HIV vaccines: Ad out?

By Michael J. Haas, Senior Writer

When **Merck & Co Inc.**'s V520 HIV vaccine failed in last year's Phase II STEP trial, it was the most advanced T cell–based HIV vaccine in development. Two research teams now have posited different theories for the failure. Whether adenovirus-induced immunogenicity is to blame, as one paper suggests, or heterologous prime-boost regimes will be needed, as the other suggests, the findings offer some hope for future development of HIV and T cell–based vaccines.

V520 was designed to induce cytotoxic T cells capable of killing HIV-infected T cells. The vaccine consisted of an adenovirus serotype 5 (Ad5) vector encoding three HIV proteins—gag, pol and nef—and was designed to be used for both primary and booster shots.

There are more than 50 adenovirus serotypes, many of which are responsible for upper respiratory infections in humans. Several serotypes—including Ad5—are endemic in human populations. Others have been used as gene vectors for a variety of applications.

> **"We're not at the end of the road for HIV vaccines or T cell–based vaccines."**
> *—Dan Barouch, Harvard Medical School*

Since terminating the V520 trial in September 2007 due to adverse results, Merck scientists have reported that the observed increase in the risk of HIV infection was greatest in men who were circumcised and in men who were already Ad5 seropositive at the beginning of the trial.[1] An unanswered question was whether the trial failure was specific to the vaccine or revealed a fundamental flaw of T cell–based vaccine strategies.

The answer could have implications for other therapeutic strategies that employ killer T cells to treat HIV—such as the direct infusion of engineered $CD8^+$ T cells (*see* **Box 1, "TCR takes down HIV"**).

Studies published in the *Journal of Experimental Medicine* and *Nature* examined the two key findings of the STEP trial: that the vaccine increased the rate of new infections in previously uninfected individuals, and that it failed to lower viral loads in infected subjects.[2,3]

Ad's T cell troubles

The team that published in *JEM* wanted to determine whether pre-existing immunity to Ad5 because of past exposure to that virus conferred an increased risk of HIV infection upon inoculation with an Ad5-based vaccine. Their *in vitro* experiments with Ad5 vector and *ex vivo* Ad5-specific T cells showed that the Ad5 antigen-antibody immune complex caused dendritic cells (DCs) to mature and induce production of Ad5-specific cytotoxic T cells.

The group theorized that an Ad5-based HIV vaccine would induce production of T cells that killed DCs stimulated in individuals with pre-existing Ad5 immunity, because the DCs would present both Ad5 and HIV antigens.

The team also suggested that Ad5-specific T cells provided a large pool of targets for HIV—increasing the risk of infection upon exposure to the virus.

Eric Kremer, a lead author on the *JEM* paper and director of research at the **Institut National de la Santé et de la Recherche Médicale** (INSERM), told *SciBX* the increased risk of HIV infection theoretically could occur in response to Ad5-based vaccines against any pathogen. This could inadvertently endanger participants in trials of Ad5-based vaccines against other infections because such trials would not necessarily track HIV infection rates, he said.

The team was led by Kremer and Giuseppe Pantaleo, chief of immunology and allergy and professor of medicine at Centre Hospitalier Universitaire Vaudois (CHUV) at the **University of Lausanne**. It included scientists from CHUV, the **Swiss Vaccine Research Institute**, the **National Center for Scientific Research** (CNRS) and the **University of Montpellier 1**.

There is at least one Ad5-based vaccine in the clinic and at least one in preclinical development.

Crucell N.V.'s VRC-EBOADV018-00-VP is an Ad5-based vaccine expressing glycoproteins from two strains of Ebola virus. It is in a Phase I trial to prevent Ebola infection. The trial is sponsored by the **National Institute of Allergy and Infectious Diseases**.

Partners **GenVec Inc.** and the **Malaria Vaccine Initiative** have GenVec Ad5-CSP:Ag2:LSA1 GenVec Ad5-AMA1:MSP1, an Ad5-based vaccine expressing five malarial antigens. It is in preclinical development to prevent malaria infection.

Does it Ad(d) up?

In the work published in *Nature*, researchers inoculated macaques with one of three prime-boost regimens. In each regimen, the prime vaccination used a different Ad serotype—Ad26, Ad35 or Ad5—as a vector to deliver the gene encoding simian immunodeficiency virus (SIV) gag. After 24 weeks, all three groups received an Ad5-gag booster. Six months after the boost, the team injected the macaques with a high dose of SIV and monitored their T cell counts and viral loads for 500 days.

Macaques receiving the heterologous Ad26/Ad5 regimen had greater decreases in viral load and AIDS-related mortality than those receiving the heterologous Ad35/Ad5 or homologous Ad5/Ad5 regimens. However, none of the regimens prevented SIV infection.

The team began their study three years ago, so "this Ad5/Ad5 result took on a whole new meaning after the STEP trial failure" because it confirmed that the Merck trial failure was specific to the vaccine, not the T cell–based vaccine strategy, according to Dan Barouch, an associate professor of medicine at **Harvard Medical School** who led the team.

Box 1. TCR takes down HIV.

In *Nature Medicine*, an international research team has described a T cell receptor (TCR) capable of binding a range of mutated HIV peptide epitopes that are presented on infected cells and normally elude T cell recognition.[7] The findings suggest TCRs could be the basis for new HIV therapeutics that circumvent a major mechanism of viral resistance. Indeed, the team plans to take a therapy based on these findings into clinical trials next year.

The researchers began by isolating a TCR with nanomolar binding affinity for HIV p17 Gag–derived antigen (SL9) from an HIV patient. SL9 is a nine-residue peptide of HIV gag that is presented by major histocompatibility complex class I, A (HLA-A) molecules on the surface of infected cells. However, the TCR had a very short interaction time with HLA-presented peptides—less than one minute—that limited its therapeutic potential.

Using phage display, the team isolated an enhanced TCR variant that had an interaction time of more than 2.5 hours and an SL9 binding affinity of less than 400 pM.

Next, the group engineered cytotoxic $CD8^+$ T cells to express the improved variant and found that the cells exhibited several advantages over T cells expressing the original TCR: they controlled HIV replication better, produced IL-2 and interferon-γ in response to lower levels of SL9, had a high affinity for several common SL9 escape mutations and replicated faster in response to antigen.

In their paper, the team noted that other groups have shown that mutations that allow HIV to escape killer T cells also can limit the virus' ability to replicate—as indicated by better viral control and increased life expectancy of the host.[8,9]

Andrew Sewell, research professor of medical biochemistry at **Cardiff University School of Medicine**, suggested that even if HIV should manage to mutate and escape the engineered $CD8^+$ T cells, it might be weakened to an extent that would slow or even prevent the onset of AIDS in infected patients.

The research team included scientists from Cardiff, **University of Pennsylvania School of Medicine**, the **University of Oxford**, **Adaptimmune Ltd.** and **Immunocore Ltd.** and was led by Sewell and James Riley, research associate and professor of pathology and laboratory medicine at UPenn.

Adaptimmune said Riley and Carl June, also an author on the paper, expect to start clinical trials in early 2009 of $CD8^+$ T cells expressing the enhanced TCR to treat HIV. June is a professor of pathology and laboratory medicine at UPenn and facility director of the human immunology core at the university's Abramson Cancer Center.

"It's important that they've selected a TCR that proved to have a strong response to escape mutants of SL9" to provide proof of concept, said Laurent Humeau, VP of R&D at **Virxsys Corp.** However, he raised questions regarding the therapy's effect on CD4 counts and immunogenicity.

"We know that HIV patients lose CD4 cells," providing one measure for the transition from HIV to AIDS, he said. "It is not clear what effect the infusion of CD8 cells will have on CD4 counts" and thus on disease progression.

Humeau also worried that the T cells could be immunogenic. The initial infusion "might have a quick flushing effect that clears out the HIV-infected cells," he said. "But once the infused cells themselves have been cleared by the immune system, the virus could be replenished from HIV reservoirs," and immunogenicity might prevent the patient from responding to additional infusions.

Virxsys's VRX496, autologous T cells treated *ex vivo* with a lentiviral vector encoding antisense RNA that targets HIVgp120, is in a Phase II trial to treat HIV.

Helen Tayton-Martin, COO of Adaptimmune, told *SciBX* her company is developing the enhanced TCRs for adoptive T cell–based therapy under an exclusive license from Immunocore, which holds patents on the TCR technology.

Adaptimmune is also exploring other improved TCRs to treat HIV and has a pipeline of cancer TCR targets, whereas Immunocore is developing improved TCRs as soluble fusion molecules to treat HIV, cancer and diabetes.

Both Adaptimmune and Immunocore spun out of **MediGene AG**'s Avidex Ltd. subsidiary this year. —*MJH*

"Now, the implication is that it's possible to have a vaccine that generates a better T cell response and viral control" than the Merck vaccine, he said. "We're not at the end of the road for HIV vaccines or T cell–based vaccines. But we have no clue yet whether our results will translate into humans."

The team included scientists from the Beth Israel Deaconess Medical Center at Harvard, the Irvine School of Medicine at the **University of California, Irvine**, **Duke University School of Medicine**, the **New England Primate Research Center**, Crucell and TNO Biosciences, a business unit of the **Netherlands Organization for Applied Scientific Research**, a not-for-profit organization.

Persistent dis-Ad-vantage

Vaccine researchers polled by *SciBX* said the significance of the *JEM* study depended on whether Ad5-specific T cells induced by vaccination or by natural exposure to Ad5 virus could persist long enough to increase the risk of HIV infection.

Pervin Anklesaria, VP of therapeutic development at **Targeted Genetics Corp.**, said she was not convinced that the effect proposed in the *JEM* study caused the increase in HIV infections in the Merck trial.

She said Ad5 antibodies in individuals with pre-existing immunity might lower the efficacy of an Ad5-based vaccine, "but we don't think this would increase HIV infections. Ad5 is not expected to have such an impact on the ability of the vaccine to control HIV infection."

Anklesaria thinks the artificial *in vitro* system used in the *JEM* study made it difficult to determine how the Ad5 antigen-antibody immune complex would localize *in vivo* to the site of an intramuscular vaccine injection.

She also said the time scale in the *JEM* study—a few hours compared with many months required for a vaccine trial—made it difficult to accept

that a pool of vaccine-induced, Ad5-specific T cells persisted long enough to contribute to an increase in HIV infections.

"I have the impression that HIV infections [in the Merck trial] did not occur within, say, 10 days or even a few months of vaccination, but much longer afterwards," Anklesaria said. She added that exposure to HIV could not reactivate Ad5-specific T cells.

"To confirm the paper's findings, you would have to repeat the study *in vivo* or look for such Ad5 immune complexes in future clinical trials—but it would be difficult to develop the necessary assays and technology," Anklesaria said. "Also, you would have to follow the trial participants for a long time and perhaps more closely" to determine whether Ad5-specific T cell counts were still higher at the time of HIV infection.

Targeted Genetics is developing two prophylactic HIV vaccines that use adeno-associated virus serotype 1 (AAV-1) and AAV-2 as vectors to deliver genes coding for HIV proteins. Unlike adenoviruses, Anklesaria said AAVs have not been associated with human disease and cannot replicate on their own in mammalian cells.

She confirmed that the company plans to begin a Phase I trial of both vaccines together in a heterologous prime-boost regimen in 2009 in collaboration with the **NIH**.

Like Anklesaria, Marc Hertz was not sure how well the *JEM* findings related to the STEP trial results. "This is certainly not the whole story," said Hertz, who is the former CEO of vaccine company Pharmexa-Epimmune, a subsidiary of **Pharmexa A/S**. He is now managing director of **Bird Rock Biotech Consultancy**.

If the hypothesis in the *JEM* study were correct, Hertz said, he would have expected higher rates of HIV infection among participants with pre-existing Ad5 immunity in both the vaccine and control arms.

He said the hypothesis might have merit if the higher rate of HIV infection in individuals with pre-existing Ad5 immunity could be attributed to the persistence of Ad5 virus and Ad5-specific T cells in individuals naturally exposed to that virus. But Hertz did not think that Ad5 virus would persist long enough to cause a significant increase in the levels of Ad5 immune complex upon vaccination.

Moreover, he expects that all vaccinated individuals would quickly develop high levels of anti-Ad5 antibodies—thereby leveling any differences among participants with and without pre-existing Ad5 immunity.

Instead, Hertz speculated that the Merck results highlighted some other difference between the immune responses of Ad5-experienced and Ad5-naïve individuals.

One obvious difference between the two groups, he said, is that naturally occurring Ad5 infections and Ad5 vaccine vectors enter the host by different physical routes. The innate immune response may play a role in the former that it does not play in the latter—and thus Ad5-experienced individuals might conceivably respond to an Ad5-based vaccine differently than Ad5-naïve individuals, Hertz said.

Until the role of innate immunity in natural Ad5 infections can be elucidated, he said, "I think it will remain difficult to develop adenovirus-based vectors."

Michael Robertson, director of infectious diseases and vaccine clinical research at Merck, said that although it was not clear whether the *JEM* results reflect what happens *in vivo*, "this is one of the first *in vitro* studies to test a possible mechanism to explain the findings in the STEP study, and these findings merit further investigation."

Booster step

Anklesaria, Hertz and Robertson all thought the *Nature* study provided clearer signposts for future HIV vaccine development.

For example, Robertson said the results demonstrated that a heterologous prime-boost regimen using adenovirus vectors could induce a better T cell response to control viral loads than a homologous regimen.

"These results suggest that more robust T cell responses may improve postinfection disease course and may form the basis of a next-generation T cell–based vaccine" that could control HIV viral loads better than the STEP trial vaccine, he said.

Anklesaria agreed. "The *Nature* paper shows that you should be able to control HIV infection with a T cell–based vaccine, but also shows that Ad5 is not as strong as other vectors in inducing the immune response to HIV antigens."

Given the six-month period between the Ad5 boost and SIV infection, she didn't think the homologous regimen induced Ad5 immunity in macaques that mimicked the pre-existing Ad5 immunity of some STEP trial participants. Rather, she thinks the low potency of Ad5—as demonstrated in the *Nature* study—"played a greater role in trial results than actual facilitation of HIV infection by pre-existing Ad5 immunity."

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Gary McGarrity, EVP of scientific and clinical affairs at **Virxsys Corp.**, disagreed with Anklesaria. He argued that an Ad5-based prime inoculation might indeed induce a pre-existing immunity that the Ad5-based boost could trigger. Thus, McGarrity said that even heterologous regimens would have to account for pre-existing immunity to the chosen Ad vector—whether that was Ad5 or another common serotype.

Virxsys' VRX1023, a lentivirus vector carrying genes that encode undisclosed HIV antigens, is in preclinical macaque studies to treat or prevent HIV infection. In those studies, VRX1023 is used as a boost inoculation, following a DNA prime inoculation that codes for the same antigens. McGarrity said that the company has also run studies of a VRX1023 prime–Ad5 boost regimen in mice.

Hertz agreed with Robertson that the *Nature* study showed that a heterologous vaccine regimen is better than a homologous one. He added that the study demonstrated the need for models—such as the one Barouch's team used—that are more stringent than the one Merck used in the preclinical studies of its vaccine.

Barouch's team used the SIVmac251 model in which macaques lacking the protective major histocompatibility complex class I (MHC I) alleles *Mamu-A*01* and *Mamu-B*17* were challenged with SIV. Merck used a SHIV-89.69 model in which macaques were challenged with a chimeric simian-human immunodeficiency virus (SHIV), without regard to which *Mamu* alleles they had.

"We wanted a better SIV-challenge model, in which it was more difficult to show protection" against infection because the animals lacked the protective MHC I alleles, Barouch told *SciBX*.

"Vaccines that failed in the clinic are failing in the SIVmac251 model but succeeded, in some cases, in the previous SHIV-89.69 models," Hertz noted.[4–6] "The SIVmac251 model might be more predictive of what happens in humans because it appears to be more stringent."

Laurent Humeau, VP of R&D at Virxsys, said his company is studying VRX1023 in macaques that lack the protective *Mamu-A*01* allele.

He said it was important to remove the protective *Mamu* alleles from the equation because "otherwise you can't distinguish the protective effect of the alleles from any protective effect of your vaccine."

Barouch agreed. "Our results are the first to show that viral load can be controlled in an SIV-challenge model, indicating this model is more stringent" and therefore should become the model of choice, he said.

Future prevention

For now, both the *JEM* and *Nature* teams are building upon their published studies.

Kremer said his team's study showed that the combination of anti-Ad5 antibodies and Ad5 sequences in the vector increased the rate of HIV infection in vaccinated individuals. "Potentially we can modify one or both of these factors to circumvent the effect caused by the Merck vaccine," he said.

To that end, Kremer's team is conducting studies to identify which Ad5 sequences stimulate pre-existing Ad5 immunity and to identify other adenovirus serotypes as safer vectors.

Kremer said the findings reported in *JEM* are not patented.

Barouch said his team has an Ad26-based HIV vaccine in a Phase I trial. The NIH is sponsoring the trial, and vaccine manufacturing has been subcontracted to Crucell.

He declined to disclose the IP status of the *Nature* findings but said anyone interested in the status should contact him directly.

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