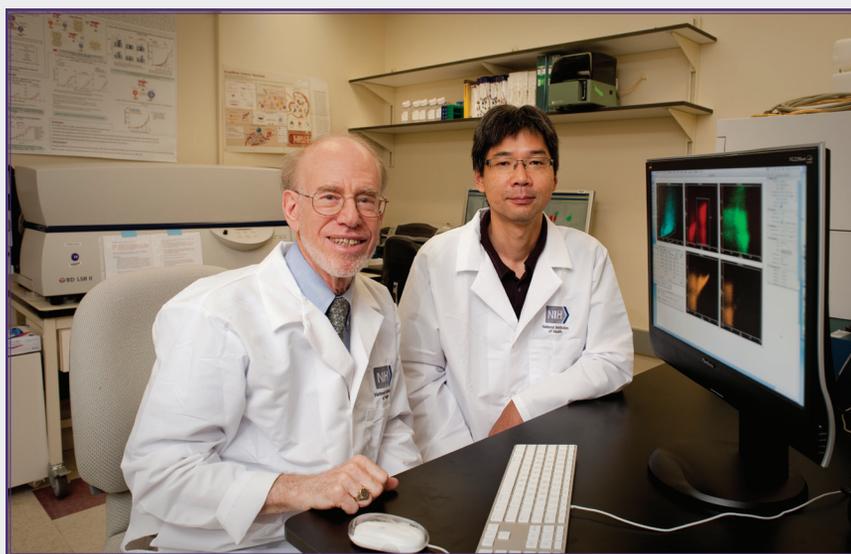


# Vaccines 2.0

*In 1974, Jay A. Berzofsky, M.D., Ph.D., now Chief of CCR's Vaccine Branch, came to NIH to study protein folding. His curious mind and collaborative spirit quickly led him into the intertwined fields of immunology and vaccine development. With close to 500 publications to his name, Berzofsky has pioneered the characterization of B- and T-cell epitopes and their modification to make vaccines directed against cancer and chronic infectious diseases. He has also characterized and taken advantage of the cellular and molecular regulators of immune responses in order to enhance tumor immunity and vaccine efficacy. In the last several years, he has translated many of these strategies into promising clinical trials. From the microcosm of his laboratory, he brings the same spirit of cross-fertilizing, bench-to-bedside research to leading the Vaccine Branch as a whole.*

Conventional vaccines have been extremely effective against disease agents, especially viruses, which cause acute, self-limited infection. In unvaccinated populations, diseases such as smallpox and measles are sometimes fatal, but if the immune system clears the virus, individuals usually have long-lasting immunity against future infection. Vaccine development, therefore, has classically been directed by virologists, who design attenuated agents that are safer than the original virus, but are capable of recreating the immune response.

In the case of both cancer and HIV, vaccine development has proven much more challenging, and, according to Berzofsky, the challenges are for similar reasons. "Both cancer and HIV cause chronic disease," said Berzofsky. "If the immune response to the virus could clear HIV, it would have done so. Therefore, the natural virus itself is not an adequate vaccine. Similarly, it may be that a lot of cancers are eliminated by the immune system, but for the ones that are



(Photo: R. Baer)

Jay A. Berzofsky M.D., Ph.D., and Masaki Terabe, Ph.D.

not, we need better stimulation than the tumor provides. Vaccine development has shifted from the domain of the virologist to that of the immunologist."

Berzofsky's interest in vaccine development evolved from studying enzymes and protein structures. Arriving at the NIH for a postdoctoral fellowship in the laboratory of Alan Schechter, M.D., and Chris Anfinsen, Ph.D., just

two years after Anfinsen won the Nobel Prize for his work on protein folding, Berzofsky got involved in a collaboration with David Sachs, M.D., then a new investigator in NCI's Immunology Branch. "I became fascinated with immune response genes," said Berzofsky.

Immune response genes are now known as major histocompatibility complex (MHC) genes, and in humans the MHC molecules are

“...the immune system has this exquisite specificity to see differences in single amino acids in a protein.”

given the name human leukocyte antigen (HLA) molecules because of the way they were first discovered. These molecules bind fragments of pathogens, i.e., epitopes, and present them on the cell surface for recognition by T-cell receptors (TCRs). In the 1980s, Berzofsky began collaborating with Charles DeLisi, Ph.D., in NCI's Mathematical Biology Branch, on algorithms for predicting which amino acid sequences would serve as epitopes by binding to MHC molecules and TCRs, and developed one of the first successful ones. To complement the modeling studies, they started doing amino acid substitution and binding studies to dissect out which residues interacted with MHC molecules and which interacted with TCRs.

“In the course of that work, we discovered that, in some cases, we could improve on epitopes and make them even more potent by getting rid of or substituting a side chain,” said Berzofsky. “If you imagine the peptide is like a hotdog in a bun—between the MHC and the TCR—and you can change just the amino acids on one side of the bun that will improve binding to the MHC, then you could have more potent antigen-eliciting T-cell responses, which would also be effective against the native protein.”

## Immune Activation

And thus, the concept of epitope enhancement was born. Berzofsky's laboratory applied this strategy to many infectious diseases, including malaria, collaborating with Lou Miller, M.D., Section Chief in the National Institute of Allergy and Infectious Diseases' (NIAID's) Laboratory of Malaria and Vector

Research. Around this time, HIV was discovered. While working with the NCI laboratory of Robert Gallo, M.D., and in collaboration with Gene Shearer, Ph.D. in NCI's Immunology Branch, Berzofsky's group obtained unpublished sequences from HIV proteins and described some of the first T-cell epitopes for the virus. Collaborating with Stephen Feinstone, M.D., at the U.S. Food and Drug Administration, Berzofsky's team published one of the first epitope-enhancement papers as a strategy to improve a vaccine against hepatitis C.

“We were working on viruses, then viruses that caused cancers, and, eventually, we started looking at cancer antigens themselves,” said Berzofsky. “I had the idea that you should be able to target an antigen that is unique to cancer. Most conventional chemotherapies are poisons, but the immune system has this exquisite specificity to see differences in single amino acids in a protein. We could have a more effective and much safer therapy for cancer that wouldn't have side effects. That's what really excited me.”

Berzofsky started looking for tumor antigens. He formed a collaboration with John Minna, M.D., then at NCI, and his fellow, David Carbone, M.D., Ph.D., to study mutations in RAS and p53, which led to a proof-of-concept human trial in which the group created an individualized peptide vaccine based on sequences from each patient's tumor biopsy. Working with John Morris, M.D., Staff Clinician in CCR's Metabolism Branch, Berzofsky's team created another vaccine that they have just

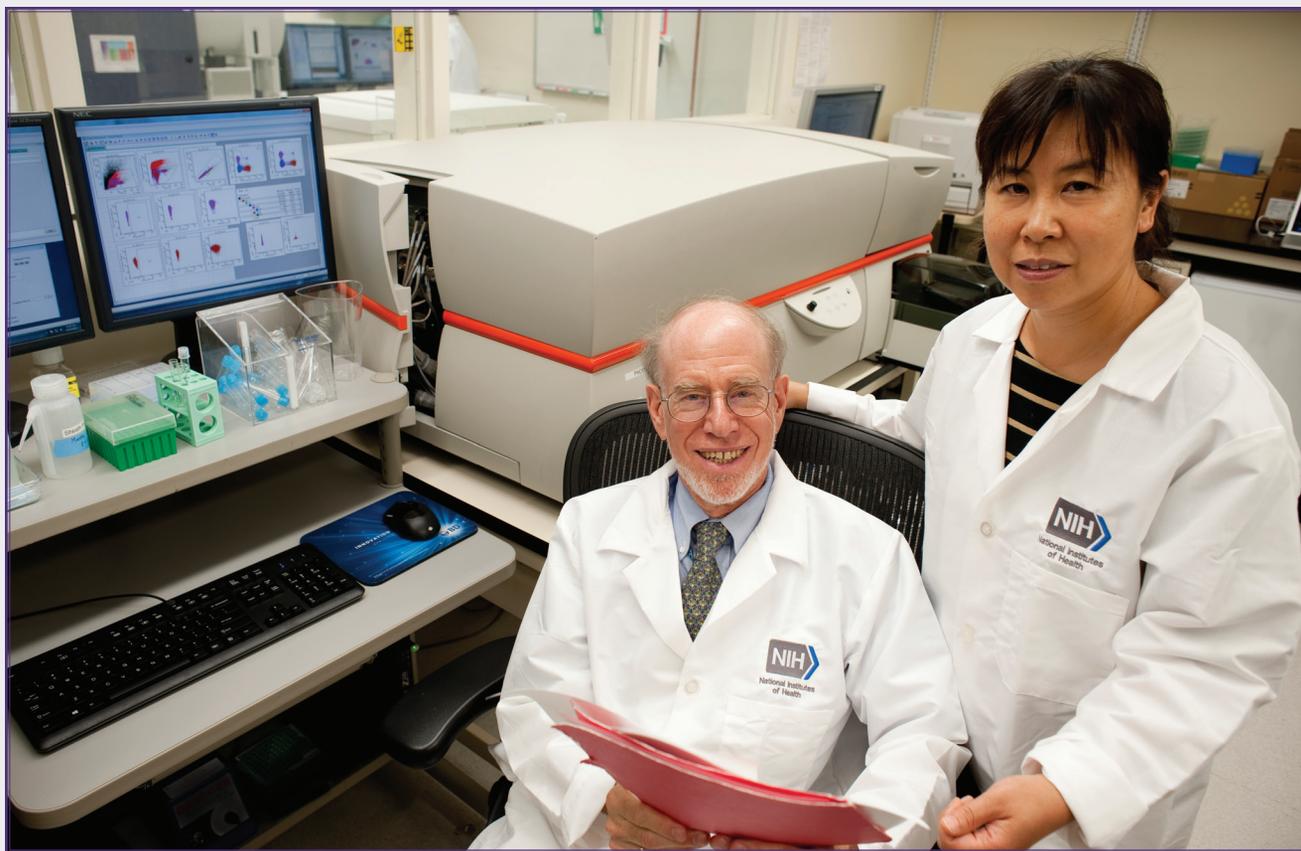
translated to a clinical trial run by Lauren Wood, M.D., Head of the Vaccine Branch Clinical Trials Team, based on an adenovirus that expresses nononcogenic domains of HER2, the receptor expressed in 25–30 percent of breast cancers as well as a smaller percentage of many other cancer types. Meanwhile, they also started working with Ira Pastan, M.D., Co-Chief of CCR's Laboratory of Molecular Biology, who was mining a database of genes expressed in cancer for a different purpose, namely to find cell-surface targets for immunotoxins.

“Ira gave us sequences of the tumor antigens he discovered, and we mapped epitopes initially presented by HLA-A2 because it is the most common. We applied epitope enhancement to improve binding to the HLA-A2 molecule and enhance immunogenicity. One of these cancer antigens was TARP,” said Berzofsky.

TARP (T-cell receptor gamma chain alternate reading frame protein) is expressed in the normal prostate and overexpressed in prostate and breast cancers. In 2004, the team published preclinical evidence from transgenic mice and in cells from a patient with prostate cancer, showing that their TARP epitopes could stimulate T-cell responses that killed human tumor cells.

“It took a number of years to translate those results into human clinical trials, but we have recently completed one trial and are starting another,” said Berzofsky. “We are very excited about the possibilities for the TARP vaccine.”

For their phase I trial, Wood enrolled 40 patients with stage D0 prostate cancer for treatment with TARP-primed autologous cells. At stage D0, the primary tumor has been removed or treated with radiation to completely destroy the tumor and the prostate. A certain



(Photo: R. Bauer)

Jay A. Berzofsky M.D., Ph.D., and Yongjun Sui, Ph.D.

fraction of patients are cured, but many are not.

“After a year, we had a decreased tumor growth rate in about three-quarters of our patients. Based on these promising results, we’ve set up a randomized placebo-controlled phase 2 study, which is currently in progress. If we confirm what we saw in phase 1, we hope to be able to try the vaccine in more advanced prostate cancers,” said Berzofsky. “It’s very gratifying to see work we did at a very basic level, beginning with epitope mapping 30 years ago, now translated into human clinical trials that could benefit patients.”

## Immune Regulation

“The very first work I did here at NCI was to understand how the immune response against cancer is regulated, and the result we got was totally unexpected,” said Masaki Terabe, Ph.D., who arrived in Berzofsky’s laboratory as a postdoctoral fellow

in 1999 and is now Deputy Section Chief. “Even Jay was surprised.”

Working with the laboratory of Ron Germain, M.D., Ph.D., then a Principal Investigator in NIAID’s Laboratory of Immunology, Berzofsky’s lab had created a mouse model in which tumors would grow, start to regress spontaneously, and then recur. Not surprisingly, they found, through cell-depletion experiments, that the regression was dependent on CD8+ T cells, but they were surprised to find that the recurrence of the cancer was due to CD4+ T cells, specifically a subset defined as type II NKT cells, which recognize lipid antigens presented by a nonclassical MHC molecule.

“Masaki made the groundbreaking discovery that NKT cells could inhibit tumor immunity,” said Berzofsky. “And he discovered the mechanism involved IL-13 and TGF- $\beta$ . This really opened up a whole

new area of immune regulation for our laboratory.”

After Terabe showed that TGF- $\beta$  was a critical mediator of immune suppression in this system, the team began using an antibody against TGF- $\beta$  to prevent growth of tumor models, both independently and in synergy with cancer vaccines. As a result, Berzofsky has worked first with Genzyme and now with Xoma to bring anti-TGF- $\beta$  into the clinic. A phase 1 clinical trial for melanoma sponsored by Genzyme gave encouraging results, but changes in corporate priorities ended that line of investigation. Now, Berzofsky has a Cooperative Research and Development Agreement (CRADA) with Xoma, under which his lab is completing preclinical mouse studies with a new set of anti-TGF- $\beta$  antibodies.

“Our work with TGF- $\beta$  is one part of an overall ‘push-pull strategy’ to improve the T-cell response to cancer. We are using defined molecular

adjuvants to push and steer the response in the right direction and the blockade of negative regulators to take the brakes off the response and thus ‘pull’ it forward,” said Berzofsky. A key molecular adjuvant is IL-15, which Berzofsky studied in collaboration with Thomas Waldmann, M.D., now Chief of CCR’s Lymphoid Malignancies Branch, who was Berzofsky’s former mentor (See “IL-15 Prepares for Its Clinical Debut,” *CCR connections* Vol. 5, No.2).

Meanwhile, at a more fundamental level, Terabe and Berzofsky are working on finding a good marker for type II NKT cells. “They are very rare; if you draw blood, you find only one in a thousand to ten-thousand cells,” said Terabe. “We are trying to establish a method to identify them reliably. We still know very little about these cells.”

## Immune Compartmentalization

Even while cancer immunology has blossomed in Berzofsky’s laboratory, HIV vaccine research has not languished. What began as the study of T-cell epitopes in unpublished sequences from

the Gallo laboratory has become focused on ways to defeat HIV at the mucosal membrane.

“Eighty-five percent of HIV/AIDS is transmitted vaginally or rectally. HIV is a mucosal disease,” said Yongjun Sui, Ph.D., Staff Scientist leading Berzofsky’s HIV mucosal vaccine team. “There are systemic antibodies in the blood that could protect against flu or other infectious diseases, but the first line of defense for HIV is at the mucosal surface. Our idea is to develop the T-cell or antibody response at the point of entry.”

In the 1990s, Berzofsky’s team showed that T cells present locally in the mucosa could protect against HIV in a mouse model. They translated their work into rhesus macaques and showed that intrarectal immunization was much more effective than systemic administration against infection with the species’ equivalent virus, SIV. But, intrarectal immunization was criticized as impractical.

Searching for a new approach, Berzofsky’s team worked with Nanotherapeutics to develop a nanoparticle vaccine that could

encapsulate HIV epitopes, shield them from interaction with the stomach or small intestine, and only gradually dissolve them in the large intestine to release the vaccine into the colon.

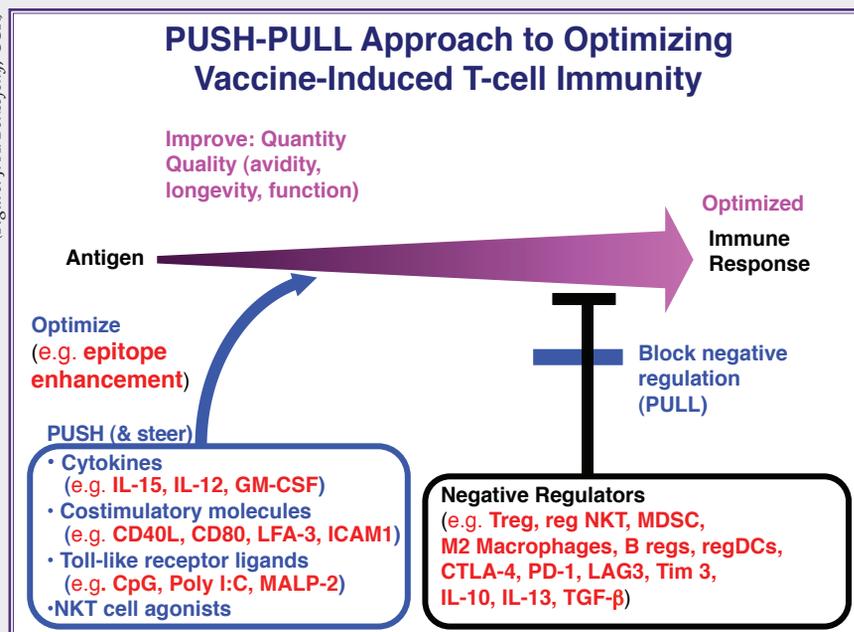
Sui is now translating that research from mouse to macaque. In the first cohort, the vaccine clearly protects the animals from rectal challenge with SIV. The second cohort is still under study. “If everything goes as expected, we calculate the efficacy as 40–50 percent protective,” said Sui. “For a vaccine to be considered for potential use in man, we will need to show 60–70 percent efficacy. Our plan is to add a topical microbicide.”

Meanwhile, the team published a paper earlier this year in *Nature Communications*, in which they demonstrated that vaginal immunity to HIV could be induced locally in the vaginal mucosa. “This goes against textbook dogma, which states that dendritic cells must pick up an antigen and carry it through the lymphatic system to induce immunity in T cells at the lymph nodes, which then circulate back through the blood stream,” said Berzofsky. “The vaginal mucosa has no organized lymphoid tissue.”

Thus, their work on the HIV vaccine has led them back into the basics of mucosal immunology.

“Our laboratory is multi-dimensional: on the one hand, we have cancer, on the other, HIV. Then, we have basic research, which we translate into animal models and then into the clinic,” concluded Berzofsky. “Rather than spreading ourselves thin, each of our activities reinforces and feeds the others.”

(Figure: J. A. Berzofsky, CCR)



To learn more about Dr. Berzofsky’s research, please visit his CCR website at <http://go.usa.gov/3JwJW>.