



What's in the Pipeline: New HIV Drugs, Vaccines, Microbicides, HCV and TB Treatments in Clinical Trials

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e thymidine • BI-201 • Racivir (PSI 5004) • TMC-278 • Diarylpyrimidine (DAPY) • 640385
• Reverset (D-D4FC) • JTK-303 • UK-427 (maraviroc) • Amdoxovir • AMD-070 • Vicriviroc
LIPO-5 • GTU-Multi-HIV • pHIS-HIV-B • rFPV-HIV-B • ADMVA • GSK Protein HIV Vaccine
TBC-M335 (MVA) • TBC-F357 (FPV) • TBC-F349 (FPV) • LIPO-4T (LPHIV-1) • LFn-p24 • H
G • Oligomeric gp140/MF59 • VRC-HIVDNA-009-00-VP • PolyEnv1 • ISS P-001 • EP HIV-
• BufferGel • Lactin-V • Protected Lactobacilli in combination with BZK • Tenofovir/PMPA G
ulose acetate/CAP) • Lime Juice • TMC120 • UC-781 • VivaGel (SPL7013 gel) • ALVAC
Ad5 • Autologous dendritic cells pulsed w/ALVAC • Autologous dendritic cell HIV vaccination
x • Tat vaccine • GTU-nef DNA vaccine • Interleukin-2 (IL-2) • HE2000 • Pegasys (peginter
L-4/IL-13 trap • Serostim • Tucaresol • MDX-010 anti-CTLA4 antibody • Cyclosporine A •
96 • HGTV43 • M87o • Vertex • VX-950 • Idenix • Valopicitabine (NM283) • JTK-003
implant • Albuferon • Celgosivir (MBI-3253) • IC41 • INN0101 • Tarvicin • ANA971 (oral)
floxacin, Tequin • J, TMC207 (ex R207910) • LL-3858 • M, moxifloxacin, Avelox • PA-824

Immune-Based Therapies and Preventive Technologies Pipeline

by Richard Jefferys

The Current Landscape

The development of immune-based HIV therapies, preventive HIV vaccines, and microbicides is hampered by a common problem: the lack of any effective precedent. All three fields are currently attempting to build on a barren landscape—a challenge not faced by developers of new antiretroviral, hepatitis C, and TB treatments. This makes investment in this research high-risk and highly dependent on public funding. Many of the products in the pipeline are the fruits of collaborations between academic and/or government investigators, small companies, and non-profit organizations. Even if proven successful, obtaining regulatory approval and scaling up manufacturing to allow widespread distribution will likely depend on additional investments, the prospects for which are uncertain. In short, the path through these particular pipelines is tortuous and difficult. Additionally, preventive vaccines and microbicides must confront a problem also faced by new treatments for TB: the vast majority of the potential market for these products is primarily in the developing world where people have the least ability to pay. Again, this puts the onus on public-private collaborations to walk a landscape where most major pharmaceutical companies fear to tread.

Preventive Technologies: Vaccines

Over the last five years, the once-dry preventive vaccine pipeline has begun to fill with new candidates. This upsurge can be traced to improvements in techniques for inducing T-cell (also known as cell-mediated) immune responses. Initial efforts to develop an HIV vaccine focused primarily on candidates designed to induce neutralizing antibodies against the virus. These efforts, however, were severely compromised by the discovery that, while HIV viruses grown in the lab could be easily neutralized, viruses taken from infected people (called primary isolates) are highly resistant to antibody-mediated neutralization. The relevance of these observations was confirmed by the failure of an early antibody-based vaccine candidate (AIDSVAX) to protect against HIV infection in two large efficacy trials (Flynn 2005; Pitisutithum 2004).

While researchers continue to work on strategies for inducing neutralizing antibodies against HIV, only a minority of the vaccines currently in human trials are antibody-based. Instead, the leading candidates have drawn on evidence that T-cell responses may play an important role in controlling HIV infection (Pantaleo 2004). T-cell responses are comprised of both CD4 “helper” and CD8 “killer” T cells. The ability of scientists to dissect T-cell responses has improved vastly over recent years due to new technologies that allow the numbers, specificity (i.e., the pathogen they are targeting), and functional properties of CD4 and CD8 T cells to be evaluated. Most researchers believe it is unlikely that a T-cell-based vaccine will completely protect against HIV infection, but there is some optimism that it could slow or prevent disease progression in an immunized individual who subsequently becomes HIV-infected. Also, if vaccination could reduce post-infection viral load, the risk of onward transmission of the virus might also be reduced.

The leading vaccine strategies for inducing HIV-specific T-cell responses involve the use of naked DNA (genetic code engineered to make selected HIV proteins when delivered into the body) and viral vectors (harmless viruses altered so that they carry the genetic code for making selected HIV proteins when delivered into the body). Other approaches being investigated in human clinical trials include lipopeptide and whole protein vaccines.

Desired Elements

The ideal attributes of a preventive HIV vaccine can be quickly summarized:

- Complete protection against HIV infection in as many recipients as possible
- Effective against multiple HIV clades
- Long-lasting immunity
- Safe
- Easy to deliver (e.g., a single shot)
- Cheap
- Easy to manufacture on a large scale
- Easy to ship and distribute globally

A Note on Antigens and Clades

An important aspect of vaccine design is deciding which parts of HIV (in immunological parlance, HIV “antigens”) the vaccine should induce immune responses against. HIV contains a total of nine genes (*env*, *gag*, *pol*, *nef*, *tat*, *rev*, *vpr*, *vif*, *vpu*), all of which encode proteins that are potential targets for the immune system. Specialized immune system cells called antigen-presenting cells (APCs) break down proteins into small slices called epitopes that can be recognized by individual T cells, and some vaccines include known epitopes from particular HIV proteins rather than (or in addition to) using the whole protein. As is evidenced by the vaccine pipeline table, a diverse array of HIV antigens are being employed in current HIV vaccine studies. For example, Merck has selected the *gag*, *pol* and *nef* genes for their Ad5 vaccine candidate. This decision was based on extensive studies of HIV-specific T cell responses in infected individuals which showed that the proteins encoded by these genes are the most frequently targeted (Coplan 2005).

Merck also based their decision on the relative conservation of these genes across different HIV clades. Clades are a way of classifying HIV based on the virus’s genetic make up; for example most viruses from North America and Europe are genetically similar and are said to belong to HIV clade B. Many viruses found in Africa are also similar, but show distinct genetic differences from HIV clade B and are therefore classified as belonging to different clades (clades A, C and D are the most common in Africa). Mixes between different clades are called circulating recombinant forms (CRFs), for example the prevalent HIV in Thailand was once classified as belonging to clade E but is now designated as a mix between clades A and E called CRF01_AE. The genetic variability of HIV globally presents a major challenge for vaccine developers because immune responses that recognize HIV from one clade may fail to recognize viruses from other clades. Hence Merck’s focus on genes from HIV that are very similar from clade to clade (in general, HIV’s *env* gene varies the most while *gag* is the most conserved). While initially most vaccine candidates were based on HIV clade B, an increasing number are now including HIV components from alternative or multiple clades.

Table 1. Preventive Vaccines Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|---------------------------------------|
| ALVAC vCP1521 | Canarypox vector | Aventis Pasteur | Phase III |
| AIDSVAX B/E (booster only) | Gp120 envelope recombinant protein | VaxGen | Phase III |
| MRKAd5 | Adenovirus serotype 5 vector containing <i>gag/pol/nef</i> genes from HIV-1 clade B | Merck | Phase IIb |
| LIPO-5 | 5 lipopeptides containing CTL epitopes (from <i>gag</i> , <i>pol</i> , and <i>nef</i>) | ANRS; Aventis | Phase II (on hold due to toxicity) |
| GTU-Multi-HIV | DNA vaccine containing <i>nef</i> , <i>rev</i> , <i>tat</i> , <i>gag</i> , <i>pol</i> , <i>env</i> , and CTL epitopes | FIT Biotech | Phase I/II |
| pHIS-HIV-B rFPV-HIV-B | DNA vaccine + fowlpox boost containing <i>gag</i> , <i>rev</i> , <i>tat</i> , <i>vpu</i> , and truncated <i>env</i> genes from HIV-1 clade B | University of New South Wales, Australia, Virax | Phase I/II |
| ADMVA | MVA vector containing <i>env/gag-pol</i> , and <i>nef-tat</i> genes from HIV-1 clade C | Aaron Diamond AIDS Research Center (ADARC), IAVI, IDT | Phase I |
| GSK Protein HIV Vaccine | Recombinant Tat, Nef, and gp120 proteins in ASO2A adjuvant | GlaxoSmithKline | Phase I |
| VRC-HIVADV014-00-VP | Adenovirus serotype 5 vector containing <i>gag/pol</i> genes from HIV-1 clade B and <i>env</i> genes from clades A, B, and C | NIH Vaccine Research Center | Phase I (both alone and as a booster) |
| AdVax 101 (VEE) | Venezuelan Equine Encephalitis virus vector containing the <i>gag</i> gene from HIV-1 clade C | AlphaVax | Phase I |
| VRC-HIVDNA016-00-VP | DNA vaccine containing <i>gag</i> , <i>pol</i> , and <i>nef</i> genes from HIV-1 clade B, and <i>env</i> genes from clades A, B, and C | NIH Vaccine Research Center | Phase I |
| TBC-M358 (MVA) TBC-M335 (MVA) TBC-F357 (FPV) TBC-F349 (FPV) | MVA and fowlpox vectors encoding <i>env</i> , <i>gag</i> , <i>tat</i> , <i>rev</i> , <i>nef</i> , and reverse transcriptase genes from HIV-1 clade B | NIAID, Therion | Phase I |
| LIPO-4T (LPHIV-1) | 4 lipopeptides containing CTL epitopes (from <i>gag</i> , <i>pol</i> -RT, <i>pol</i> , and <i>nef</i>) | ANRS, Biovector SA | Phase I |
| LFn-p24 | Anthrax-derived polypeptide LFn Gag p24 protein | AVANT, NIAID, WRAIR | Phase I |

Table 1. Preventive Vaccines Pipeline (Cont.)

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------|
| HIV CTL MEP | DNA vaccine containing CTL epitopes from <i>env</i> or <i>gag</i> | NIAID, Wyeth | Phase I |
| DNA + Protein Vaccine Combination | DNA vaccine containing a <i>gag</i> gene (from HIV-1 clade C) and 5 <i>env</i> genes (from clades A, C, and E + two from clade B), plus a protein boost using recombinant gp120 proteins from the same 5 isolates that supplied the <i>env</i> genes for the DNA component | University of Massachusetts Medical School, Advanced BioScience Laboratories, Inc. | Phase I |
| tgAAC09 AAV | Adeno-associated virus vector containing <i>gag</i> , protease, and reverse transcriptase genes from HIV-1 clade C | Targeted Genetics, IAVI | Phase I |
| ADVAX DNA | DNA vaccine containing <i>gag</i> , <i>env</i> , <i>pol</i> , <i>nef</i> , and <i>tat</i> genes from HIV-1 clade C | IAVI, ADARC, Vical | Phase I |
| DNA/PLG Oligomeric gp140/MF59 | DNA vaccine containing <i>gag</i> and <i>env</i> genes from HIV-1 clade B, plus a protein boost containing a gp140 protein also from clade B | Chiron | Phase I |
| VRC-HIVDNA-009-00-VP | DNA vaccine containing <i>gag</i> , <i>pol</i> , and <i>nef</i> genes from HIV-1 clade B, and <i>env</i> genes from clades A, B, and C, together with an adjuvant gene encoding an IL-2 fusion protein | VRC, HVTN, Vical | Phase I |
| PolyEnv1 | Vaccinia viruses expressing 23 different <i>env</i> genes | St. Jude's Children's Hospital | Phase I |
| ISS P-001 | Recombinant Tat protein from HIV-1 clade B | ISS, Excell | Phase I |
| EP HIV-1090 DNA | DNA vaccine containing 21 CTL epitopes from <i>gag</i> , <i>pol</i> , <i>env</i> , <i>nef</i> , <i>rev</i> , and <i>vpr</i> (HIV-1 clade B) | NIAID, Epimmune | Phase I |

ALVAC (Aventis Pasteur)

ALVAC is an HIV vaccine candidate manufactured by Aventis Pasteur that uses a bird virus called canarypox as a vector. ALVAC has the dubious distinction of being the longest-studied viral vector vaccine candidate, with more than 1,000 people having participated in phase I and II studies over the last ten years. Unfortunately, ALVAC induces persistent HIV-specific CD8 T-cell responses in only around 10–20% of recipients (Nitayaphan 2004; Russell 2005), leading to considerable skepticism about its potential efficacy. A number of ALVAC variants have been developed in an effort to improve the response rate (known as the vaccine's immunogenicity), but none have

proved successful. ALVAC version vCP1521 (which encodes HIV-1 CRF01_AE *env* and clade B *gag*, the protease-encoding portion of the *pol* gene and a synthetic polypeptide encompassing several known CD8 T-cell epitopes from the Nef and Pol proteins) is undergoing an efficacy evaluation in a huge 16,000-person trial in Thailand. Results should be available within the next five years. Many leading HIV vaccine scientists have harshly criticized the planning and design of this trial, as has TAG (Burton 2004; Jefferys 2004).

AIDSVAX: A Dishonorable Mention

In the early years of HIV vaccine development, when it was thought that antibodies to the viral envelope protein gp120 might be successful, several companies made constructs using recombinant gp120 proteins. Among them is the AIDSVAX vaccine (manufactured by a company called VaxGen) that failed to protect against HIV infection or slow disease progression in two phase III trials. Initial claims by VaxGen that the vaccine had shown some protection in people from particular ethnic backgrounds were quickly revealed to be spurious (Follman 2004). Although the trial results indicate that AIDSVAX should have been flushed from the vaccine pipeline, it is still being used as a booster in the Thai ALVAC trial mentioned above.

Adenovirus-Based Vaccines

Merck created a splash in 2001 when it announced the launch of a clinical development program for an HIV vaccine candidate based on two platforms: naked DNA and a viral vector using adenovirus serotype 5 (Ad5). Ad5 was chosen in part due to its salutary ability to target dendritic cells, the initiators of T-cell responses. The original plan, based on promising immunogenicity studies in macaques, was to utilize the DNA vaccine as a “prime” (to generate low-level HIV-specific T-cell responses) followed by the Ad5 vaccine as a “boost” (to raise the HIV-specific T-cell responses to much higher levels). Human trials have since shown that the DNA vaccine is only marginally immunogenic, leading Merck to focus on the Ad5 construct. The data to date on Ad5 (still unpublished) appear to be highly promising, with one very significant caveat. The good news is that Ad5 can induce HIV-specific CD8 T-cell responses in up to 60–70% of recipients, a far superior showing compared to the previous best achieved by ALVAC (Isaacs 2004). The bad news is that this robust immunogenicity is seen only in people that lack significant levels of neutralizing antibodies against Ad5 (in its natural form, Ad5 is a common cause of severe colds and many people have been exposed to it). It’s estimated that about a third of the North American population has high levels (a titer of over 200) of neutralizing antibodies against Ad5; in the developing world the proportion approaches 90% (Kostense 2004). In such individuals, Ad5 is essentially about as immunogenic as ALVAC.

As a result of this dilemma, Merck is currently collaborating with the HIV Vaccine Trials Network (HVTN) on a phase IIb “proof of concept” efficacy trial that restricts enrollment to individuals with low levels of anti-Ad5 antibodies. The primary goal of the study is to evaluate whether the HIV-specific T-cell responses induced by the vaccine (which encodes the *gag*, *pol*, and *nef* genes from HIV clade B) can offer protection against HIV infection and/or disease progression. Results are expected by 2007. Because so many current candidates aim to induce T-cell responses, the study is addressing a critical question for the future of HIV vaccine research; if the results are positive, alternative versions of the vaccine will have to be developed in order to circumvent the neutralizing

antibody problem. Merck is looking at less common Ad5 serotypes such as 11 and 35. Work is also underway to determine whether the Ad5 vaccine can be modified to evade preexisting antibody responses by altering the Ad5 hexon protein, which is the major target of these antibodies (Sumida 2005).

The NIH's Vaccine Research Center (VRC) has developed another Ad5 candidate, which is in human trials. VRC is testing this vaccine both alone and as a booster to its DNA vaccine. VRC's long-term plan is to conduct a three-arm 16,000-person efficacy trial that will compare Ad5 to DNA + Ad5 to placebo, powered to detect efficacy in any vaccine arm. However, as with the Merck construct, the issue of pre-existing antibodies to Ad5 will need to be addressed.

Lipopeptides

Lipopeptides comprise synthetic fragments of viral proteins associated with lipids that facilitate the induction of T-cell immune responses. Lipopeptide vaccines have induced HIV-specific CD8 T-cell responses in around 50% of recipients (Salmon-Ceron 2002). Lipopeptides are difficult to manufacture on a large scale, making it uncertain whether they could ever be produced commercially. These vaccines are being developed by the French research agency ANRS, but studies are currently on hold after a serious adverse event (spinal cord inflammation) in a trial participant.

The Travails of MVA

Modified Vaccinia Virus Ankara strain (MVA) is an attenuated, nonpathogenic derivative of the cowpox virus. An MVA-based HIV vaccine candidate designed by Andrew McMichael and Tom Hanke from Oxford University has undergone extensive human testing with the support of the International AIDS Vaccine Initiative (IAVI). Unfortunately, the immunogenicity was disappointing, with persistent HIV-specific CD8 T-cell responses detectable in just 10–20% of recipients (Guimaraes-Walker 2004; Jaoko 2004). As a result, IAVI is not pursuing further studies of this construct. Two other MVA-based HIV vaccine candidates (one manufactured by Therion and the other developed by the Aaron Diamond AIDS Research Center) remain in human studies, but it is unclear whether they will prove more immunogenic. The problem with MVA may be its large size; immune responses targeting the vector appear to dominate at the expense of responses targeting the HIV components that the vector encodes (Harrer 2004).

A Multitude of DNAs

DNA vaccines are perhaps the simplest, cheapest approach for inducing T-cell responses. These constructs have proven immunogenic in mice and monkey studies, and a few years ago there was considerable optimism that they would be efficacious in humans. Data from human trials have since dimmed that optimism, with only a minority of recipients displaying low-level T-cell responses to the vaccines. Scientists speculate that the problem may be a matter of size and dose: humans are simply much larger than the animals used in preclinical studies, and the dose of DNA vaccine that can be delivered is limited by the fact that the DNA becomes an unwieldy goo (that is difficult and painful to inject) at doses much above 5 mg. Nevertheless, multiple DNA vaccines continue to undergo human testing. In some cases, as with the candidate being developed by

VRC, the intent is to use the DNA vaccine as a “prime” before boosting with a viral vector vaccine. Another strategy under study involves adding adjuvant components (such as cytokines like IL-2, IL-12, or IL-15) to the DNA vaccine that may boost the T-cell response. (For a detailed review of DNA vaccine development in HIV, see Giri 2004.)

Recombinant Proteins

Shortly after Merck announced the launch of its HIV vaccine program, GlaxoSmithKline (GSK) chimed in with a press release touting its own “new” HIV vaccine program. Jaded observers of the field rapidly noticed that the construct—recombinant HIV Nef, Tat, and gp120 proteins in a proprietary ASO2A adjuvant—was a candidate developed and then shelved by SmithKline Beecham, the company with which Glaxo had just merged. SKB discontinued developing the vaccine due to conflicting results from macaque challenge experiments and its apparent inability to induce CD8 T-cell responses. GSK chose to put a positive spin on these studies and has advanced the construct into phase I human testing. Preliminary results have demonstrated decent HIV-specific CD4 T-cell responses, but no vaccine-induced CD8 T cells were detected (Horton 2004).

Three other vaccine candidates undergoing human trials also use recombinant protein components. Chiron is employing an oligomeric envelope protein (gp140, with the V2 region deleted) as a booster following immunization with a DNA vaccine. An oligomeric protein is composed of multiple protein chains as compared to a monomeric protein, which contains a single chain (for example, AIDSVAX is a monomeric gp120 protein). Chiron is hoping that this protein will stand a better chance of inducing neutralizing antibodies against HIV. Macaque studies demonstrated induction of antibodies capable of some degree of neutralizing activity against four of five primary HIV isolates tested, but this activity was seen only at high antibody concentrations (Srivastava 2003).

Advanced Bioscience Laboratories (in collaboration with the University of Massachusetts and CytRx, and with NIH support under the HIV Vaccine Design and Development Team program) are using recombinant gp120 proteins from multiple clades (A, C, E, and two from B) as a booster following a DNA vaccine encoding the same *env* genes along with HIV clade B *gag*. The approach, based on unpublished animal data, suggests that immunization with gp120 proteins from multiple clades may induce qualitatively superior antibody responses compared to those induced by gp120 from a single clade.

Maverick Italian researcher Barbara Ensoli has long been pursuing the hypothesis that a recombinant HIV Tat protein could prove effective as a vaccine. Ensoli and colleagues published a controversial study in cynomolgus macaques many years ago that claimed successful protection against a SHIV89.6P challenge using this approach (Cafaro 1999). A subsequent attempt to confirm these findings by David Watkins was unsuccessful, although the construct and approach used were not exactly matched (Allen 2002). Ensoli has now successfully moved the Tat protein vaccine into phase I human testing in Italy.

Adeno-Associated Virus (AAV)

One of the more intriguing new viral vectors to enter human trials is Adeno-Associated Virus (AAV). AAV is a parvovirus that is dependent on adenovirus for replication; the vector has been

further modified so that it is completely replication-incompetent. Developed by Phil Johnson at the Children's Research Institute in Columbus, Ohio, in collaboration with Targeted Genetics (with the support of IAVI), AAV displays some unique features that could prove extremely advantageous for an HIV vaccine. Specifically, AAV appears to persist and express its HIV protein payload for months after a single immunization. This feature offers the hope that, if successful, AAV could be used as a single-shot immunization. It has taken many years to advance this candidate to human testing due to concerns that it may integrate into human DNA, but extensive safety studies in animals have now reassured regulatory authorities that it is safe to test in humans; AAV appears able to persist in the episomes of cells without integration. Immunogenicity results in macaques were impressive, showing a robust, dose-dependent induction of HIV-specific T-cell responses and anti-Gag antibodies (Schulz 2004). A phase I human trial began in Germany at the end of 2003, and results are anticipated by mid-2005.

Venezuelan Equine Encephalitis Virus (VEE)

Originally developed by the U.S. military as a potential biological weapon, an attenuated form of VEE is in testing as a potential HIV vaccine vector under the aegis of the biotech company AlphaVax and NIH (early work on the vector was sponsored by IAVI but they recently ended their relationship with AlphaVax). VEE belongs to a family known as alphaviruses and, like adenovirus, targets dendritic cells. Only limited immunogenicity data are available from macaques (Davis 2000); human data from a phase I trial initiated in South Africa in 2002 are pending.

Preventive Technologies: Microbicides

Microbicides are substances that aim to prevent HIV infection (and possibly other STDs) by being applied topically to the vaginal or rectal surface prior to sex. One major advantage to such an intervention, if one could be successfully developed, is that it could potentially be used by women who may not be able to control whether or not their partner uses a condom. After a period in which microbicide research seemed to wander in something of a scientific wilderness, the past few years have witnessed a new and broadening enthusiasm for the field. As a result, the microbicide pipeline has swelled, and a number of phase III efficacy trials have recently got underway.

Desired Elements

As outlined in a recent review, the four guiding principles of microbicide design are "cheap, safe, effective, acceptable" (Moore 2003). It would also be highly advantageous if a microbicide could be used without detection by the sexual partner. A rectal product is also desirable, but no candidates are yet in human trials. The microbicide field therefore faces the challenge of not just finding compounds, but developing user-friendly delivery methods (a science in itself). A key long-term goal is the development of formulations or devices (such as intravaginal rings) that can facilitate the slow release of a microbicide over a period of days or months (Woolfson 2000).

Table 2. Microbicides Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|---------------------------------------------------------------------|---------------------------------|--------------------------------------------------|---------------------------------------------------------------------|
| Carraguard® | Adsorption inhibitor | Population Council | Phase III |
| Cellulose sulfate (Ushercell®) | Adsorption inhibitor | CONRAD/Polydex Pharmaceuticals Limited | Phase III |
| PRO 2000/5 Gel | Adsorption inhibitor | Indevus Pharmaceutical, Inc. | Phase III, Phase II/IIb (with BufferGel), Phase II (with tenofovir) |
| Savvy (C31G) | Surfactant | Biosyn, Inc. | Phase III |
| BufferGel™ | Acid-buffering agent | Reprotect, LLC | Phase II/IIb (with PRO2000) |
| Lactin-V | Vaginal defense enhancer | Osel, Inc. | Phase II |
| Protected Lactobacilli in combination with BZK | Acid-buffering agent/surfactant | Biofem, Inc. | Phase II |
| Tenofovir/PMPA Gel | Reverse transcriptase inhibitor | Gilead Sciences, Inc. | Phase II (alone and with PRO2000) |
| Invisible Condom | Entry/fusion inhibitor | Laval University (Division of Microbiology) | Phase I/II |
| ACIDFORM Gel | Acid-buffering agent | Global Microbicide Project | Phase I |
| Cellulose acetate 1, 2-benzenedicarboxylate (cellulose acetate/CAP) | Adsorption inhibitor | Aaron Diamond AIDS Research Center | Phase I |
| Lime Juice | Acid-buffering agent | University of Melbourne | Phase I |
| TMC120 | Reverse transcriptase inhibitor | International Partnership for Microbicides (IPM) | Phase I |
| UC-781 | Reverse transcriptase inhibitor | Biosyn, Inc. | Phase I |
| VivaGel (SPL7013 gel) | Entry/fusion inhibitor | Starpharma Ltd. | Phase I |

The microbicides that have advanced into efficacy trials fall into the following categories:

Surfactants

Surfactants are detergent-like chemicals that disrupt the lipid membranes of cells and the envelope of HIV. Nonoxynol-9 (N-9) is a surfactant with anti-HIV activity that was tested for efficacy as a potential microbicide in a phase III trial sponsored by the United Nations Joint Programme on HIV/AIDS, but results showed that it marginally increased the risk of HIV infection (Van Damme 2002), likely due to its demonstrated capacity to induce vaginal inflammation (Stafford 1998).

Results from this trial strongly suggest that, to be successful, a microbicide will have to be almost totally devoid of vaginal toxicity. A newer and putatively less toxic surfactant named SAVVY has been developed by the company Biosyn (Krebs 2000); SAVVY is currently undergoing evaluation in a phase III efficacy trial in West Africa.

Adsorption Inhibitors

Adsorption inhibitors block the binding of HIV to target cells. Candidates currently being studied belong to a group of chemicals called polyanions (which include dextran sulfate, proposed as an HIV treatment in the 1980s), which have too high a molecular weight to be absorbed orally. Three adsorption inhibitors are being assessed as microbicides in phase III efficacy trials: PRO 2000 (a naphthalene sulphonate polymer), carrageenan (trade name Carraguard, a naturally occurring sulphated sugar polymer), and cellulose sulphate (trade name UsherCell). All three are highly active against both R5 and X4 HIV isolates in vitro and have low toxicity. A small macaque study demonstrated protection against SHIV89.6PD infection in 4/8 animals using PRO2000 (Weber 2001), but there are no published challenge experiments using Carraguard or UsherCell.

Acid-Buffering Agents

A key aspect of vaginal health is the maintenance of a low pH by hydrogen-peroxide-producing lactobacilli. Several microbicides are designed to maintain the acidity of the vagina, thereby making it toxic to viruses like HIV. One such agent, BufferGel (Mayer 2001), is being studied in a phase IIb efficacy trial with PRO2000.

Microbicides: The Next Generation

In earlier phases of human trials are a number of microbicides with direct antiretroviral effects mediated by blocking viral fusion with, or entry into, target cells. There are also several reverse transcriptase inhibitors, including a gel form of the drug tenofovir that is currently in phase II trials (alone and in combination with PRO2000).

Preventive Technologies: Pre-Exposure Prophylaxis (PrEP)

Table 3. Pre-Exposure Prophylaxis (PrEP) Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|-------------------------|---------------------------------|-----------------------|----------|
| Tenofovir (Viread, TDF) | Reverse transcriptase inhibitor | Gilead Sciences, Inc. | Phase II |

PrEP is the moniker given to drug therapies that may prevent HIV infection when taken prior to exposure. Currently there is only one candidate being evaluated as PrEP, the nucleotide reverse transcriptase inhibitor tenofovir (trade name Viread). There are several ongoing and planned trials designed to evaluate both the safety and efficacy of this approach (three sponsored by the US Centers for Disease Control, two by Family Health International and the Bill & Melinda Gates Foundation). Recent criticisms of the ethics of these trials have led to the suspension of a study site

in Cameroon and the termination of a proposed study among sex workers in Cambodia. A number of issues have been raised by critics, including the long term safety of tenofovir (side effects, albeit rare, include bone loss and kidney damage), the quality of safe sex counseling, provision of clean needles to intravenous drug users, plans for condom provision to participants and provision of care for participants that seroconvert and/or experience tenofovir-related toxicities. Discussions among the various stakeholders are now occurring in the hopes that these issues can be addressed.

Immune-Based Therapies

Despite more than two decades of research, there is as yet no approved immune-based therapy (IBT) for HIV infection, and while antiretrovirals continue to course through the developmental pipeline, relatively few potential immunologic interventions are dripping their way toward efficacy trials. This imbalance is partly due to an incomplete understanding of HIV's effects on the human immune system compared to our detailed knowledge of the viral life cycle. Absent this information, targets for IBTs are typically based on theories regarding pathogenesis and thus are susceptible to failure if a particular theory turns out to be incorrect. In contrast, a new antiretroviral compound can be targeted to a well-understood step in the HIV replication process. In addition, several IBTs (including Jonas Salk's ill-starred therapeutic vaccine candidate, Remune, and the bone marrow stimulant GM-CSF) have progressed to phase III efficacy trials but have failed to show clinical benefit (Kahn 2000; Angel 2000), making industry leery of pursuing compounds that risk a similar fate. It is also difficult to assess the prospective market for IBT, given that none are available, while there are years of accumulated data on the sales of antiretroviral drugs.

Desired Elements

There are a number of settings where IBTs could potentially prove useful. It is estimated that perhaps 5–10% of recipients experience a discordant response to HAART wherein viral load is successfully suppressed but CD4 T-cell counts do not increase (Carcelain 2001). An IBT that could speed immune reconstitution in such individuals would be highly desirable. An IBT that delayed, or allowed prolonged interruptions of, HAART could potentially reduce both the cost and toxicity of drug therapy. Recently, some researchers have proposed using IBTs to specifically target drug-resistant HIV (Stratov 2005). Beyond these potential uses, the desired characteristics of an IBT would be much the same as other therapies: broadly effective, safe, cheap, and convenient.

Therapeutic Vaccines

The advent of HAART has led to a resurgence of interest in therapeutic immunization, based on the idea that viral suppression and the attendant immune reconstitution may provide an opportunity to induce new and more effective T-cell responses targeting HIV. As with preventive vaccines, the field has been aided by improved tools for evaluating the functional properties of HIV-specific T cells. These tools have identified a number of properties that are associated with control of HIV viral load such as IL-2 production and proliferation (Pantaleo 2004; Boritz 2004; Migueles 2002), but it remains to be seen whether the induction of these T-cell responses by vaccination will prove beneficial. The primary goal of therapeutic immunization is to maintain better control of viral

Table 4. Therapeutic Vaccines Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------|
| ALVAC (vCP1452) | Canarypox vector encoding <i>env</i> , <i>gag</i> , the protease-encoding portion of the <i>pol</i> gene and CTL epitopes from the <i>nef</i> and <i>pol</i> gene products | Aventis Pasteur | Phase II |
| Lipopeptides | Peptides from Gag, Nef and Pol proteins | Aventis Pasteur/ANRS | Phase II |
| VRC-HIVDNA009-00-VP | DNA vaccine encoding <i>gag</i> , <i>pol</i> , <i>nef</i> , and multiclade (A, B, and C) <i>env</i> genes | VRC/NIAID | Phase I |
| MVA-BN- <i>nef</i> | MVA vector encoding clade B HIV <i>nef</i> gene | Bavarian Nordic | Phase I |
| MVA-mBN32 | MVA vector encoding multiple CTL epitopes | Bavarian Nordic/Epimmune | Phase I |
| MRKAd5 | Adenovirus serotype 5 vector encoding <i>gag</i> | Merck | Phase I/II |
| Autologous dendritic cells pulsed w/ALVAC | | ACTG/Aventis | Phase I |
| Autologous dendritic cell HIV vaccination w/conserved HIV-derived peptides | | University of Pittsburgh | Phase I |
| Multi-epitope DNA | 21 CTL epitopes and proprietary, non-HIV derived "universal" CD4 T-cell epitope | Epimmune | Phase I |
| DNA/MVA | DNA vaccine and an MVA vector encoding <i>gag</i> and multiple CTL epitopes | Cobra Pharmaceuticals, Impfstoffwerk Dessau-Tornau GmbH (IDT), Oxford University/MRC | Phase I/II |
| Remune +/- AmpliVax | Whole-killed clade A/G recombinant HIV isolate depleted of gp120 | Immune Response Corporation | Failed phase III; remains under investigation in context of STIs |
| Tat vaccine | Recombinant protein | Aventis Pasteur | Phase I |
| GTU- <i>nef</i> DNA vaccine | DNA encoding the clade B <i>nef</i> gene | FIT-Biotech | Phase I |

replication during HAART interruptions, thereby reducing dependence on drug therapy over the long term. Although this is certainly a desirable outcome, there are as yet no convincing human data showing that it is achievable. Some researchers are optimistic about the prospects for this approach, while many others remain profoundly skeptical.

There is extensive overlap between the therapeutic and preventive vaccine fields, with many of

the same candidates being studied in both settings. ALVAC is a stalwart of therapeutic vaccine studies, despite its poor immunogenicity. A recent ANRS study found a statistically significant difference in post-treatment interruption control of viral load among recipients of a regimen that included ALVAC, lipopeptides, and IL-2 compared to participants receiving HAART alone. The numbers were small, however, and the average time to restarting HAART differed by only a matter of weeks (Levy 2005). Merck's Ad5 vaccine candidate is also being evaluated as a therapeutic in an ongoing ACTG study, although the construct being used encodes only the HIV *gag* gene and not the *pol* and *nef* genes that are included in the preventive trial.

Jonas Salk's Remune, amazingly, is edging its way towards two decades in human trials. Remune underwent a mild renaissance in the post-HAART era due to its one demonstrable talent, which is to induce HIV-specific CD4 T-cell responses capable of proliferating and producing IL-2 (Maino 2000). A tiny pilot study has hinted that these responses may also improve HIV-specific CD8 T-cell proliferation (Lichterfeld 2004), and trials using Remune in the context of treatment interruptions are continuing, although recent results from a study in acute HIV infection failed to show an effect of immunization on viral load (Perrin 2004). Recently initiated trials are also investigating the effects of delivering Remune with a CpG-based adjuvant called AmpliVax (CpG motifs are immune-stimulating stretches of DNA).

A new strategy for therapeutic immunization involves the use of dendritic cells (DCs). The job of DCs is to process and present small protein slices (called epitopes) of pathogens to T cells, thereby initiating an immune response (the job is known as antigen presentation). Several research groups are conducting trials wherein DCs are taken from an individual's blood and mixed with HIV proteins or epitopes, then reinjected to act as a vaccine. A small, uncontrolled pilot study recently claimed an immunologic and virological benefit to the approach in participants with early HIV infection who had yet to start HAART (Lu 2004), but these results await confirmation in larger controlled studies. It is unclear whether the approach can be rendered practical and cheap enough for widespread use.

Cytokines, Immunomodulators & Gene Therapy

Interleukin-2

Another category of IBTs comprises candidates intended to improve overall immune function as opposed to just HIV-specific immunity. The hardy perennial of this class of therapies is interleukin-2 (IL-2), which has been in trials since the mid-1980s. IL-2 belongs to a family of chemical messengers called cytokines, which transmit signals among the cells of the immune system. Initially dubbed "T-cell growth factor" due its ability to induce T-cell proliferation, IL-2 is now understood to have more complex effects, including an unexpectedly important role in programmed T-cell death (Waldmann 2001).

Many studies have demonstrated that IL-2, administered either intravenously or subcutaneously, can increase peripheral blood CD4 T-cell counts in people with HIV infection (De Paoli 2001). Questions persist, however, about the functionality of these IL-2-induced CD4 T cells, with one recent ACTG study finding that they did not appear to improve (and may in some cases have diminished) the response to a variety of routine vaccinations such as hepatitis A vaccine (Valdez 2003).

Table 5. Cytokines and Immunomodulators Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|---------------------------------|-------------------------------|--------------------------------------------------|---------------------------------|
| Interleukin-2 (IL-2) | Cytokine | Chiron | Phase III |
| HE2000 | DHEA derivative | Hollis Eden | Phase II |
| Pegasys (peginterferon alfa-2a) | Cytokine | Roche Pharmaceuticals | Phase Ib/II |
| BAY 50-4798 | Modified IL-2 | Bayer | Phase I/II |
| HRG214 Passive Immunotherapy | HIV-specific goat antibodies | Virionyx Corporation Ltd | Phase I/II |
| Interleukin-7 (IL-7) | Cytokine | Biotech Inflection Point | Phase I |
| IL-4/IL-13 trap | Anti-cytokines | Regeneron Pharmaceuticals | Phase I |
| Serostim | Human growth hormone | Serono | Phase not specified (ACTG 5174) |
| Tucaresol | Schiff base forming drug | GlaxoSmithKline | Phase I |
| MDX-010 anti-CTLA4 antibody | Monoclonal antibody | Medarex | Phase I |
| Cyclosporine A | Immunosuppressant | Novartis | AIEDRP AIN501/ ACTG A5216 |
| Zenapax (daclizumab) | Anti-CD25 monoclonal antibody | Intramural NIH Program, Roche Pharmaceuticals | Phase I |

The mechanism of IL-2's effect is also uncertain, with recent evidence suggesting that over the long term it decreases T-cell proliferation and increases T-cell survival (Sereti 2004). Side effects such as fever, chills, and malaise are also typically associated with IL-2 administration. Nevertheless, it remains possible that the CD4 T-cell increases associated with IL-2 therapy will lead to long-term clinical benefit by delaying HIV-induced CD4 T-cell depletion, and this hypothesis is being investigated in two large clinical endpoint trials: SILCAAT and ESPRIT. IL-2's manufacturer, Chiron, originally sponsored SILCAAT, but in 2001 it pulled its support, and NIH (already the sponsor of the ESPRIT trial) had to step in to prevent the trial's termination. Preliminary results from the two trials should be available in 2005. An engineered and potentially less toxic form of IL-2 known as BAY 50-4798 is also under investigation in a phase I/II trial.

IL-7

IL-7 is a cytokine that plays a key role in T-cell development and naïve and memory T-cell proliferation and survival (Fry 2005). IL-7 studies in SIV-infected rhesus macaques have shown dramatic increases in peripheral blood CD4 and CD8 T-cell counts, without a concomitant increase in SIV replication (Fry 2003; Nugeyre 2003). Although it was originally thought that IL-7 might stimulate thymic production of new T cells, the increases in the macaque studies appeared to result from peripheral naïve and memory T-cell proliferation. The ACTG has recently initiated a phase I trial in HIV infection.

Anti-IL-4 and IL-13

Another IBT strategy involves blocking potentially harmful cytokines. A small Biotech company called Regeneron is developing a product called IL-4/IL-13 Trap based on the idea that these cytokines inhibit virus-specific CD8 T-cell responses. Results from a phase I dose-ranging trial in HIV-negative volunteers were presented at the 2004 Retrovirus conference, showing that the construct was well tolerated with a long half-life of 13 days (Parsey 2004). Further studies in HIV-infected individuals are planned.

Human Growth Hormone

One of the more surprising proposed IBTs is human growth hormone (HGH, Serostim), which is better known as an approved treatment for AIDS wasting syndrome. Several years ago, studies in mice indicated that HGH increased the size of the thymus. As a result, researchers became interested in the potential for HGH to speed naïve T-cell reconstitution in people with HIV. Mike McCune's research group at the Gladstone Institute measured thymus size and naïve T-cell counts in five individuals who were receiving HGH as a treatment for wasting and found that thymic mass did indeed increase, and that this was associated with a rebound in naïve T-cell numbers (Napolitano 2002). The ACTG is now enrolling a larger study involving over 100 participants that will prospectively evaluate the impact of HGH on thymus size and naïve T-cell reconstitution.

Tucaresol

Tucaresol is a relatively obscure IBT candidate that has languished in GlaxoSmithKline's HIV drug portfolio since the early 1990s. The drug appears to enhance interactions between antigen-presenting cells and T cells and has been shown to boost cell-mediated immune responses both in mice and in humans. Preliminary data from a phase I trial in 17 HIV-infected individuals were presented at the 2004 Retrovirus conference, demonstrating increases in naïve CD4 T-cell counts and the number of T cells containing TRECs (a potential marker for T cells recently produced by the thymus) in the group of participants receiving HAART treatment (Gazzola 2004).

MDX-010, Zenapax

Some experimental IBTs aim to influence T-cell function by interacting with signaling molecules on the T-cell surface. One such molecule is CTLA-4, which is upregulated on T cells in HIV infection and associated with the induction of T-cell unresponsiveness or anergy (Leng 2002). In June 2003, the Biotech company Medarex launched a phase I trial of an anti-CTLA-4 antibody dubbed MDX-010 in heavily treatment-experienced HIV-infected individuals who were failing HAART, with the aim of blocking the suppressive activity of CTLA-4 and thus improving HIV-specific immunity. Results from this study have not yet been presented.

A monoclonal antibody targeting another signaling molecule, CD25 (the IL-2 receptor) is also under evaluation as an HIV therapeutic. Roche manufactures this antibody under the trade name Zenapax (generic name daclizumab) and it was approved in 1997 for the prevention of kidney transplant rejection. A small phase I trial of Zenapax in HIV infection is being conducted by the NIH intramural research program.

Pegylated Alpha Interferon

Straddling the boundary between antiretrovirals and IBTs is the approved hepatitis C treatment, pegylated alpha interferon. Alpha interferon appears to have direct antiviral effects and also enhances cell-mediated immune responses in humans. The unpegylated form of alpha interferon was studied for many years as a potential HIV therapy, but eventually abandoned due to underwhelming results. The newer pegylated form is now once again being studied as an adjunct to HAART and in the context of treatment interruptions.

HE2000

Another proposed enhancer of cell-mediated immune responses is the DHEA derivative HE2000, but no data have been published on this IBT, and many distrust the drug's developer, Hollis Eden, which has hyped the results from a small phase II South African study without ever managing to get them into the scientific literature. The company's web site currently states that they "are pursuing public/private partnerships to conduct a phase II/III clinical trial in infectious disease."

Immunosuppressive Agents

The association between heightened levels of immune activation and HIV disease progression has led some researchers to pursue studies of several drugs that are typically referred to as "immune suppressants." These drugs include cyclosporine, prednisone, hydroxyurea, and mycophenylate mofetil. All are approved for other indications, and none of the manufacturers are specifically developing these compounds as IBTs. Academic researchers nonetheless continue to evaluate their potential, typically as an adjunct to HAART or in the context of treatment interruptions. It seems unlikely that any of these agents will be approved for the treatment of HIV/AIDS, and there are no known novel drugs being evaluated (a lone cyclosporine derivative from Sandoz never made it to human trials).

CD4 Reinfusion

There is a grab bag of approaches involving infusing CD4 T cells that are isolated from HIV-infected individuals, expanded, in some cases genetically modified in the laboratory, and then reinfused as a potential IBT. NeoProbe's Activated Cellular Therapy (ACT) does not involve genetic modification, but expands CD4 T cells isolated from the lymph nodes using a technique designed to select the cells secreting HIV-suppressing factors such as beta-chemokines. Despite the publication of intriguing data from a pilot study of this approach (Tiozzi 1999), the development of ACT is currently on hold pending identification of commercial partners that might support further research. At least three different biotech companies are attempting to genetically modify CD4 T cells in the lab in order to enhance their resistance to HIV infection, subsequently reinfusing them into a matched HIV-infected donor. A similar approach modifies both CD4 and CD8 T cells in an attempt to improve their ability to restrict HIV replication. Preliminary results from trials of these approaches have shown some limited promise (Deeks 2002; Amado 2002), but it remains very uncertain whether any of these gene therapy/IBT combinations will eventually enter efficacy trials.

Table 6. Gene Therapies Pipeline

| PRODUCT | TYPE | MANUFACTURER | STATUS |
|--------------------------------------|-----------------------------------------------------------------------------------------------------|-------------------|----------|
| CD4zeta modified CD4 and CD8 T cells | <i>Ex vivo</i> T cell Modifier | Cell Genesys | Phase II |
| Ribozymes (RRz2) | Antiviral ribozyme targeted against the <i>tat</i> gene, introduced into CD4 T cells via stem cells | Johnson & Johnson | Phase II |
| VRX496 | Lentiviral vector encoding antiretroviral antisense, introduced into CD4 T cells <i>ex vivo</i> | VIRxSYS | Phase I |
| HGTV43 | Vector encoding antiretroviral antisense, introduced into CD4 T cells <i>ex vivo</i> | Enzo Biochem | Phase I |
| M87o | Entry inhibitor gene encoded by a lentiviral vector, introduced into CD4 T cells <i>ex vivo</i> | EUFETS AG | Phase I |

Online Resources

Alliance for Microbicide Development Microbicide Research Portal
<https://secure.microbicide.org/DesktopDefault.aspx>

Global Campaign for Microbicides Pipeline Fact Sheet
<http://www.global-campaign.org/clientfiles/FS3-Pipeline-May05.pdf>

HIVInsite: Clinical Trials Databases and Lists
<http://hivinsite.ucsf.edu/InSite?page=li-04-24>

HIVInsite/HIV Vaccine Trials Network Pipeline Project
<http://chi.ucsf.edu/vaccines/>

International AIDS Vaccine Initiative: IAVI database of AIDS vaccines in human trials
<http://www.iavireport.org/trialsdb/>

NIH/National Library of Medicine Clinical Trials Database
<http://clinicaltrials.gov/>

TAG Immune-Based Therapy Pipeline Chart
<http://www.aidsinfonyc.org/tag/science/IBTpipeline.html>

References

- Allen TM, Mortara L, Mothe BR, et al. Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. *J Virol* 76(8):4108-12, 2002
- Amado R, Mitsuyasu R, Rosenblatt J, et al. Development of genetically protected T-lymphocytes from transduced hematopoietic progenitors in human immunodeficiency virus-1 infected patients. Abstract # LbOr10, XIV International AIDS Conference, Barcelona, Spain, July 7-12 2002
- Angel JB, High K, Rhame F, et al. Phase III study of granulocyte-macrophage colony-stimulating factor in advanced HIV disease: effect on infections, CD4 cell counts and HIV suppression. *AIDS* 14;4:387-395, 2000
- Boritz E, Palmer BE, Wilson CC. Human immunodeficiency virus type 1 (HIV-1)-specific CD4+ T cells that proliferate in vitro detected in samples from most viremic subjects and inversely associated with plasma HIV-1 levels. *J Virol* 78;22:12638-46, 2004
- Burton DR, Desrosiers RC, Doms RW, et al. Public health: A sound rationale needed for phase III HIV-1 vaccine trials. *Science* 303;5656:316, 2004
- Cafaro A, Caputo A, Fracasso C, et al. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat Med* 5;6:643-50, 1999
- Carcelain G, Debre P, Autran B. Reconstitution of CD4+ T lymphocytes in HIV-infected individuals following antiretroviral therapy. *Curr Opin Immunol* 13(4):483-8, 2001
- Coplan PM, Gupta SB, Dubey SA, et al. Cross-reactivity of anti-HIV-1 T cell immune responses among the major HIV-1 clades in HIV-1-positive individuals from 4 continents. *J Infect Dis* 191;9:1427-34, 2005
- Davis NL, Caley JJ, Brown KW, et al. Vaccination of macaques against pathogenic simian immunodeficiency virus with Venezuelan equine encephalitis virus replicon particles. *J Virol* 74;1:371-8, . 2000
- Deeks SG, Wagner B, Anton PA, et al. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. *Mol Ther* 5;6:788-97, 2002
- De Paoli P. Immunological effects of interleukin-2 therapy in human immunodeficiency virus-positive subjects. *Clin Diagn Lab Immunol.* 8;4:671-7, 2001
- Follmann D, Gilbert P, Self S, et al. An Independent Analysis of the Effect of Race in VAX004. Abstract #106, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco CA, February 8-11, 2004
- Fry TJ, Mackall CL. The Many Faces of IL-7: From Lymphopoiesis to Peripheral T Cell Maintenance. *J Immunol* 174(11):6571-6, 2005
- Fry TJ, Moniuszko M, Creekmore S, et al. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. *Blood* 101;6:2294-9, 2003
- Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF; The rgp120 HIV Vaccine Study Group. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* 191;5:654-65, 2005
- Gazzola L, Marchetti G, Bandera A, et al. Dynamics of T Cells Homeostasis Induced by Tucaresol. Abstract #523, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco CA, February 8-11, 2004
- Giri M, Ugen KE, Weiner DB. DNA vaccines against human immunodeficiency virus type 1 in the past decade. *Clin Microbiol Rev* 17;2:370-89, 2004

Guimaraes-Walker A, Mackie N, McMichael A, et al. Priming with a candidate HIV-1 clade A DNA vaccine followed by booster with HIV-1 Clade A MVA vaccine in volunteers at low risk of HIV infection. Abstract #55, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Harrer EG, Schmitt-Haendle M, Petzold B, et al. Phase-I-study with a MVA-BN-Nef vaccine in HIV-1 negative volunteers. Abstract# ThPeA7007, XV International AIDS Conference, Bangkok, Thailand, 2004

Horton H, Beckham C, Stucky J, et al. Induction of IL-2 secreting CD4+ T-cells capable of proliferation in seronegative subjects receiving the HIV-1 gp120/NefTat subunit vaccine. Abstract #54, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Isaacs R. Impact of pre-existing immunity on the immunogenicity of adenovirus serotype 5-based vaccines. Abstract #69, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Jaoko W, Omosa G, Bhatt K, et al. Safety and immunogenicity of DNA and MVA HIVA vaccines in phase I HIV-1 vaccine trials in Nairobi, Kenya. Abstract#56, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Jefferys R, Harrington M. Outstanding questions on HIV vaccine trial. *Science* 305;5681:180, 2004

Kahn JO, Cherng DW, Mayer K, Murray H, Lagakos S. Evaluation of HIV-1 immunogen, an immunologic modifier, administered to patients infected with HIV having 300 to 549 x 10(6)/L CD4 cell counts: A randomized controlled trial. *JAMA* 284(17):2193-202, 2000

Kostense S, Koudstaal W, Sprangers M, et al. Adenovirus types 5 and 35 seroprevalence in AIDS risk groups supports type 35 as a vaccine vector. *AIDS* 18;8:1213-6, 2004

Krebs FC, Miller SR, Catalone BJ, et al. Sodium dodecyl sulfate and C31G as microbicidal alternatives to nonoxynol 9: Comparative sensitivity of primary human vaginal keratinocytes. *Antimicrob Agents Chemother* 44: 1954–1960, 2000

Leng Q, Bentwich Z, Magen E, et al. CTLA-4 upregulation during HIV infection: association with anergy and possible target for therapeutic intervention. *AIDS* 16;4:519-29, 2002

Levy Y, Gahery-Segard H, Durier C, et al. Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. *AIDS* 19;3:279-86, 2005

Lichterfeld M, Kaufmann DE, Yu XG, et al. Loss of HIV-1-specific CD8+ T cell proliferation after acute HIV-1 infection and restoration by vaccine-induced HIV-1-specific CD4+ T cells. *J Exp Med* 200;6:701-12, 2004

Lu W, Arraes LC, Ferreira WT, Andrieu JM. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat Med* 10;12:1359-65, 2004

Maino VC, Suni MA, Wormsley SB, Carlo DJ, Wallace MR, Moss RB. Enhancement of HIV Type 1 Antigen-Specific CD4+ T Cell Memory in Subjects with Chronic HIV Type 1 Infection Receiving an HIV Type 1 Immunogen. *AIDS Res Hum Retroviruses* 16;18:2065-2066, 2000

Mayer KH, Peipert J, Fleming T, et al. Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. *Clin Infect Dis* 32: 476–482, 2001

Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol* 3;11:1061-8, 2002

Moore JP, Shattock RJ. Preventing HIV-1 sexual transmission--not sexy enough science, or no benefit to the bottom line? *J Antimicrob Chemother* 52;6:890-2, 2003

Napolitano LA, Lo JC, Gotway MB, et al. Increased thymic mass and circulating naive CD4 T cells in HIV-1-infected adults treated with growth hormone. *AIDS* 16;8:1103-11, 2002

Nitayaphan S, Pitisuttithum P, Karnasuta C, et al. Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. *J Infect Dis* 190;4:702-6, 2004

Nugeyre MT, Monceaux V, Beq S, et al. IL-7 stimulates T cell renewal without increasing viral replication in simian immunodeficiency virus-infected macaques. *J Immunol* 171;8:4447-53, 2003

Pantaleo G, Koup RA. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med* 10;8:806-10, 2004

Parsey M, Fauci G, Dunn J, et al. Human Pharmacokinetic Evaluation of the IL-4/13 Trap: a Novel Immunomodulatory Agent for the Treatment of HIV Disease. Abstract #522, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco CA, February 8-11, 2004

Perrin, L. Data on Quest Therapeutic Vaccination. Abstract # 31, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Petrovas C, Mueller YM, Bojczuk P, et al. IL-15 Treatment of SIV-infected Non-human Primates. Abstract #512, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco CA, February 8-11, 2004

Pitisuttithum, P. Efficacy of AIDS VAX B/E Vaccines in Injecting Drug Use. Abstract #107, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco CA, February 8-11, 2004

Russell ND, Graham BS, Keefer M, et al. A Phase II Double Blind Randomized Trial to Evaluate an HIV-1 Canarypox Vaccine (vCP1452) Alone and in Combination with Rgp120: Low Frequency T cell Responses. Abstract #408, Keystone Symposia on HIV Vaccines: Current Challenges and Future Prospects, Banff, Alberta, Canada, April 9-15, 2005

Salmon-Ceron D, Gahery H, Pialoux G, et al. Lipopeptides trials in non-HIV infected volunteers. Session V, XIIIth Cent Gardes Symposium on HIV and AIDS, Annecy, France, October 27-29, 2002

Schultz AM, Connell MM, Koff WC, Wyand M, Anklesaria P, Johnson, PR. Immunogenicity of two different AAV-based HIV vaccine candidates in non-human primates. Abstract# 16, AIDS Vaccines 04, Lausanne, Switzerland, August 30-September 1, 2004

Sereti I, Anthony KB, Martinez-Wilson H et al. IL-2-induced CD4+ T-cell expansion in HIV-infected patients is associated with long-term decreases in T-cell proliferation. *Blood* 104;3:775-80, 2004

Srivastava IK, VanDorsten K, Vojtech L, Barnett SW, Stamatatos L Changes in the immunogenic properties of soluble gp140 human immunodeficiency virus envelope constructs upon partial deletion of the second hypervariable region. *J Virol* 77(4):2310-20, 2003

Stafford MK, Ward H, Flanagan A, et al. Safety study of nonoxynol-9 as a vaginal microbicide: Evidence of adverse effects. *J Acquir Immune Defic Syndr Hum Retrovirol* 17: 327-331, 1998

Stratov I, Dale CJ, Chea S, McCluskey J, Kent SJ. Induction of T-cell immunity to antiretroviral drug-resistant human immunodeficiency virus type 1. *J Virol* 79;12:7728-37, 2005

Sumida SM, Truitt DM, Lemckert AA, et al. Neutralizing Antibodies to Adenovirus Serotype 5 Vaccine Vectors Are Directed Primarily against the Adenovirus Hexon Protein. *J Immunol* 174;11:7179-85, 2005

Triozzi PL, Aldrich W, Bresler HS, et al. Cellular immunotherapy of advanced human immunodeficiency virus type 1 infection using autologous lymph node lymphocytes: effects on chemokine production. *J Infect Dis* 179;1:245-8, 1999

Valdez H, Mitsuyasu R, Landay A, et al. Interleukin-2 Increases CD4+ lymphocyte numbers but does not enhance responses to immunization: results of A5046s. *J Infect Dis* 187(2):320-5, 2003

Van Damme L, Ramjee G, Alary M, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: A randomised controlled trial. *Lancet* 360: 971–977, 2002

Waldmann TA, Dubois S, Tagaya Y. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. *Immunity* 14(2):105-10, 2001

Weber J, Nunn A, O'Connor T, Jeffries D, et al. 'Chemical condoms' for the prevention of HIV infection: evaluation of novel agents against SHIV(89.6PD) *in vitro* and *in vivo*. *AIDS* 15(12):1563-8, 2001

Woolfson AD, Malcolm RK, Gallagher R. Drug delivery by the intravaginal route. *Crit Rev Ther Drug Carrier Syst* 17;5:509-55, 2000