus in plasma.

Another piece of evidence in support of antibody-based vaccines is based on the
observation that infected individuals are capable of making antibodies that can neutralize HIV. However, most of those antibodies are narrowly directed against the infecting strain, and the virus continually mutates to escape the neutralizing antibodies. A handful of human monoclonal antibodies against HIV-1 do neutralize a broader range of HIV-1 strains, and are thought to represent the type of antibody that an HIV vaccine should elicit.

CAVD researchers are taking a number of approaches to breaking down the barriers that HIV-1 has thrown in the way of antibody-eliciting vaccines. These approaches include:

- Rational antibody vaccine design based on induction of broadly neutralizing antibodies in HIV-infected individuals,
- Elicitation of antibodies that target broadly conserved regions of HIV-1,
- Novel immunogens that mimic HIV-1 envelope proteins bound to CD4+ T-cell receptors,
- Antigens that mimic the functional envelope protein, such as stabilized trimeric constructs; and finally,
- Stimulation of antibodies against the V3 loop.

**Rational Antibody Vaccine**

Broadly neutralizing antibodies identified in infected individuals may help researchers identify specific regions of the viral envelope that elicit those antibodies during the course of natural infection. By studying these epitopes, researchers hope to “reverse engineer” a vaccine that can elicit these antibodies.

When the CAVD first began, investigators knew of only five naturally occurring broadly neutralizing monoclonal antibodies to HIV-1. These antibodies recognize the viral envelope proteins gp120 and gp41 in their natural shape on the virus or, in the case of some gp41 antibodies, a form of the envelope that appears only briefly as the virus infects a new cell.

Over the past two years, CAVD researchers have provided more than twice that number of newly identified broadly neutralizing monoclonal antibodies to the scientific community.

**Robin Weiss** at the University College London heads one of the Vaccine Discovery Consortia. These researchers are applying “reverse vaccinology” approaches to finding ways to induce antibodies that can neutralize the HIV-1 subtypes most common in Africa.

Researchers of the Weiss-led VDC have isolated 58 new monoclonal antibodies from the blood of HIV-infected donors, including 25 that neutralize more than one HIV-1 subtype, or clade. They have selected five of these for further study. This work has greatly expanded the research tools available to the HIV-vaccine community both within CAVD and worldwide.

The Weiss VDC has also identified new HIV-1 antibodies in two animal models, llamas and humanized mice. Llamas are particularly attractive for this purpose because they make very small antibodies that are amenable to screening by today’s high-throughput technologies. To date the researchers have produced eight groups of broadly neutralizing
monoclonal (highly specific) antibodies in llamas as well as 43 new mouse-derived monoclonal antibodies, two of which neutralize more than one clade.

With this profusion of new monoclonal antibodies against HIV-1, the researchers can screen proteins and smaller molecules for their ability to act as epitopes and bind the antibodies. The targets are HIV-1 envelope proteins and also peptide subunits tethered to scaffolds that hold them in the optimal position for presentation to the host immune system. This “reverse vaccinology” approach uses natural anti-HIV-1 antibodies to uncover novel immunogens for use in vaccine candidates. Another important approach is to make virus-like-particles bearing variants of the envelope proteins.

If these proteins, peptide-scaffold molecules or virus-like-particles bind the HIV-1 monoclonal antibodies in the screening assay, then the researchers know that they’ve found a candidate that might be a good immunogen in a vaccine. The investigators can then test the ability of selected molecules to elicit neutralizing antibodies in rabbits. The best candidates will be tested in macaques to see if the antibodies can indeed protect against infection. In addition to these studies, the Weiss VDC is systematically evaluating novel immune-stimulating molecules called adjuvants to enhance the efficacy of the vaccine candidates.

The Weiss VDC includes collaborations with leading researchers at the Biomedical Primate Research Centre, the Institute for Research in Biomedicine, the Medical Research Council, Pepscan Systems BV, Polymun GmbH, Prince Leopold Institute of Tropical Medicine, Queen Mary University of London, Imperial College London, University of Cambridge, Université Joseph Fourier in Grenoble, University of Oxford, the University of Regensburg’s Institute of Medical Microbiology and Hygiene, and the University of Utrecht.
Targeting Broadly Conserved Regions of HIV-1

Another research group taking a reverse engineering approach to developing vaccines is the one led by Leonidas Stamatatos of the Seattle Biomedical Research Institute (SBRI). The Stamatatos-led VDC brings together expertise from diverse disciplines, such as computational biology, structural biology, virology and immunology. These researchers are using computational methods to create novel protein constructs that could act as immunogens to stimulate the body to produce antibodies against HIV-1.

The researchers are developing non-HIV proteins that present structurally accurate HIV-1 epitopes known to be targets of the human broadly neutralizing antibodies. To learn more about these antibodies, the researchers analyzed antibodies in human HIV-infected plasma samples collected from AIDS-free individuals. The researchers identified antibodies specific to the CD4 binding site as well as other epitopes. Their work indicates that there are multiple epitopes to target and multiple ways to elicit neutralizing antibody responses by vaccination.

One epitope of interest to the Stamatatos VDC researchers is located near the membrane spanning domain of the gp41 subunit of the envelope protein. Previous work has shown that the broadly neutralizing monoclonal antibody 4E10 binds to this epitope, which is located close to the viral membrane. This membrane proximal external region (MPER) is relatively highly conserved across the clades (or subtypes) of HIV-1 and is involved in HIV’s fusion with host cells. Another of the naturally occurring human neutralizing antibodies, 2F5, also binds to this region.

The team led by Stamatatos is making protein scaffolds incorporating epitopes that can bind to the monoclonal antibody 4E10, which is thought to be the most broadly cross-reactive of the known human neutralizing antibodies. Of the 226 scaffold sequences evaluated, 26 were able to bind to 4E10, although they did not elicit neutralizing antibodies in rabbit models. The researchers are exploring what is happening in the immune system that is preventing the protein scaffold from eliciting neutralizing antibody responses that target conserved epitopes.

In addition to the reverse-engineering approach, the Stamatatos VDC researchers are engineering novel trimeric gp140 HIV envelope proteins composed of non-identical gp140 protomers (gp140 heterotrimers). They are also defining the crystal structure of the immunogens. In a related project, the researchers are isolating and characterizing broadly reactive neutralizing antibodies from non-human primates infected with SHIV. Finally, the researchers are taking steps to optimize the immunogens to elicit the broadest possible neutralizing antibody responses.

The Stamatatos VDC includes collaborators at the California Institute of Technology, the Fred Hutchinson Cancer Research Center, Tulane University, and the University of Washington.

Eliciting neutralizing antibodies to the MPER of gp41 is also the focus of a new VDC led by Ellis Reinherz at the Dana-Farber Cancer Institute in Boston. Funded in September 2008, the Reinherz VDC is characterizing the
the MPER structure and exploring ways to elicit broadly neutralizing antibodies against it. The researchers have created lipid-coated nanoparticles that they hope to use to present the MPER to B-cells, which in turn will secrete anti-MPER antibodies. The nanoparticles will concomitantly deliver adjuvants, substances that stimulate immunity. In the future the researchers plan to use the nanoparticles also to deliver epitopes that stimulate T-cell immune responses.

The Reinherz-led VDC operates in collaboration with the Massachusetts Institute of Technology.

Novel Immunogens

Novel immunogens based on native structures are one of the research imperatives of the VDC led by Barton Haynes at Duke University in Durham, North Carolina. Researchers in the Haynes VDC have designed stable, homogenous preparations of the gp41 intermediate conformation of the envelope protein. This construct is being used as an immunogen with lipids and an antigen-specific detection reagent to isolate B-cells capable of producing neutralizing antibodies to the gp41 MPER.

The Haynes-led VDC is also making peptide-lipid conjugates that bind with high affinity to the 2F5 or 4E10 monoclonal antibodies. They have demonstrated that the binding of these monoclonal antibodies to lipids is critical for their ability to neutralize HIV-1. What is more, the binding to lipid, a self-antigen, is direct evidence of the role of B-cell tolerance in the regulation of antibodies against HIV-1.

The Haynes VDC is exploring the possibility that HIV-1 has exploited the normal mechanisms that eliminate B-cells reactive to self-antigens, called tolerance. The researchers have developed a mouse model to definitively show that the B-cell response to the gp41 MPER region in mouse is regulated by bone
marrow tolerance mechanisms. Novel mimics of the gp41 conserved neutralization regions coupled with adjuvants are being tested to stimulate B-cells capable of making broadly neutralizing antibodies.

In a related project, researchers in the Haynes VDC have discovered that anti-lipid antibodies in fact may play a role in HIV-1 protection. The investigators found that anti-lipid antibodies produced in autoimmune disease have the capacity to broadly inhibit the infection of peripheral blood mononuclear cells by virtually all CCR5-dependent HIV-1 primary isolates. They are working now to translate this finding into a novel strategy of HIV-1 vaccine development.

The Haynes VDC brings together researchers at the Albert Einstein College of Medicine, Beth Israel Deaconess Medical Center, Children’s Hospital Boston, Duke University Medical Center, National Cancer Institute, Tropical Diseases Research Center (Zambia), Tulane University, University of Alabama at Birmingham, University of Kansas, and University of Texas Southwestern Medical Center at Dallas.

Stabilized Transition State Immunogens

One reason that vaccine-induced antibodies fail to protect against HIV infection may be that viral epitopes on the envelope of HIV are not exposed on virus particles that are circulating in the bloodstream. Some researchers have speculated that epitopes located in and around gp120 are only transiently exposed when the gp120 attaches to the CD4 receptor on the T-cell surface. Robert Gallo of the Institute of Human Virology in Baltimore leads a VDC that is designing vaccines based on these gp120-CD4 epitopes, known as CD4-induced (CD4i) epitopes or stabilized transition-state immunogens.

The CD4i epitopes are some of the most conserved structures of the HIV-1 envelope. Therefore antibodies to CD4i epitopes should be highly cross-reactive among viral strains. Monkeys vaccinated with the CD4i immunogen, called Rhesus Full Length Single Chain (rhFLSC) complex, and then challenged with SHIV, exhibited significantly accelerated clearance of plasma viremia and an absence of long-term tissue viremia compared with unvaccinated animals. The Gallo-led VDC aims to optimize administration to see if they can enhance protection by varying the immunization schedule, delivering the vaccine directly to the mucosa, or via passive immunization with monoclonal antibodies specific for CD4i epitopes.

In a related project, researchers at the Gallo VDC are isolating monoclonal antibodies specific for CD4i epitopes from humans and rhesus macaques. Gallo and his collaborators have developed a novel way of isolating human monoclonal antibodies in a short time. The innovation is based on the realization that memory B-cells, which produce antibodies, serve as a cellular archive of past antibody responses. Gallo’s team has established conditions that activate these memory B-cells and coax them to produce antibodies. The investigators can then screen the antibodies for specificity against a certain pathogen, in this case HIV-1. They can clone the genes for these antibodies and produce laboratory quantities of antibody for analysis. With this method they
have identified sixteen new human monoclonal antibodies specific for CD4i epitopes, with many more on the way.

**V3 as a Potential Epitope**

Researchers are studying many other regions of gp120 and gp41 for epitopes that can elicit antibodies to neutralize HIV-1. One region of gp120 that researchers have identified as a potential protective epitope is the third hypervariable loop of gp120 (V3 loop). The group led by Susan Zolla-Pazner of the New York University School of Medicine is applying reverse-engineering approaches to explore the V3 loop as a potential vaccine immunogen. To do so, researchers in the Zolla-Pazner-led VDC are characterizing natural human anti-V3 neutralizing antibodies derived from HIV-infected individuals, studying their neutralizing capability, and designing vaccine candidates that can elicit such antibodies.

Previous studies had focused on the diversity of sequences found in the V3 loop among strains of HIV-1, but the Zolla-Pazner VDC has found that there is a conserved structure to the V3 loop, explaining how anti-V3 antibodies produced by HIV-infected human subjects can be broadly reactive with the V3 loops of viruses belonging to multiple HIV-1 clades. A challenge to be solved is that the V3 loop is not exposed on the surface of the virus.

By studying the crystallized structure of the V3 loop, the researchers rationally designed new V3 immunogens. These V3 immunogens are attached to non-HIV protein scaffolds. To test them, the investigators immunized rabbits in a two-step process by first priming rabbits with plasmid DNA expressing gp120 and then boosting with V3 presented on various recombinant scaffold proteins. The regimen induced antibodies that displayed low levels of cross-clade neutralizing activity. The best candidates will be tested in non-human primate and human trials.

The Zolla-Pazner VDC is collaborating with Molsoft LLC, the University of Massachusetts Medical School, and the University of Medicine and Dentistry of New Jersey as well as several sites in Cameroon and India.

**T-cell Approaches**

Vaccines that boost T-cell responses to HIV-1 have the potential to prevent HIV infection, slow disease progression by suppressing viral multiplication, and reduce the risk of HIV transmission.

A successful T-cell vaccine would induce HIV-1-specific CD4+ T-cells and CD8+ cytotoxic T-lymphocytes (CTLs). CD4+ T-cells secrete cytokines that recruit immune cells including CTLs that kill infected cells, preferably before the cell’s internal machinery produces and releases a burst of new viral particles. HIV-1-specific CTLs could play a role as early defenders against HIV-1 by patrolling the gastrointestinal tract, genital tract and lymphoid tissue.

Evidence that vaccines can elicit specific HIV-1 T-cell protection comes from several sources. Highly exposed persistently seronegative (HEPS) individuals often have high levels of HIV-1-specific T-cells although these seem to be
directed to specific epitopes. Studies in macaques provide evidence that T-cell-based vaccines can keep viral load in check, and even reduce it to undetectable levels, whereas removing the T-cells causes viral loads to skyrocket.

Several technical challenges must be overcome, however. The virus can mutate into variants not recognized by vaccine-induced T-cells, a phenomenon known as viral escape. Long-term non-progressors eventually go on to develop AIDS later in life despite having HIV-1 specific T-cells. To keep viral loads in check, vaccines must induce long-term responses that can be maintained in immunological memory cells, so that CTLs can be mobilized rapidly when an individual is exposed to HIV-1. Finally, researchers must be conscious of the fact that the viral vector used in the T-cell vaccine can generate its own immune response.

T-cell vaccines have tremendous potential once researchers optimize these vaccines. In 2007, however, a promising T-cell vaccine candidate using an Ad5 vector failed to reduce HIV acquisition in a large clinical study known as the STEP trial. CAVD researchers are working on understanding why this vaccine failed and finding ways to improve viral vectors, HIV-1 gene inserts, and other properties that will enhance vaccine efficacy. The grantees are working on improving T-cell vaccines using the following strategies:

• Developing new and improved viral vectors
• Optimizing T-cell vaccine inserts
• Improving T-cell vaccine delivery
• Learning lessons from the STEP trial

New and Improved Viral Vectors
CAVD researchers are pursuing a number of strategies toward improving existing vectors or developing new ones.

Giuseppe Pantaleo at the Centre Hospitalier Universitaire Vaudois (CHUV) in Lausanne, Switzerland heads a T-cell VDC aimed at improving the immunogenicity of the poxvirus vectors, in particular the New York Vaccinia Virus (NYVAC), by deleting virus-specific genes encoding proteins that are suspected of being involved in evasion of host immune responses. The researchers are also working on generating a replication-competent version of the vaccinia NYVAC strain. A vector capable of replicating would be an advantage because HIV-1 epitopes would be amplified. The researchers are also creating a combined (multiple gene deletion mutant plus replication competent) poxvirus vector.

To date, the researchers in the Pantaleo-led VDC have found a good safety profile with the replication-competent NYVAC in a newborn mouse model. They have developed in vitro human immunological assays to help guide selection of the newly developed vectors. These tests have been instrumental for the selection of modified NYVAC viral vectors with improved immunogenicity. They are proceeding with the evaluation of a replication-competent NYVAC vector in a non-human primate animal model.

The Pantaleo VDC is collaborating with researchers from Arizona State University, Biomedical Primate Research Centre, CHU Henri Mondor, University Paris 12, Consejo Superior de Investigaciones Científicas, Fred
Another new vector is Newcastle Disease Virus (NDV), under study by researchers at the VDC led by David Ho at the Aaron Diamond AIDS Research Center in New York City. NDV infects avian species and since humans are not usually infected with NDV, they have no preexisting immunity to it. The researchers are currently experimenting with viral gene inserts to optimize the immunogenicity of an NDV vector vaccine.

The Ho-led VDC focuses on several research avenues that span a variety of HIV-1 vaccine strategies, including not only new viral vectors but also adjuvants and targeting of dendritic cells, which are immune system cells that present antigens to the immune system. See the section “New Approaches” for more research by the Ho VDC. The VDC collaborates with Academia Sinica in Taiwan, Mount Sinai Medical Center, and the Rockefeller University.

New and improved vectors are also a goal of the group led by Norman Letvin at the Beth Israel Deaconess Medical Center in Boston. Researchers at the Levin-led VDC are exploring ways to make recombinant adenovirus (rAd) and mycobacterial vectors that can escape preexisting vector immunity and can elicit durable systemic and mucosal immune responses. Investigators in the Letvin VDC are generating novel rAd vectors from strains of Ad that are not commonly found in the human population. Using rare strains is advantageous because many individuals have preexisting immunity to common viral strains, such as Ad serotype 5. Some researchers suspect that preexisting antiviral vector immunity can impact the performance of
the HIV-1 vaccine, perhaps even enhancing an individual’s risk of acquiring HIV-1. (See STEP Trial: Lessons Learned).

The Letvin VDC is also exploring recombinant mycobacteria (rMyco) as novel vectors with enhanced immunogenicity. The researchers are screening mutant rMyco strains with deletions in various immune evasion genes for their immunogenic potential. Once confirmed, these genetic mutations will be combined and tested in nonhuman primates. The Letvin VDC studies demonstrate that a prime-boost regimen using rMyco vectors as a prime followed by rAd boost may offer an effective vaccine strategy for eliciting robust cellular immune responses against HIV-1 antigens.

The Letvin VDC collaborators are from Albert Einstein College of Medicine, Duke University Medical Center, and the Vaccine Research Center, NIAID, NIH.

The discovery and improvement of novel viral vectors is the focus of the VDC led by Timothy Zamb at the International AIDS Vaccine Initiative (IAVI) in New York. Researchers at the Zamb-led VDC are taking a number of approaches, including the pursuit of recombinant adeno-associated viruses (AAV) and naked DNA plasmids (as priming agents), the creation of highly novel chimeric viruses that are structurally indistinguishable from HIV, vectors that target mucosal surfaces, vectors that elicit persistent immunological responses, and tailoring gene inserts to optimize immunogenicity, plus gene delivery via electroporation. These last two topics are covered later in this report.

One way the immunogenic potential of HIV-1 vaccines could be enhanced is via the creation of non-pathogenic HIV-like chimeric viruses. Researchers in the Zamb VDC have created a chimera of HIV-1 and the Venezuelan equine encephalitis virus (VEEV). The chimeric virus combines HIV-1 genes with VEEV’s replication machinery. The result is a virus that appears to be morphologically identical to HIV-1 but which contains the replication machinery of
VEEV, making these HIV-like viruses incapable of integration into the host cell genome. The investigators plan to test the chimeric viruses in non-human primates in 2009.

The Zamb VDC collaborates with researchers from Children’s Hospital of Philadelphia, Global Vaccines, Inc., Harvard University, Oregon Health Sciences University, Scripps Research Institute, and the University of Wisconsin-Madison.

Optimizing T-cell Vaccine Inserts

The genes carried by T-cell vector-based vaccines are crucial for initiating a host immune response that is specific to HIV-1. Typically vectors carry HIV-1 genes such as gag, which codes for viral capsid proteins. Several groups are working on optimizing the combination of HIV-1 genes introduced via the vaccine. But HIV-1 genes are not the only type of gene that could provide immune stimulation. The Zamb VDC is designing vectors that carry genes that express proteins with adjuvant, or immune-stimulating, activity. These “adjuvanting” genes encode cytokines, proteins that stimulate innate and adaptive immunity, and gene products that induce programmed cell death, which can stimulate CD8+ T-cell activation. [See more about stimulating innate immunity with adjuvants in the “New Approaches” section.]

Another novel approach to improving the genes carried by vectors is being explored at the VDC led by Steven Patterson at Imperial College London. One hypothesis to explain the limited diversity of T-cell responses in response to natural infection or vaccination is competition for space on the surface of the antigen-presenting cells, such as dendritic cells, which present antigens to the immune system. The Patterson-led VDC researchers are trying to overcome this problem by dividing the HIV-1 vaccine genes into a series of mini-genes and incorporating them into separate vectors. This strategy will reduce the number of HIV-1 epitopes expressed by any single presenting cell and could thus broaden the response. Steady progress has been made in mini-gene vector construction. Another strategy under study at the Patterson VDC is to construct adenovirus vectors coding for fusion proteins between ubiquitin and HIV-1 gag. These fusion vectors have been shown to enhance targeting to the proteasome and increase the level of viral peptide presentation by specialized proteins on the antigen-presenting cell surface.

T-cell Vaccine Delivery

The Patterson VDC is also working on enhancing the delivery of T-cell vaccines. Mass vaccination in developing countries could be improved if the vaccine could be delivered via a skin patch that does not require refrigeration. Additionally, strategies that help the vector evade adenoviral-neutralizing antibodies that blunt the effectiveness of vaccination in people previously exposed to adenovirus are needed.

To address these concerns, the Patterson VDC members are exploring delivery of adenovirus vector vaccines inside a polymer coating, delivered to dendritic cells via sugar microneedles embedded in a skin patch. The patch would consist of roughly 25 to 40 sugar microneedles, each up to 1500 micrometers in length. On application
the sugars dissolve releasing the vaccine in close proximity to the dendritic cells. The investigators have demonstrated that adenovirus vector embedded in dry sugar can enter the cells and induce expression of encoded transgenes after one month of storage at 40°C. Mice vaccinated with skin patches containing dried adenovirus vector coding for ovalbumin were able to develop ovalbumin-specific CD8 T-cell responses.

The Patterson VDC's collaborators include researchers at Hybrid Systems, Ltd., King's College London, National Institute for Biological Standards and Control (NIBSC), Royal Holloway and Bedford New College, TheraJect, Inc., and the University of Washington.

Another way to introduce vaccines into the body is via pulsed electrical stimulation, or electroporation. Electroporation allows delivery of DNA to non-dividing muscle tissues and can enhance robust immunological responses to the encoded products. The Ho VDC is leading a clinical trial to test the effect of electroporation on enhancing the immune response to the ADVAX(TM) DNA vaccine candidate in HIV-uninfected volunteers. In this Phase I study, the vaccine is injected directly in the muscle immediately followed by an electrical pulse delivered to the muscle. All volunteers have been enrolled and have successfully received two vaccinations.

Another consortium working on vaccine targeting and delivery is the VDC headed by Zamb at IAVI. The Zamb team is also exploring electroporation for delivery of viral vectors and naked DNA. In addition, the researchers are creating viruses capable of targeting the mucosal lining [see section on mucosal immunity].

**STEP Trial: Lessons Learned**

An open question in T-cell vaccine research is whether preexisting immunity to the Ad5 viral vector used in the STEP trial enhanced HIV-1 susceptibility. The STEP trial vaccine failed to reduce HIV-1 acquisition in trial participants. Some participants became more susceptible to HIV-1 after vaccination. Researchers have speculated that pre-existing exposure to the vaccine vector adenovirus serotype 5 (Ad5) from natural infection could have contributed to HIV-1 risk.

To explore this question, researchers at the Letvin VDC examined cryopreserved samples from humans who received the rAd5 HIV-1 vaccine candidate in a number of early phase vaccine studies. Results from the analysis of anti-rAd5 vector-specific cellular and humoral immune responses do not support the hypothesis that the enhancement of HIV-1 acquisition was due to the induction of Ad5-specific T-cell responses in individuals with detectable preexisting anti-Ad5 antibodies. When the anti-Ad5 antibodies were analyzed, they found that neutralizing antibodies generated by natural Ad5 infection and by rAd5 vaccination targeted different viral proteins. Furthermore, preexisting immunity from natural infection reduced the T-cell responses to specific HIV antigens in a particular prime-boost regimen. These data indicated significant differences in Ad5 viral antigen presentation between natural infection and vaccination, which might have important implications for vaccine design. The investigators are continuing to study the biology of anti-vector immunity and the differences in immune system responses between natural infection by adenovirus and vaccination with rAd vectors.
New Approaches

Classical vaccine design has so far been inadequate to tackle HIV-1’s immune system-evading maneuvers. New approaches that complement the antibody-based and T-cell-based vaccine initiatives are discussed in this section.

Mucosal Immunity

HIV usually invades at the mucosal surfaces of the body and researchers are learning much about the body’s immune response at the mucosal surfaces in the gastrointestinal and genital tracts. Since HIV establishes a reservoir in gut-associated lymphoid tissues, mucosal immunity appears to play a larger role than previously suspected in protecting against HIV.

Mucosal surfaces are protected by a combination of local innate and adaptive immune responses. Innate immunity is the body’s first line of defense against invaders and can involve relatively non-specific antibody and T-cell responses that patrol for foreign microbes and also play a role in shaping the subsequent adaptive immune response. Adaptive immunity involves antibody-producing cells (B-cells) trained to recognize a specific pathogen such as HIV-1, or epitope-specific T-cell activation, or both, at the mucosal surface and in the surrounding lymphoid tissues.

Innate Immunity

In recent years, great strides have been made in our understanding of innate immunity, and vaccine researchers are drawing on this knowledge. Innate immunity can be elicited through the use of adjuvants, which are substances and formulations that increase and shape the innate immune response to an antigen. Although they are widely used in today’s vaccines, adjuvants have been discovered largely through trial and error. It is only in recent years that researchers have begun to discover how and why they work to boost innate immunity.

The VDC led by Juliana McElrath at the Fred Hutchinson Cancer Research Center in Seattle is taking a comprehensive and systematic
approach to cataloguing the precise molecular pathways of innate immunity stimulated by adjuvants and vectors. The investigators are looking for signatures of innate immunity by transcriptional analysis using human peripheral blood samples obtained from individuals being vaccinated intramuscularly. With the results they are building a comprehensive matrix of the innate signatures of candidate adjuvant and vaccine formulations that can lead to optimal T- and B-cell memory responses. This platform is ideally suited to examine promising regimens emerging from the CAVD.

One of the key mechanisms of innate immunity is the activity of special receptors that can bind to ligands found in the pathogen but not the host. One family of these receptors is the Toll-like receptor (TLR) family. When a pathogen binds to a TLR, the result is the stimulation and activation of antimicrobial or antiviral molecules. A new line of inquiry in HIV-1 vaccine research is whether certain TLR ligands could induce an innate immune response. Examples of TLR ligands being studied for their use as adjuvants in HIV-1 vaccines include flagellin, double-stranded RNA, and bacterial CpG oligodeoxynucleotides (CpG ODN).

One way to improve the immunological response of vectors is by including adjuvanti ng genes for cytokines such as IL-2 and TLR ligands such as flagellin that are co-expressed with the viral genes. Researchers in the McElrath VDC have found that the priming regimen of HIV-1 protein with the TLR ligand Poly I:C induces strong multifunctional T-cells and antibodies. The researchers have tested the regimen in Rhesus macaques and plan to begin testing it in Phase I trials in the near future.

The McElrath-led VDC is also investigating how priming with different vectors can impact the effector and memory T-cell responses to an antigen. They have found that recombinant Listeria/HIV-1 regimens can induce strong multifunctional T-cells and antibodies. They are considering next steps to move the Listeria vector toward clinical Phase I trials with HIV-1 gene inserts.

The McElrath VDC is collaborating with researchers from Emory University, the Institute for Systems Biology, New York University, Oregon Health Sciences University, and the University of Washington.

In other adjuvant research, Ho VDC investigators are exploring the use of another TLR ligand, bacterial flagellin, which binds to TLR-5 and primes the immune system to elicit immune responses. The Ho VDC scientists have shown that HIV-1 proteins fused to flagellin could enhance vaccine immunogenicity. Other adjuvants that the Ho VDC team are exploring include novel glycolipids.

Adaptive Immunity

Mucosal immunity can also be adaptive. A vaccine that induces HIV-specific antibodies in the mucosa may provide better protection than antibodies produced elsewhere in the body. An HIV-1 vaccine that protects mucosal surfaces could block HIV-1 from crossing into the body and reaching target CD4+ cells.

Several lines of evidence support the idea of mucosa-targeted HIV-1 vaccines. For example, HEPS (highly exposed persistently seronegative)
individuals seem to have local mucosal HIV-specific T-cells and antibodies.

Some strategies for delivering vaccines to the mucosa are to use bacterial or viral vectors that localize to the mucosal tissue, co-administration with adjuvants that are active at mucosal surfaces, hitching immunogens to carrier molecules that travel to mucosal sites, and placing antigens in microparticles or other vehicles that travel to mucosal sites.

One viral vector that targets mucosal tissue is the reovirus. This virus infects gastrointestinal M-cells that transfer viruses across mucosal barriers. The Zamb VDC is exploring how a reovirus-based vaccine could stimulate an immune response against HIV-1 directly at the site of initial infection and replication in the gut-associated lymphoid tissue. Other viral vectors of interest to the Zamb team are Newcastle Disease Virus (NDV) and canine distemper virus (CDV). Both of these paramyxoviruses are pathogens of the upper respiratory tract and thus should target the mucosa. Once the team has produced vectors encoding protective SIV immunogens, they will test these vaccine candidates in non-human primates in 2009.

Another approach to target vector-based vaccines to the intestinal mucosa is being pursued by the Letvin VDC. A team of scientists is developing chimeric rAd vectors that target the intestinal mucosa. When these vectors were delivered directly to the small intestines in mice, the vectors elicited systemic and mucosal immune responses that were enhanced by a later intramuscular injection. The group plans to test this prime-boost regimen in an SIV challenge model in nonhuman primates.

Dendritic Cells

An exciting frontier of HIV-1 vaccine research involves a key immune system cell called the dendritic cell. The cell’s job is to capture viruses and antigens, transport them to the draining lymph nodes, and present them to the CD4+ lymphocytes so that the lymphocytes can mobilize a response to the invading pathogens. In the case of HIV-1, the dendritic cells may inadvertently be ferrying infectious HIV-1 particles directly to a massive concentration of the very CD4+ cells that HIV infects.

The Ho VDC investigators have produced the first dendritic cell-targeted HIV vaccines. These researchers are designing vaccines consisting of protein antigens fused to monoclonal antibodies that target dendritic cell receptors. So far, the researchers have produced a dendritic cell-targeted vaccine candidate containing HIV-1 gag protein. The Ho VDC members also have created mouse monoclonal antibodies to other dendritic cell receptor targets.

The existence of dendritic cells was co-discovered by Ralph Steinman, a CAVD collaborator, with Zanvil A. Cohn at the Rockefeller University. Steinman and his team, funded through the foundation’s Grand Challenges in Global Health program, is engineering monoclonal antibodies capable of targeting the DEC-205 receptor on dendritic cells. The researchers use poly I-C as an adjuvant to stimulate dendritic cell maturation. Studies in mice indicate that antibodies against human DEC-205 bound to HIV-1 gag protein plus poly I-C enhanced the CD4+ T-cell and CD8+ T-cell responses.
The Patterson VDC is targeting dendritic cells through the use of sugar microneedles embedded in skin patches. [See previous section on “T-cell vaccine delivery”]

Clues from HEPS and Long-Term Controllers

Much can be learned from studying the immune system of people that apparently control HIV-1. The team led by Francis Plummer at the University of Manitoba, Winnipeg, Canada, is studying mechanisms of HIV-1 resistance in highly exposed persistently seronegative (HEPS) women, with funding from the foundation’s Grand Challenges in Global Health program. Plummer and his team are studying a group of commercial sex workers in Kenya who do not become infected with HIV-1 despite repeated exposure. Plummer’s group has identified altered HIV-1-specific CD4+ and CD8+ T-cell responses in HIV-1-resistant women and significantly reduced expression of the innate immune system receptors such as TLR 7 and TLR 8.

Analyzing the immune responses of long-term controllers could yield clues about how to defeat the virus. The VDC led by Bruce Walker at the Partners AIDS Research Center at Massachusetts General Hospital in Boston is studying 1300 patients who spontaneously control infection. The researchers want to determine the breadth and specificity of the HIV-1-specific CTLs in viral controllers versus progressors. They are exploring the role of adaptive T-cell responses, the interaction of innate and adaptive immunity, viral evolution, and antigen-processing activities, and are trying to define the host genetic factors that impact control over HIV-1 replication.

A key question is whether HIV-1-controllers have different T-cell responses than progressors. They found that CD8+ T-cells from HIV-1 controllers preferentially target the HIV-1 gag protein over other proteins, whereas responses are more broadly distributed in persons with progressive infection. Compared to progressors, elite controllers (defined as HIV-infected individuals not taking antiretroviral drugs who maintain nearly undetectable viral loads) had significantly more CD4+ and CD8 T+ cells secreting the cytokines interferon-gamma and interleukin-2, as well as lower HIV-1 neutralizing antibody responses.

Researchers in the Walker-led VDC have also discovered that specific types of natural killer (NK) cells are expanded during the acute phase of HIV-1 infection. NK cells are part of the innate immune response and they act as quality control for all cells, including the important dendritic cells, by eliminating defective ones. Walker is exploring how HIV-1’s impact on NK cells might affect the progression of infection.

When the immune system detects foreign antigens, dendritic cells and other immune cells engulf the viral particles, degrade them, and display the viral epitopes on the cell surface in a specialized protein structure called the Major Histocompatibility Complex (MHC), or in humans the Human Leukocyte Antigen (HLA) complex. The Walker VDC investigators found that antigen-processing activities are significantly higher in controllers, especially those expressing HLA types B27 or B57, as compared to progressors with high viral loads.

Several studies have indicated that some HLA types, or alleles, appear to protect against disease progression while others seem to contribute to
The Walker VDC researchers found to their surprise that the antigen-processing activities of controllers expressing protective alleles were significantly different from those expressing non-protective HLA alleles.

The Walker VDC collaborates with researchers at Jessen-Jessen Medical Practice, Massachusetts Institute of Technology, the SAIC-Frederick, and the University of KwaZulu-Natal.

**Allogeneic Vaccines**

One CAVD-project is exploring vaccine candidates that target HLA, a group of human proteins. The team lead by Thomas Lehner of King’s College London is testing a vaccine consisting of antigens to HLA-class I and HLA-class II in combination with epitopes made of trimeric HIV-1 gp140, SIVp27, and heat shock protein 70 (HSP70). The HSP70 is an adjuvant that elicits innate immunity. The antigens are linked to dextran and administered with an adjuvant. Preliminary results suggest that macaques develop high levels of antibodies to the antigens. When challenged with SHIV, preliminary results indicate that the macaques experience a decrease in viral load or prevention of infection. The Lehner VDC study indicates that complement-dependent neutralizing activity to HIV-1 can be achieved by a combination of HLA-class II or class I, HIV-1gp120 and HSP70.

The Lehner VDC vaccine candidates harness both innate and adaptive immunity through this multifunctional approach. The researchers are targeting both mucosal and systemic immunity by inoculating non-human primates vaginally and intramuscularly. They are also determining correlates of protection in their macaque studies by evaluating the innate, non-cognate, and adaptive mucosal mechanisms.

The Lehner-led VDC’s collaborators are researchers at Dako Denmark A/S, LIONEX Diagnostics and Therapeutics GmbH, National Center for AIDS/STD Control and Prevention of China, the NIH’s National Institute of Allergy and Infectious Diseases, Sanquin Blood Supply Foundation, and the Swedish Institute for Infectious Disease Control.

**Central Service Facilities: Improving the Infrastructure for Evaluating Vaccines**

The CAVD has established five central service facilities (CSF) that provide central services and expertise to the consortia for the evaluation of vaccine candidates and the analysis of data. These include two laboratory networks for measuring the immune responses elicited by vaccine candidates, a mouse immunology facility, a research specimen repository, and a data and statistical management center. In addition to these services, scientists in these CSFs conduct novel research to improve the vaccine effort.

**The Comprehensive Antibody Vaccine Immune Monitoring Consortium**

The Antibody Vaccine Immune Monitoring Consortium (Ab VIMC) led by David Montefiori at Duke University in Durham, North Carolina, is evaluating the antigenic diversity of HIV-1 as it relates...
Section 3 | Scientific Updates

to neutralizing antibody-based vaccines. The goal is to provide a strong foundation to assess vaccine-elicited neutralizing antibody responses and to identify key epitopes using new technical, analytical and computational tools.

The Ab VIMC provides standardized and validated assays to evaluate the binding and neutralizing capabilities of antibodies under good clinical laboratory practices (GCLP) and with high throughput capacity. The technologies were recently expanded to include the capacity to measure antibody-dependent cell-mediated viral inhibition and a new peripheral blood mononuclear cell (PBMC) assay technology for neutralizing antibodies. The consortium is currently developing new methods to measure antibody-dependent cellular cytotoxicity and inhibition of cell-to-cell transmission.

To enhance the rapid assessment of vaccine-elicited neutralizing antibodies and study broadly neutralizing antibodies, the Ab VIMC has created over 330 reference Env clones of various genetic subtypes from diverse geographic locations. The Ab VIMC has initiated a new program for high-capacity Env-pseudotyped virus production in collaboration with the team lead by Hagen von Briesen at the CAVD’s HIV Specimen Cryorepository that promises to enhance the quality and reproducibility of neutralizing antibody assay results across laboratories. A standardized TZM-bl assay proficiency-testing program was finalized and will be implemented in the first quarter of 2009.

In addition to the Ab VIMC’s role in providing technical assistance to the CAVD community, the researchers conduct novel research. In one such project, the researchers found that there is indeed a correlation between an individual’s strain or clade of HIV-1 and the types of antibodies he or she develops. Previously researchers had not been able to detect a clade-specific genetic signature that correlated with the types of antibodies that individuals developed. Now the Ab VIMC researchers have identified 33 amino acid signatures that are associated with different neutralization phenotypes. Genetic signatures of neutralization susceptibility may yield new insights for improved vaccine designs.

Additionally, the consortium is embarking upon a large-scale study to determine the optimal choice of reference strains of HIV-1 to be used in neutralizing antibody assays. The Neutralization Serotype Discovery Project involves conducting 90,000 neutralization assays in serum from 300 HIV-1 infected individuals representing all major genetic viral subtypes. The results will be used to delineate neutralization serotypes and genetic signatures of broadly neutralizing antibody responses.

The Montefiori-led Ab VIMC collaborates with researchers at Beth Israel Deaconess Medical Center, Institute de Salud Carlos III, Los Alamos National Laboratory, the Henry M. Jackson Foundation, Makerere University Walter Reed Project, McMaster University, National AIDS Research Institute of India, National Center for AIDS/STD Control and Prevention of China, National HIV Repository and Bioinformatic Center (Thailand), National Institute for Communicable Diseases, National Institute of Allergy and Infectious Diseases, Torrey Pines Institute for Molecular Studies, Tulane University, the University of Alabama at Birmingham, the University of Cape Town, the University of North Carolina at Chapel Hill, the U.S. Army Medical Component, Armed Forces
Research Institute of Medical Sciences, Thailand Vaccine Research Center, NIH, and the YRG Centre for AIDS Research and Education.

The Comprehensive T-cell Vaccine Immune Monitoring Consortium

The T-cell Vaccine Immune Monitoring Consortium (T-cell VIMC) led by Richard Koup at the National Institute of Allergy and Infectious Disease’s Vaccine Research Center in Bethesda, Maryland, has created a consortium of immune testing laboratories within the larger HIV-1 vaccine pre-clinical and clinical trials networks. The Koup-led CSF is bringing together prominent scientists to identify new targets and assays for use in T-cell immune monitoring and to optimize and standardize essential assays for use in comparing different vaccine platforms.

An important accomplishment was establishing the equivalence of two different validated ELISpot assays. A common sentiment among researchers is that two different laboratories performing different, though similar, ELISpot assays were unlikely to generate comparable data. However, the T-cell VIMC showed that different assays, if properly controlled and validated, run in different laboratories, can and will yield the same results. This allows either assay in either lab to be used in the evaluation of CAVD vaccines with the knowledge that the results will be comparable.

Another important validation was to establish a peptide set that could be used to compare immunogenicity of vaccines across different products that might contain different inserts.

The clade-specific T-cell discovery group developed a peptide set that incorporates sequences from multiple different clades into a single collection of peptides. These have now been used to evaluate the T-cell immunogenicity of multiple products in comparison to peptides that match the vaccine inserts. HIV-1 vaccine researchers have adopted this testing platform as the current standard for cross-product immunogenicity comparisons.

The Koup T-cell VIMC researchers are also developing a multicolor intracellular cytokine staining (ICS) assay using commercially available reagents, collaborating with multiple non-human primate laboratories to coordinate ELISpot and ICS testing, determining the utility of Potential T-cell Epitope (PTE) peptides for cross-trial comparisons, optimizing a cultured ELISpot assay, assessing the importance of Th17 cells in the mucosal response to gut flora, establishing genomic profiles relevant to several vaccine platforms and trials, and establishing a collaboration to study the genomics of exposed-uninfected cohorts.

The Koup T-cell VIMC researchers also engage in new research to identify new T-cell functions that correlate with protection from disease and progression. Recent data suggest that

“We have established a successful collaboration with the Central Service Facilities, particularly with Duke University’s Antibody Vaccine Immune Monitoring Center (led by David Montefiori), which has run neutralization confirmation tests with excellent quality and speed.” —Robin Weiss
the ability of CD8+ T-cells to express certain forms of perforin, a protein that CTLs and NK cells use to lyse cells, strongly predicts the degree to which those T-cells can control HIV-1 replication. This new T-cell parameter is being tested against other assays to determine its utility in monitoring future T-cell vaccines.

The Koup T-cell VIMC collaborates with researchers at BD Biosciences, Beth Israel Deaconess Medical Center, Biomedical Primate Research Centre, Centre Hospitalier Universitaire Vaudois, Duke University Medical Center, Fred Hutchinson Cancer Research Center, Foundation for the National Institutes of Health, The Henry M. Jackson Foundation, Institut National de la Santé et de la Recherche Médicale, International AIDS Vaccine Initiative, McMaster University, the NIH’s National Institute of Allergy and Infectious Diseases, University of Montreal, University of Oxford, and Wits Health Consortium.

Mouse Immunology Laboratory

Despite more than a decade of research on T-cell HIV vaccines, researchers know relatively little about the requirements for an effective response in terms of what cell types are involved, in what parts of the body the responses need to localize and/or accumulate, and what magnitude is essential. To study these cellular immune responses, researchers at the Mouse Immunology Laboratory led by Philip Greenberg at the University of Washington in Seattle are developing transgenic mouse models for efficiently studying T-cell responses. Researchers will be able to inject the mouse with various vaccine candidates and “read out” the magnitude, localization, phenotype, and function of donor T-cell responses, particularly in mucosal sites where initial exposure to HIV is most likely to occur. Investigators will also be able to evaluate the pathways involved or not...
engaged in different vaccine responses by using mice deficient in specific immune response cell subsets or signaling pathways, and will use this information to facilitate development of more effective vaccine regimens/reagents.

Now in development, mice are being designed to express T-cell receptors (TCRs) that recognize specific HIV-1 epitopes. The Greenberg-led CSF researchers are using prototype vaccines based on DNA, MVA, and Ad5 vectors expressing HIV proteins gag, env, pol, and rev, as a representative set of immunogens to generate T-cell responses. The researchers identified the epitopes derived from these HIV proteins recognized by the most effective T-cells, cloned and expanded the T-cells specific for these epitopes, and isolated and validated the genes for the TCRs from these T-cells. They are using these TCR genes to generate the needed TCR-transgenic mice by injecting the TCR gene constructs via pronuclear injection.

In addition to the transgenic T-cell mice, the Greenberg CSF has begun generating a second model for antibody responses. This model will be testing the ability of candidate HIV-1 vaccines to engage B-cells that are capable of producing neutralizing antibodies specific for a known HIV structure, and to generate B-cell memory responses and antibody-producing plasma cells. When finished, both the T-cell and B-cell mouse models should be able to provide direction as to which candidate vaccines should be advanced to further pre-clinical and clinical studies. It may also suggest potential improvements to vaccine candidates.

The Greenberg CSF is currently using the developed model systems to evaluate candidate HIV vaccines developed by other VDCs. For example, the Greenberg CSF has helped the McElrath VDC evaluate the relative immunogenicity of its vaccine constructs in systemic and mucosal sites. In collaboration with the Zamb VDC, the Greenberg CSF evaluated the immunogenicity of adenovirus and adenovirus-associated virus vaccines expressing the gag protein and TLR receptor ligand flagellin.

The Greenberg CSF collaborates closely with researchers at the Fred Hutchinson Cancer Research Center and others.

**Vaccine Immunology Statistical Center**

One goal of the Vaccine Immunology Statistical Center (VISC) led by Steven Self at the Fred Hutchinson Cancer Research Center in Seattle is to develop novel statistical methods that will improve the efficiency of CAVD study designs and data analyses.

In the past year, VISC statisticians have developed a novel approach for quantitation of titration-based virus neutralization assays that extends the limits of quantitation to levels that are much lower than is possible with the current standard methods. This approach, based on a summary measure that is a scaled area under the titration curve, has a simple interpretation, is more robust to non-standard patterns of neutralization than the standard method, has an integrated, statistically-rigorous method for making positive/negative calls and correlates very strongly with the standard method over the range of higher levels of neutralization. In an attempt to more fully understand the basis for viral neutralization,
some CAVD groups are investigating patterns of neutralization at very low levels with the notion that, if amplified, these mechanisms of neutralization may confer protection. The new analytic method has now provided an ability to rigorously distinguish low-level signals from noise and quantitate those signals, thereby removing a barrier to their research.

Another important thrust of CAVD research is the evaluation of candidate vaccine regimens in non-human primate models with respect to their impact on susceptibility to infection. Over the past five years, the repeated low-dose challenge model has been employed in such studies, as it may more faithfully reflect the challenges to humans by HIV through sexual exposure. However, the statistical properties of this relatively new challenge model have not been fully elucidated, nor have the most efficient choices of design parameters for these studies been fully investigated.

In collaboration with CAVD researchers, VISC statisticians have performed a detailed analysis of the statistical properties of the repeated low-dose challenge design and developed specific recommendations for both the design of these studies and for methods to be used in data analyses and interpretation. An online tool has been developed and implemented that provides direct access to CAVD researchers to design calculations for these studies that allows direct exploration of the impact of changing design parameters such as sample size, probability of infection per challenge, maximum number of challenges per animal and the fraction of animals that may be naturally resistant to infection from the low-dose challenge protocol. This work makes available to CAVD researchers the ability to identify the most efficient study designs to meet their research objectives and a plan for how to best analyze data from these studies.

**HIV Specimen Cryorepository**

Access to human and animal specimens has been identified as one of the great needs of the worldwide effort to develop an AIDS vaccine. Hagen von Briesen at the Fraunhofer Institute for Biomedical Engineering (IBMT) in Sulzbach, Germany heads the HIV Specimen Cryorepository (HSC) central service facility. In just two years his team has established a high-quality cryorepository for use by all CAVD members. The group has developed novel cryoequipment to improve the cryobanking process, including a freezer handling box and an access tower that allow for high throughput and processing at highest quality. They are also developing a unique cryocontainer, which is 100% mismatch safe and suited for automation. The first industrially tailored devices have been delivered to the Medical Virology laboratory at Stellenbosch University, Cape Town, where the devices are currently evaluated.

During the first year, the need for centralized production of defined reference strains of HIV-1 env-pseudoviruses emerged at the Ab VIMC. A cooperative agreement was initiated in January 2008 and the production of the first pseudovirus stocks and the distribution to regional labs for proficiency testing has been completed.

The von Briesen-led HSC collaborates with researchers at the Division of AIDS, NIH AIDS Reference Reagent Program, Fondazione Centro
San Raffaele del Monte Tabor, the Ivanovsky Institute of Virology, Lund University, the National Institute for Biological Standards and Control, the Oswaldo Cruz Foundation (Fiocruz), Stellenbosch University, the University of Saarland, the University of Washington, and the World Health Organization.

Conclusion

The scientists of the Collaboration for AIDS Vaccine Discovery are at the forefront of HIV vaccine research. These dedicated individuals are exploring a range of innovative approaches aimed at enhancing immune responses with the goal of preventing people from acquiring HIV or lessening the adverse effects of infection. The collaborative nature of the consortia promotes the overall efficiency of HIV vaccine research by providing opportunities to share information among research teams and compare results using standardized methods. The central facilities provide centralized expertise and facilities, and they engage in research to improve current methods of vaccine evaluation, specimen storage, and data analysis. With this united research effort, the CAVD grantees have made solid progress toward the creation of an HIV vaccine that has the potential to save millions of lives.
Our definition of success is the development of a safe and effective vaccine that can be used to stop the human suffering caused by the worst worldwide pandemic of recent history. Although no one can guarantee success in any defined period of time, we do believe that success is possible.
The ultimate goal of the CAVD is to develop a vaccine that prevents HIV infection or disease—anything less than that can be characterized as progress, but not success. Generating new scientific knowledge, conducting carefully designed clinical trials that answer critical questions for the field, and standardizing laboratory assays that allow for the comparative evaluation of those trials are progress. Learning to work together as a global community of scientists committed to the development of an HIV vaccine is also progress. But our definition of success is the development of a safe and effective vaccine that can be used to stop the human suffering caused by the worst worldwide pandemic of recent history.

Although no one can guarantee success in any defined period of time, we do believe that success is possible. Success requires discipline. It requires that we keep our eyes on the “prize,” avoiding unnecessary distractions and side projects, no matter how exciting and appealing they may be. We must stay focused on the critical path to success. And we should conduct our work with the necessary sense of urgency.

The CAVD was conceived as a translational program that harnesses existing or new science with the objective of developing candidate vaccines to be tested in proof-of-concept clinical trials. In the creation of the CAVD, scientists acknowledged that the path to success is incompletely defined, and that innovation is needed throughout the process. Exploring innovative approaches can be risky, but such innovation will likely be required to develop an effective vaccine.

The CAVD is exploring diverse vaccine concepts, and while we expect progress in every project, not every project will succeed. In a best-case scenario the vaccine may be developed by one of the CAVD groups. But success could come from combining elements from different CAVD efforts. When success is finally achieved, it will be the success of the CAVD as a whole, given the nature of the collaborations and the contributions of each consortium to this network of researchers.

“Humanity’s greatest advances are not in its discoveries, but in how those discoveries are applied to reduce inequity.” —Bill Gates

The CAVD is a dynamic research network, and we expect new research groups to come to the CAVD, while others will leave after making important contributions to the global search for an HIV vaccine. The CAVD will evolve as science evolves. Future consortia may be organized with scientists who today are in another consortium, or with the new investigators as required to confront new challenges. We expect that the basic knowledge that is generated by the CAVD, or by other partners of the Enterprise, will inform the design of novel vaccine candidates and the conduct of carefully coordinated clinical trials. That is why we encourage even the most basic research projects to consider a clinical development path.

As Bill Gates said, “Humanity’s greatest advances are not in its discoveries, but in how those discoveries are applied to reduce inequity.” The challenge of CAVD scientists is to harness science to achieve success. And success is an HIV vaccine.
The CAVD includes over 700 individuals — senior and junior scientists, project managers, administrators, legal representatives and more.
CAVD Principal Investigators

Robert Gallo  University of Maryland, Baltimore, USA
Philip Greenberg  University of Washington, Seattle, USA
Barton Haynes  Duke University, Durham, USA
David Ho  Aaron Diamond AIDS Research Center, New York, USA
Richard Koup  Vaccine Research Center, Foundation for the National Institutes of Health, Bethesda, USA
Thomas Lehner  King's College London, UK
Norman Letvin  Beth Israel Deaconess Medical Center, Boston, USA
Juliana McElrath  Fred Hutchinson Cancer Research Center, Seattle, USA
David Montefiori  Duke University, Durham, USA
Giuseppe Pantaleo  Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
Steven Patterson  Imperial College London, UK
Francis Plummer (GCGH)  University of Manitoba, Winnipeg, Canada
Ellis Reinherz  Dana-Farber Cancer Institute, Boston, USA
Steve Self  Fred Hutchinson Cancer Research Center, Seattle, USA
Leonidas Stamatatos  Seattle Biomedical Research Institute, Seattle, USA
Ralph Steinman (GCGH)  The Rockefeller University, New York, USA
Hagen von Briesen  Fraunhofer Institut für Biomedizinische Technik, Sulzbach, Germany
Bruce Walker  Massachusetts General Hospital, Boston, USA
Robin Weiss  University College London, UK
Timothy Zamb  International AIDS Vaccine Initiative, New York, USA
Susan Zolla-Pazner  New York University, New York, USA
CAVD Collaborating Institutions

To date, a total of 95 institutions in 21 countries have signed the CAVD Data and Materials Sharing Agreement.

Aaron Diamond AIDS Research Center, USA
Academia Sinica, Taiwan
Albert Einstein College of Medicine, USA
Arizona State University, USA
BD Biosciences, USA
Beth Israel Deaconess Medical Center, USA
Biomedical Primate Research Centre, The Netherlands
California Institute of Technology, USA
Centre Hospitalier Universitaire Vaudois, Switzerland
Children’s Hospital Boston, USA
Children’s Hospital of Philadelphia, USA
CHU Henri Mondor, University Paris 12, France
Consejo Superior de Investigaciones Científicas, Spain
Dako Denmark A/S, Denmark
Dana-Farber Cancer Institute, USA
Division of AIDS, (NIH AIDS Research and Reference Reagent Program), USA
Duke University, USA
Emory University, USA
Fondazione Centro San Raffaele del Monte Tabor, Italy
Foundation for the National Institutes of Health (Vaccine Research Center), USA
Fraunhofer—Institut für Biomedizinische Technik, Germany
Fred Hutchinson Cancer Research Center, USA
The General Hospital Corporation (Massachusetts General Hospital), USA
Global Vaccines, Inc., USA
Harvard University, USA
The Henry M. Jackson Foundation, USA (US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Thailand)
Hybrid Systems, Ltd., UK
Imperial College London, UK
Institut National de la Santé et de la Recherche Médicale, France
Institute for Research in Biomedicine, Switzerland
Institute for Systems Biology, USA
Instituto de Salud Carlos III, Spain
International AIDS Vaccine Initiative, USA
IPPOX Foundation, Switzerland
Ivanovsky Institute of Virology, Russia
Jessen-Jessen Medical Practice, USA
King’s College London, UK
Leiden University Medical Centre, The Netherlands
Lionex Diagnostics and Therapeutics GmbH, Germany
Los Alamos National Laboratory, USA
Lund University, Sweden
Makerere University Walter Reed Project, Uganda
Massachusetts Institute of Technology, USA
McMaster University, Canada
Medical Research Council, UK
Molsoft LLC, USA
Mount Sinai School of Medicine, USA
Murdoch University, Australia
Appendix 2 | Collaborating Institutions

National AIDS Research Institute, India
National Cancer Institute, USA
National Center for AIDS/STD Control and Prevention, China CDC
National HIV Repository and Bioinformatic Center, Thailand
National Institute for Biological Standards and Control, UK
National Institute for Communicable Diseases, South Africa
National Institute of Allergy and Infectious Diseases, NIH, USA
New York University, USA
Oregon Health Sciences University, USA
Oswaldo Cruz Foundation (Fiocruz), Brazil
Pepscan Systems BV, The Netherlands
Prince Leopold Institute of Tropical Medicine, Belgium
Queen Mary University of London, UK
The Rockefeller University, USA
Royal Holloway and Bedford New College, UK
Sanofi Pasteur, Canada
Sanquin Blood Supply Foundation, The Netherlands
Seattle Biomedical Research Institute, USA
The Scripps Research Institute, USA
Stellenbosch University, South Africa
The Swedish Institute for Infectious Disease Control, Sweden
TheraJect, Inc., USA
Torrey Pines Institute for Molecular Studies, USA
Tropical Diseases Research Center, Zambia
Tulane University, USA
Université Joseph Fourier, France
University College London, UK
University of Alabama at Birmingham, USA
University of Cambridge, UK
University of Cape Town, South Africa
University of Kansas, USA
University of KwaZulu-Natal, South Africa
University of Manitoba, Canada
University of Maryland, Baltimore (Institute of Human Virology), USA
University of Massachusetts, USA
University of Medicine and Dentistry of New Jersey, USA
University of Montreal, Canada
University of North Carolina at Chapel Hill, USA
University of Oxford, UK
University of Regensburg, Germany
University of Saarland, Germany
University of Texas Southwestern Medical Center at Dallas, USA
University of Washington, USA
University of Wisconsin-Madison, USA
Wits Health Consortium, South Africa
World Health Organization, Switzerland
YRG Centre for AIDS Research and Education, India

To the best of our knowledge, this list is up to date at the time this report went to print in February 2009. The institutions signed on to the CAVD DMSA change over time with some being removed and others being added as the research progresses. Please check the CAVD Web site for the most up-to-date list.
Appendix 3 | Site Map

Countries with CAVD Collaborating Institutions

- Australia
- Belgium
- Brazil
- Canada
- China
- Denmark
- France
- Germany
- India
- Italy
- Russia
- South Africa
- Spain
- Sweden
- Switzerland
- Thailand
- The Netherlands
- UK
- USA
- Uganda
- Zambia

Location of CAVD Grantee Institutions

1. Saarland, Germany
2. Lausanne, Switzerland
3. London, UK
1. Baltimore, USA
3. New York, USA
3. Boston, USA
4. Seattle, USA

University of Washington, Seattle, USA
Fred Hutchinson Cancer Research Center, Seattle, USA (two grants)
Seattle Biomedical Research Institute, Seattle, USA
Beth Israel Deaconess Medical Center, Boston, USA
Dana-Farber Cancer Institute, Boston, USA
Massachusetts General Hospital, Boston, USA
Aaron Diamond AIDS Research Center, New York, USA
International AIDS Vaccine Initiative, New York, USA
New York University, New York, USA
Vaccine Research Center, Foundation for the National Institutes of Health, Bethesda, USA
University of Maryland, Baltimore, USA
Duke University, Durham, USA (two grants)
CAVD Composition

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Appendix 4 | Publications

CAVD Publications

The following is a list of all publications emanating from research conducted as part of the CAVD. To the best of our knowledge, this list is up to date at the time this report went to print in February 2009. Please check the CAVD Web site for the most current list of publications.


### Abbreviations

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<th>Abbreviation</th>
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<tbody>
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<td>Ab</td>
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