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By D. Woods

SUMMARY

Participants appeared most interested in:

♦ Genomic research, including reverse vaccinology.

♦ Advances in vaccines for Ebola, menococcal and rotaviruses, SARS, and tuberculosis.

♦ New methods of delivery, including needle-free vaccines and mucosal immunization.

♦ Fast-track vaccine licensing.

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7TH ANNUAL CONFERENCE ON VACCINE RESEARCH
NATIONAL FOUNDATION FOR INFECTIOUS DISEASES (NFID)

May 24-26, 2004
Arlington, Virginia

The 7th Annual Conference on Vaccine Research focused on recent scientific advances in vaccine development, including genomic research, SARS, and vaccination technologies. William Egan PhD, acting director of the FDA's Center for Biologics Evaluation and Research (CBER) Office of Vaccines Research and Review, said, "I am quite excited about the rotavirus vaccines. Also, HPV (human papilloma virus) vaccines will be very important in the U.S. and in the developing world, where screening programs are not in place." Dr. Rino Rappuoli, vice president of vaccine research at Chiron, said, "The Ebola vaccine is very exciting, now that we know that it is technically feasible. We know that we are finally overcoming the challenges to creating vaccines, and, with new regulations and rules, overcoming the logistical problems. The menococcal vaccines will cure all types of menococcal diseases. Reverse vaccinology is making available a lot of vaccines that were previously technically impossible. There also is great effort and strives in new delivery systems." Dr. Rappuoli said participants had agreed not to discuss HIV at the conference.

GENOMIC RESEARCH

The completion of the human genome sequence last year opened new areas for scientists to explore, including a human baplotype map and reverse vaccinology, presenters at the conference agreed. Dr. Francis Collins, of NIH's National Human Genome Research Institute, said, "This is a field that is in its nascent stages…We now have the capacity to generate DNA sequence on lots and lots of organisms…Sequencing pathogens is a powerful pathway to vaccine development…(The challenge ahead is to) sequence lots of additional genomes, develop new technologies for sequencing, genotyping, expression and analysis, and proteomics – another kind of sequencing using nanometer pores. We must also define the structure of human variation – the human baplotype map – to discover susceptibility to infectious disease and host factor information."

REVERSE VACCINOLOGY

The availability of the genomic sequence of most pathogens has resulted in the ability to discover vaccines without the need to grow pathogens. Vaccine discovery can now be done in silico, starting from computer analysis of the genomes, or reverse vaccinology. Chiron's Dr. Rappuoli said, "Reverse vaccinology is a novel, genomic approach to vaccine development." He described how reverse vaccinology was used to obtain the genomic sequence of serogroup B Neisseria meningitidis (MenB), for which there currently is no effective, universal vaccine.

Today, no vaccine project is started without knowing the pathogen's genome sequence, according to Rappuoli. Reverse vaccinology is now a routine discovery approach, having been used to discover vaccines for Group A streptococcus, pneumococcus, Yersinia pestis, Porphyromonas gingivalis, staphylococcus, and malaria, among others. However, until recently, it has not been used to discover treatments for viruses. Rappuoli said, "Viral genomes have been available for more than two decades, but we never applied the knowledge that we had. For example, with HIV, we have had some promising results with HIV early proteins – non-structural proteins, such as Tat, Rev, Pol, etc. They could possibly be used in viruses."

SPECIFIC VACCINES

CHOLERA

BERNA BIOTECH'S Orochol (CVD 103-HgR). This new-generation, single-dose, live-attenuated, oral, cholera vaccine is the only single-dose oral vaccine being manufactured in the world. It is the first recombinant bacterial vaccine to be licensed as a live vaccine, with minimal transmissibility and lack of introduction into the environment.

The estimated efficacy of the vaccine is 79%. Developers are working on a practical formulation for very young children. A scientist said, "WHO has used it several times now in outbreaks, where it couldn't give a vaccine that required more than one dose…The holy grail in the developing world is a single-dose vaccine. We found that we could coax older infants and toddlers into drinking the vaccine cocktail. Three- to five-month-olds did not like to take 70 mls or more, and that's a bit of bad news. However, the bit of good news is that when a full dose or less was swallowed, the sero conversion rate was essentially the same…It looks as if we have found a very practical way to immunize young infants."

A study is underway in Mali looking at a new formulation of the vaccine that may be ready in 2005.

EBOLA

Advances in the search for a safe and effective Ebola vaccine continue. Ebola is an emerging infectious disease and potential microbial threat, and its molecular pathogenesis is not well understood. The National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center was able to generate protective immunity to Ebola through genetic immunization, using a DNA prime followed by an adenoviral boost or an accelerated immunization using adenovirus alone. Immune responses to a vaccine expressing the nucleoprotein (NP) and different strains of glycoprotein were efficacious in protecting both guinea pigs and primates from subsequent viral challenge in models relevant to human disease. Although the results are promising, it may take many more steps and much more time to develop a successful human vaccine.

HEPATITIS C

Perhaps the best example of reverse vaccinology is Hepatitis C – a virus, which, by conventional microbiology, doesn't exist, according to Rappuoli. He said, "It's a virus with no known conventional pathology, but by following the sequencing of genome, right now an HCV vaccine is being tested – of recombinant HCV-1 gpE1/gpE32 vaccines in chimps. The combined results from homologous HCV-1 and heterologous HCV-1 challenges – hopefully this will be another product that will be coming to benefit people by the use of a genome – an HCV vaccine."

HPV

HPV (human papilloma virus) vaccines, for the treatment of the most common sexually transmitted disease in the U.S., are in development and are likely to become available in the next few years. **MERCK** is thought to be closest to market with a vaccine in Phase III trials against a strain of HPV that causes cervical cancer. This is expected to be on the market in late 2005. **GLAXOSMITHKLINE** also has an HPV vaccine in Phase III trials.

SEROGROUP B MENINGOCOCCUS (MENB)

Serogroup B meningococcus, a bacterium, is a major cause of sepsis and meningitis and has been resistant to all conventional approaches to vaccine development. Serogroup B meningococcal disease remains a serious global problem for which no effective vaccine is available. Dr. Rappuoli said, "MenB has been a problem for a long time. MenB is also a meningococcus that carries a capsulate polysaccharide but, unfortunately, the polysaccharide is a self-antigen. It is identical to polysaccharide acid and is linked to NCAM in a lot of tissues; and, therefore, we can see MenB as a bacterium. It basically uses the capsule and goes away with a coat that is one of our tissues. Our immune system doesn't recognize it as foreign; it can bypass the immune system."

Early meningococcus vaccines included:

- A vaccine developed in the 1960s did not work in children aged one to two years, and in adults it only induced a primary response, with no memory. Dr. Rappuoli said, "An early try used a MenC conjugate vaccine, which induces a high level of bactericidal antibodies in infants, but plain polysaccharide is a poor immunogen for children aged 1-2 years."

➢ A conjugate vaccine – with seven to eight years of development – was licensed in the U.K., where there were problems of meningococcus. A laboratory there confirmed cases of serogroup C meningococcal disease, and immunization with serogroup C conjugate vaccine in 15- to 17-year-olds began in 1999. By 2000, the disease was gone. It's beautiful when vaccines work."

➢ In the past 30-40 years, the best that has been developed is the OMV (outer membrane vesicle) vaccine, which is made by purifying the outer membrane of cells containing it – purified LPS (lipopolysaccharide) and depleted OMV. Dr. Rappuoli said, "The major problem is that you can use the vaccine only when you have just one strain. We've used it in the past in Cuba, where there was one strain, and right now we're working with the New Zealand government to attack a strain that has been around since 1990. This is one particular strain of MenB. However, if we were to use it in the U.S., we'd need 50-100 different strains to represent all the strains in this country. So, in effect, this particular vaccine works for a particular epidemic caused by one strain."

The companies working to develop a MenB vaccine include:

- **CHIRON**, which has Phase I trials ongoing with a recombinant vaccine derived from the reverse vaccinology approach (antigens identified from genome screening).
- **NIPH** (Norway), which has trials combining OMV vaccines from New Zealand, with those of Norway.
- **GLAXOSMITHKLINE**
- **WYETH**
- **NIVM** (Netherlands), has no trials but is working on MenB.

Within three to five years new vaccines are expected to be available globally. Among the current vaccine approaches are:

1. WYETH'S tetravalent vaccines. These MenB vaccines are in the late stages of development.

2. CHIRON'S OMV vaccine. Trials of this monovalent OMV vaccine are in clinical trials in New Zealand in conjunction with the University of Auckland. Several clinical trials of a strain-specific group B meningococcal OMV vaccine, developed in three years (compared to the usual 8-14 years it takes to develop a vaccine), have begun, and this vaccine is supposed to be on a fast track toward licensing. However, delays in the licensing procedure may force the New Zealand government to dump large quantities of the vaccine if it is not dispensed by the October 31 expiration date. The country's drug safety agency was expected to approve the vaccine in July 2004.

The vaccine was tailor-made to the specific New Zeland sero subtype based on a parent vaccine produced and tested by the Norwegian Institute of Public Health. Immunogenicity results on the New Zealand vaccine were described as promising, with the vaccine showing a similar reactogenicity profile to the parent vaccine.

More than 4,000 doses of this new vaccine have been given to >1,300 children with no serious adverse reactions, according to the scientists involved. However, there have been frequent injection site reactions. An official with the New Zealand Ministry of Health said, "The latest epidemiology study showed no increased risk of neurological events and local and systemic adverse events were low."

The first series of clinical trials involving 75 healthy adults (18-50 years old) started at the end of May 2002 and ended in the fall of 2003. Each adult received three doses of the vaccine, administered six weeks apart by a clinical trial vaccination team at Middlemore Hospital. Phase II involved 600 school children, 300 toddlers, 300 late infants, and 300 early infants. All are finished except the 300 early infants, which were started in January 2004.

After the second phase is completed, there will be a pilot rollout with a licensed vaccine before a mass vaccination campaign targeting all under-20-year-olds, starting with the most at-risk. The first goal is a 70% reduction of cases of Neisseria meningitidis B:4:P1.7b,4 in children aged 0-9 years.

MenB Vaccine Results

Measurement	Vaccine
Type	Strain B;15:P1.7.16
Dosing	Two doses at 6-8 week interval
Efficacy at 29 months	57% (27% for 13-16 year olds)
Efficacy at 10 months	86%

Adverse events:

- Local and systematic adverse events low.
- 4 cases of serious neurological disease reported in efficacy studies.
- Large epidemiological study of 345,000 teenagers showed no increased risk (149,000 vaccinated).

Provisional licensure of a new OMV vaccine in New Zealand is dependent on:

➢ An immunogenic tailor-made vaccine with:
  - Reactogenicity and safety profile.
  - Physiochemical bridging to parent.

➢ A validated assay.

➢ Intensive safety monitoring.

3. Neisseria Lactamica OMV vaccine. Some research presented at the NFID meeting suggested that development of a MenB vaccine based on Neisseria Lactamica outer membrane vesicles (OMV) is possible. U.K. scientists are planning to start clinical trials of a *N. Lactamica* OMV vaccine in early 2005. The trial will test safety and immunogenicity in young adults. A researcher said, "We get good protection using this single preparation, and we haven't found a strain we cannot protect against yet…This is a novel approach to a MenB vaccine, and we think that we have a new MenB candidate. It protects mice against diverse N. Meningitidis serogroup B strains. Protection is independent of a bactericidal antibody response. Protection is transferable, independent of serogroup, independent of the immunodominant PorA antigen. Protection may be due to broad opsonophagocytic response, and meningococcal OMV vaccines are safe."

Preliminary data suggest that the carriage of commensal Neisseria species, particularly *N. Lactamica*, is involved in the development of natural immunity against invasive meningococcal disease. *N. Lactamica* is a commensal carried in the nasopharynx by young children. It has many surface structures in common with N. Meningitidis. Like N. Meningitidis, *N. Lactamica* produces blebs (warts) of outer membrane material. It lacks a capsule as well as the immunodominant PorA protein. Pre-clinical data indicate that immunization with *N. Lactamica* OMVs protects against a diverse population of meningococcal strains, irrespective of capsular serogroup. Native and desoxycholate-extracted OMVs were made by differential centrifugation of log-phase *N. Lactamica* cultures. Mice immunized with OMVs protected against lethal i.p. challenge with meningococcal isolates from the ET-5, ET-37, lineage II and A4-cluster clonal lineages. No challenge strain tested evaded this protection. Protection was transferable impassive protections experiments, and protection was observed in the absence of a marked serum bactericidal antibody response.

Severe Acute Respiratory Syndrome (SARS)

When SARS first struck in 2002, it didn't appear that a vaccine would be possible, but it only took two years to develop vaccines for SARS. Dr. Rappuoli said, "For a year, everyone was scared about SARS. Then the question became, was it basically a corona virus? The genome was there, and vaccines could be started to be designed, and today there are several vaccines which work on SARS."

Among the SARS vaccines in development are a **nasal vaccine** developed by scientists at the National Institute of Allergy and Infectious Diseases (NIAID), which requires only one dose in monkeys. A recently reported study compared the vaccine to placebo in eight African green monkeys. At 30 days, the monkeys given the SARS vaccine showed an immune response and no evidence of viral replication.

Candidate SARS Vaccines

Developer	Type	Funding (Location)	Human trials target date
Sinovac/CAMS	Inactivated virus	China	March 2004
University of British Columbia	Inactivated virus	Canada	December 2004
University of Toronto	Recombinant	Canada	December 2004
McMaster University	Adenovirus	Canada	December 2004
Aventis Pasteur	Inactivated virus	NIAID contract (France)	Late 2005
Baxter Healthcare	Inactivated virus	NIAID contract (Austria)	Late 2005
Protein Sciences	Recombinant	NIAID contract (U.S.)	Late 2005
U.S. Vaccine Research Center	Plasmid DNA	NIAID (U.S.)	Late 2005
Chiron Vaccines	Inactivated virus	Chiron (Italy)	Not set
University of Pittsburgh	Adenovirus	NHLB/CDC (U.S.)	Not set

SARS CoV vaccines must induce immunity at mucosal sites of CoV infection and disease. Live vaccines are usually more effective. Booster doses may be necessary, but sterilizing immunity is uncommon.

Smallpox Vaccine Advances

A safer smallpox vaccine is desirable due to observed myocarditis and encephalitis associated with **Wyeth's** DryVax and other non-attenuated smallpox vaccines. **VaxGen** presented data from two animal studies showing efficacy of its live attenuated smallpox vaccine, LC16m8.

In both rabbits and mice, a single dose of the vaccine protected all animals against a lethal poxvirus challenge, and demonstrated efficacy equivalent to DryVax, the currently licensed vaccine. A Phase I/II human trial to test safety and immune response is scheduled to begin later this year. A large-scale safety trial is also scheduled for this year. LC16m8 is considered to be as effective and safer than conventional smallpox vaccines. The vaccine has been licensed in Japan since 1980 and has been shown to be safe in more than 50,000 humans (mostly Japanese children). It is the only attenuated vaccine licensed for use in humans to prevent smallpox.

LC16m8 was tested in vivo using white rabbits, which were challenged with rabbit pox, an orthopoxvirus that produces high levels of EEV. Tests showed that LC16m8 was able to protect rabbits 100% from lethal rabbit pox challenge. Three groups of 20 rabbits each were vaccinated with LC16m8, DryVax, or a placebo. Rabbits were then challenged with lethal doses of intradermal rabbit pox. All of the rabbits vaccinated with LC16m8 or DryVax survived, and all but one of the placebo recipients died.

Three groups of mice were vaccinated with LC16m8, DryVax, or saline solution. The mice were then challenged with a mouse orthopoxvirus, aerosolized extromelia. All the mice vaccinated with LC16m8 or DryVax survived, and 9 out of the 10 placebo mice died. VaxGen's Dr. Cyril Empig, said, "The route (used with the mice) mimics the possible route of smallpox released into a human population during a bio-terrorist attack. To sum up, LC16m8 protected mice against severe aerosol extromelia challenge similarly to DryVax, with comparable clinical symptoms. Weight loss was prevented and they had higher levels of ortho-specific antibodies compared to DryVax…To sum up, LC16m8 protects rabbits against lethal rabbit pox virus challenge and protects mice against severe aerosol challenge. Further animal studies, including primates, are planned, hopefully bringing us closer to licensure, and clinical studies will commence shortly.

TYPHOID

S. Typhi is the cause of typhoid fever. Attenuated S. Typhi are considered to be very promising delivery systems for expressed recombinant antigens. An attenuated strain has been demonstrated to be an excellent live delivery system for either foreign protein antigens or DNA vaccines encoding those antigens.

Several salmonella typhi (S. Typhi) vaccines have looked very good in Phase II trials. They are all well-tolerated and much more immunogenic than a single dose of Ty21, the only live bacterial vaccine licensed for use against typhoid fever. Ty21a must be administered in three oral doses, however. The most recently developed typhoid fever vaccine candidates include S. Typhi CVD 908-htrA, Ty800, and ZH9, which were created by the deletion of rational selected genes of known function from S. Typhi Ty21, the strain used to create Ty21a. Phase I and II studies evaluating the safety and immunogenicity of single, oral doses of the vaccines show that they are generally well-tolerated and immunogenic.

Interest is high in using S. Typhi as a live vector vaccine. Mucosal priming with S. Typhi based from C vaccine allows an anamnestic response to parenteral tetanus toxoid.

Typhoid Vaccines

Company	Vaccine	Status	Comments
Mediva	CVD 908-htrA	Phase II completed	Results supported further development as a single-dose vaccine against typhoid fever and as a possible live vector for oral delivery of other vaccine antigens
Avant	Ty800	Phase II ongoing	---
Wokingham Microscience (U.K.)	ZH9	Phase II	---
University of Maryland	CVD 909	Phase I	---

Researchers have adapted this for use with an anthrax construct, using a boost. Patients would take a mucosal vaccine that would prime them one week after response.

TUBERCULOSIS

Although a vaccine is available for tuberculosis (Bacille-Calmette-Guerin or BCG), it only protects against Myco-bacterium tuberculosis (TB) in newborns and does not prevent the most common form of the disease, adult pulmonary TB. One-third of the world population is infected with the etiologic agent Mycobacterium TB. Dr. Stefan Kaufmann, of the Max Planck Institute for Infection Biology, in Berlin, Germany, said, "Tuberculosis is a global threat, with nine million new cases annually and two million deaths annually. There is a link between TB and AIDS; 15 million people are co-infected, with half a million additional deaths annually."

Current vaccination strategies have to consider both pre-exposure and post-exposure vaccines. Dr. Kaufmann said, "Subunit vaccination strategies comprise protein formulations or naked DNA in an appropriate adjuvant. Viable attenuated vaccines comprise either gene deletion mutants of M. (Myco-bacterium) tuberculosis or improved recombinant BCG." Rational vaccination strategies performed in Dr. Kaufmann's lab first focused on an improved BCG, which was a recombinant BCG strain which expressed listeriolysin and induced better protection than wild type BCG. At least three improved BCG vaccine candidates are in development.

BCG TB Vaccines in Development

Vaccine candidate	Potential advantage	Potential disadvantage	Examples
r-BCG expressing cytolysin	CD4 plus CD8 T-cells, unconventional T-cells	Devoid of TB-specific antigens, safety concerns	r-BCG-listeriolysin - urease r-BCG-listeriolysin
r-BCG expressing cytokine	Improved immunogenicity	Primarily CD4 T-cells, devoid of TB-specific antigens, safety concerns	r-BCG- IL-2, IFN-γ
r-BCG over-expressing antigen	Improved immunogenicity	Primarily CD4 T-cells, safety concerns/virulence factors	r-BCG-AG85 r-BCG-RD1

Dr. Kaufmann warned against giving up on BCG vaccines, "Because BCG has its merits; it can't be given up prematurely. Therefore, prime/boost vaccinations based on BCG prime need particular consideration…BCG does the job 2.7 times better than the controls, but we are still not satisfied. BCG takes away the urea enzyme and produces a promising strain."

There is a strain of TB, now found all over the world, which was responsible for outbreaks in New York in the early 1990s, in most of Asia, and in Beijing. The response to BCG vaccination was never proven. Infected mice with the Beijing strain, which is virulent in mice, were given normal BCG, and there was no protection.

While BCG is attenuated, with an excellent safety record, there are also some problems, including:

- BCG lacks important antigens [16 regions of difference (RD) with 129 ORFs present in M. tuberculosis and absent from BCG].
- BCG fails to stimulate the "right" combination of T-cells.

TB vaccination strategies being pursued include:

1. **Life vaccine**.

2. **Subunit vaccine**. Two subunit vaccines are now in clinical trials. Dr. Kaufmann said, "With subunit vaccines, there is no down-modulation, no cross-reaction, but there are also few antigens and few T-cell populations…There is an argument for prime/boost with BCG. BCG is attenuated and has an excellent safety record, but it lacks important antigens."

3. **Combinations of life and subunit vaccines**. This may be the most promising approach. Dr. Kaufmann said, "One vaccine won't do it against TB; we need a combination approach. Rather than competing one against another, there is a need for cooperation. Together, we can have one super vaccine which will be the best candidate for boosters between five and 30 years. I'm talking about a combination super BCG and a booster."

Among the combination vaccines in development are:

- A worldwide license to the Vakzine Projekt Management GmbH (VPM) and GMP production is planned for summer/autumn 2004, with a Phase I trial planned for spring/summer 2005.
- Negotiations with AERAS (the Bill and Melinda Gates foundation) regarding iterative and combinatory strategy towards super-BCG prime/super-subunit boost.

VACCINE DELIVERY TECHNOLOGY

JET INJECTION TECHNOLOGY

Jet injection is the needle-free delivery of liquid through the skin or mucous membrane by use of a piston, pressurizing fluid in the dose chamber. The liquid is driven into the patient using compressed gas or a metal spring by use of a foot pump or electric motor. Fluid is ejected through an orifice with a diameter of about .15 mm, creating high pressure over a small surface area and penetrating the epidermis or mucous membrane. Injection can be intramuscular, subcutaneous, or intradermal. Doses available to humans are between 0.1-1.0 ml.

Jet injectors can be used for anesthetics, antibiotics, cortico-steroids, heparin, hormones, immunostimulating drugs, tuberculin, vaccines, and vitamins.

In the age of bioterrorism and pandemic threats, companies are working to develop safe, high-speed, disposable-cartridge jet injectors. Multi-use nozzle jet injectors (MUNJIs), such as **EVANS ENTERPRISES'** Med-E-Jet and **KEYSTONE'S** Ped-O-Jet are not used because of safety concerns due to the possibility of blood-borne pathogen transmissions, and current efforts to make safer MUNJIs with Russian technology have been slow and difficult. Current disposable-cartridge jet injectors (DCJIs) are considered safe but slow. Manufacturers such as **DCI (D'Antonio Consultants International), AVENTIS PASTEUR, WESTON MEDICAL,** and **PENJET** are working to develop high-speed DCJIs with both end-user-filled and manufacturer-pre-fillable cartridges. A high priority is DCJI cartridge standardization.

Vaccine Candidates

Vaccine candidate	Advantage	Disadvantages	Examples
Subunit candidates			
Antigen in adjuvant	Mild side effects	Restricted number of T-cell clones, primarily CD4 T-cells Immunogenicity depends on adjuvant type	Culture filtrate (ill-defined antigen mixture) Defined antigen: ESAT-6, Ag85, Mtb8.4 Fusion protein: Ag85-ESAT-6
Naked DNA	CD4 and CD8 T-cells	Restricted number of T-cell clones, conventional T-cells, safety concerns	Hsp60, Ag85, Mtb8.4 Therapeutic vaccination
Recombinant carrier expressing antigen	CD4/ CD8 T-cells	Restricted number of T-cell clones, safety concerns	r-Vaccinia expressing Ag85; r-Salmonella expressing Ag85
Combination candidates			
r-BCG co expressing immunomodulator plus antigen	Improved immunogenicity, protective antigens	Safety concerns	Not done
r- M. tuberculosis deletion mutant expressing immunomodulator	Improved immunogenicity	Safety concerns	Not done
Prime to boost	Improved immunogenicity	Safety concerns	BCG – to protein (Ag85) BCG – to MVA (Ag85) Naked DNA – to protein (Ag85) Vaccinia (Ag85) Naked DNA – to BCG BCG – to naked DNA (Rv 3407)

Jet Injectors

Manufacturer	Products
Activa Brand Products	GentleJet, AdvantaJet, AdvantaJet ES Needle-Free Injection System
Antares Pharma	Medi-Jector Choice and Vision, MJ6, 7, 8, 10
Aradigm (acquired from Weston)	Intra-Ject
Aventis Pasteur	Mini-Imojet, IM-O-Jet
Bioject	Biojector 2000, Vitajet 3, SeroJet (with Serono), Iject
Biovalve Technologies	Mini-Ject
Cambridge Biostability	SNAP JET
D'Antonio Consultants International	LectraJet, LectraJet HS
ENDOS Pharma (France)	VACCIJET Electrique, VACCIJET Manuel
Equidyne Systems	Injex 30 , Injex 50
Evans Enterprises	Med-E-Jet
Felton Medical International (collaboration with Russia, PATH)	BI-100
Genesis Medical Technologies	SensaJet
Keystone Industries	SyriJet, Ped-O-Jet
Mada Medical Products	MadaJetXL
Medical Intl Technologies	Med-Jet, Agro-Jet
National Medical Products	J-Tip
Nidec Tosok (Japan)	Hyjettor
PATH	MEDiVAX
PenJet Corp	PenJet, PenJet/Micro
Chiron's PowderJect Pharmaceuticals	PowderJect
Robbins Instruments (distributor for Societe AKRA DERMOJET)	DermoJet and Vacci-Jet
Serono	SeroJet
SICIM Medical Jet	SICIM Jet 2000 and DG77 injectors
WLT Distributors	Agro-Jet Low Pressure

Advantages and Disadvantages of Jet Injection

Advantages of jet injection	Disadvantages of jet injection
Administers existing, off-the-shelf vaccines	Increased pain for irritating medications (e.g., adjuvanted vaccine)
Long history of efficacious use with many vaccine types	Potential laceration injury from improper technique
Avoids need and delay in reformulating and licensing new vaccines intended for other needle-free methods	Potential blood cross-contamination via MUNJI-type devices
Both end-user filling and manufacturer pre-filling possible	
Eliminates needle stick dangers and sharps waste burden	
Potential high rates of vaccination (more than 600 patients per hr)	
Can respond to pandemics and regional/local epidemics	
Rapid response to bioterrorism	
Can give parenteral vaccines such as anthrax, plague, quickly	

Immune Responses

Immune responses jet injection technology are generally equivalent to or better than needle syringes, possibly because of the effect of antigen-presenting dendritic (Langerhans) cells in the skin. Inactivated vaccines are usually more reactogenic than live vaccines. Pain is generally less than, or similar to, needle syringes.

Live vaccines	Inactivated vaccines
Bacille-Calmette-Guerin	Botulism
Measles	Cholera
Mumps	Diphtheria-Tetanus-Pertussis
Measles-Mumps-Rubella	Hepatitis A and B
Measles-Smallpox (vaccinia)	Influenza
Rubella	Meningococcus A and C
Smallpox (vaccinia)	Plague
Yellow fever	Polio
	Tetanus
	Tularemia-Typhoid

Adverse Events

Jet injections are slightly more reactogenic than needle syringes, with immediate local reactions such as erythema, hematoma, and delayed local reactions, including soreness, induration/edema, and ecchymosis. Other local adverse events include bleeding at the injection site, laceration if there is movement during injection, and, occasionally, traumatic injury.

Immediate local reactions	Delayed reactions	Other local adverse events
erythema	soreness	bleeding at injection site more common than with needle syringe
hematoma	ecchymosis	laceration if movement occurs during injection
	induration/edema	traumatic injury (e.g. neuropathy) reported

Next Generation Jet Injectors

The need for high-speed injection technology for mass vaccination programs and emergency response is driving manufacturers to develop high-speed devices, as well as pre-fillable or end-user-fillable disposable cartridges. The next target for mass vaccination/eradication is measles, according to the Centers for Disease Control.

DCJIs should be:

- **Safe** – using disposable, single-use, auto-disabling cartridges, allowing a clean, fingers-free end-user filling of the cartridges with vaccine as well as vaccine manufacturer pre-filling; fingers-free loading-ejection of the cartridges; all sterile components provided to avoid any field sterilization requirements; no sharps waste;

reduce volume of medical waste; interlocking mechanism to prevent unintended firing.

- **Quick**, which means a speed of more than 600 injections per hour or 10 per minute.
- **Competitively priced** compared to disposable syringes, which the system is intended to replace.

Efforts to re-engineer a safer MUNJI are slow and difficult. **FELTON MEDICAL INTERNATIONAL**, using Russian technology and PATH assistance, is developing the BI-100, a new multi-use nozzle jet injector with a disposable safety cap.

New generation slow-speed DCJIs, are usually filled by the end-user from "off-tool" single or multi-dose vials. Manufacturers include:

- **ANTARES PHARMA'S** Medi-Jector Choice.
- **BIOJECT'S** Biojector 2000 and Vitajet.
- **EQUIDYNE SYSTEMS'** Injex (diabetic and human growth hormone).
- **NATIONAL MEDICAL PRODUCTS'** J-Tip.

Some investigational devices are intended for pre-filling by the manufacturer and may be high-speed:

- **ARADIGM'S** Intra-Ject.
- **AVENTIS PASTEUR'S** Mini-Imojet.
- **PENJET'S** PenJet.

High-Speed DCJIs

DCI is the only company in an advantaged stage for a high-speed DCJI for use in mass campaigns for bioterror defense, pandemic or epidemic response, and global eradication programs. The LectraJet c2 AE is also capable of slow-speed use. DCI has completed laboratory and animal trials to assess injection depth.

- **DCI** also makes the LectraJet HS, which uses auto disabling single-shot disposable cartridges loaded on-site from the vaccine vial. The investigational LectraJet (not the HS) is filled from multi-dose vial "off-tool."
- **BIOJECT** is planning to work on a slow/high-speed DCJI, but has not yet produced working prototypes.

Need for Universal Standards for DCJIs/Cartridges

An ongoing initiative sponsored by WHO and the CDC is looking at ways to achieve common and universal standards for DCJI cartridges. The CDC and **CREARE** are developing manufacturer-pre-filled auto-reconstitution disposable cartridges:

- Nozzle ends with orifice (peelable front seals).
- Lyophilized vaccine cake reconstituted with dilutent.
- Pre-filled dilutent before reconstitution with vaccine.
- After injection, obturator jams to prevent refilling.

The Imule cartridge/vial is being developed for use with **AVENTIS PASTEUR'S** Mini-Imojet, and PATH is contracting with DCI to make a disposable cartridge that can be filled onsite or pre-filled.

MUCOSAL IMMUNIZATION

Mucosal (oral and nasal) vaccines are globally preferred because they have fewer safety problems, are easily delivered, and can stimulate all arms of the immune system, including CTL, ADCC, serum antibodies, and mucosal SigA. Most mucosal vaccines can elicit long-lived immunity (>7 years). Multiple serotypes of live viral or bacterial vaccines delivered mucosally have had a good record, including those for polio, rotavirus, and shigella. Several typhoid vaccines have looked very good in Phase II trials. Despite advances, however, there is diminished immunogenicity of oral vaccines in infants and children living under disadvantaged conditions in developing countries; vaccines don't work as well on these children as they do on children living in the developed world.

Despite the withdrawal in 1999 of **WYETH'S** RotaShield rotavirus vaccine, for the treatment of childhood diarrhea, interest is still high in developing mucosal vaccines, especially for infants and children in developing countries. Advantages include practicality and increased compliance; barriers include some safety issues and lower immunogenicity of some oral vaccines.

GLAXOSMITHKLINE and **MERCK** are thought to be closest to finding a new attenuated rotavirus vaccine with several new genetically engineered attenuated S. Typhi live oral vaccines, including CVD 908-htrA, Ty800, and ZH9.

- **MERCK'S RotaTeq** is in Phase III trials and approval is predicted for 2005 if the trials are successful. The vaccine is based on a bovine strain of rotavirus in order to avoid the adverse affects associated with RotaShield.
- **GLAXOSMITHKLINE'S Rotarix** vaccine is also in Phase III trials and the vaccine may be licensed outside of the U.S. in the coming months. Compared to RotaTeq, Rotarix has a higher incidence of low grade fever as a side effect.

Other advances:

- Big breakthroughs in shigella vaccines include attenuated shigella strains WRSS1, SC602, and CVD 1208S, developed at Walter Reed Army Institute of Research, the Institut Pasteur, and NIH.

Rotavirus Vaccines

Company	Rotavirus vaccine	Status	Concept
Bharat Biotech	116E, I321	Phase I	Monovalent vaccines, human neonatal strain and natural human/bovine reassortant
Biofarm Indonesia	RV3	Phase II	Monovalent vaccine, human neonatal strain
GlaxoSmithKline	Rotarix	Phase III	Monovalent vaccine, symptomatic human rotavirus strain 89-12
Lanshou Institute of Biologic Products	LLR	Licensed in China in 2000	Monovalent vaccine, lamb rotavirus
Merck	RotaTeq	Phase III	Pentavalent vaccine modified WC3-QV to also contain VP7 gene from human serotype G4
Merck	WC3-QV	Phase III	Quadravalent vaccine, human-bovine reassortants, bovine parent strain (WC3) with 3 VP7 and 1 VP4 genes from human strains

TRANSCUTANEOUS IMMUNIZATION (TCI)

Transcutaneous immunization, the delivery of vaccine antigens through intact skin, is considered to be one of the most promising areas of research and development. Possible vaccination applications include Hepatitis B, HIV, HSV, etc. Potential advantages of TCI include:

- Needle-free (no risk of unsafe injections)
- No infectious waste
- Logistically simple
- Minimizes local and systemic reactions
- More predictable absorption (in theory)
- None of the problems associated with oral vaccine barriers (i.e., intake problems, vomiting, problems with food, enzymes, peristalsis, mucosal injury; nor with nasal vaccine barriers such as rhinitis.
- More controlled entry of vaccine/drug
- Better compliance – a more pleasant vaccine experience.

Transdermal Drug Delivery Products

Company	Product	Drug
Boehringer Ingelheim	Catapres TTS	Conidine
GlaxoSmithKline	NicoDerm	Nicotine
Johnson & Johnson/Alza	Testoderm	Testosterone
Nitrates Topical (Systemic)	Transderm Nitro	Nitroglycerine
Novartis	Transderm Scop	Sclopalamine
Novartis	Estraderm	Estradiol
Watson Pharmaceuticals	Oxytrol	Oxybutynin

Patches are the most common form of transdermal delivery. The main problem with this type of direct delivery is limitations in the type of molecules that can cross the skin. Several approaches are being investigated to overcome this problem, including:

Liposomes. New ways of getting substances through the skin include liposomes. These are tiny vesicles, smaller than skin cells, that can carry polar and nonpolar drugs, but they can become trapped in the upper stratum corneum. A new technique transfers some liposomes with proprietary molecular "edge activators," which can squeeze through micropores in the stratum corneum. However, this technique has not been tested yet.

Nanospheres. These use a timed release approach. Some products such as **CHIRON/ POWDERJECT'S** Powderject force molecules through the skin. Solid microparticles are fired through the stratum corneum using a supersonic shock wave of helium.

Microporation. With this new technique tiny areas of the stratum corneum are removed or pierced, allowing access to the underlying epidermis. One patch contains hundreds of solid silicone needles that are coated with an agent, or hollow metal needles filled with a solution. The patch pierces only the stratum corneum, causing no pain, because it is superficial to nerves. **JOHNSON & JOHNSON/ALZA** is one of the area leaders with its Macroflux patch delivery system.

Permeation enhancements. These include alcohol, polyethylene glycol.

Controlled heat-aided drug delivery (CHADD). With this technique a heat-generating patch is placed over the drug delivery patch, keeping temperatures in a narrow range for up to 24 hours and increasing drug permeability.

Proof of immunogenicity

- TCI is able to elicit immune responses to an adjuvant (CT or LT) which are ADP-ribosylating exotoxins.
- TCI elicits immune responses to antigens, but requires co-administered adjuvants.
- Previous exposure to adjuvant doesn't interfere.
- Multiple arms of immune system are activated.

PULMONARY INHALATION OF AEROSOL OR DRIED POWDERS: THE MEASLES MODEL

WHO has launched the Measles Aerosol Product Development Group with the goal of developing and licensing at least one method for respiratory delivery of currently licensed measles vaccinations by 2007. Such devices include small and large volume nebulizers, ultrasonic nebulizers, and other devices, such as metered dose inhalers. Aerosol type methods include liquid aerosol and dry powder. Devices

currently available are nasal spray systems, ultrasonic nebulizers, jet nebulizers, dry powder inhalers, and metered dose inhalers.

Liquid Aerosol	Dry Powder
Multiple patient doses --continuous flow --spacer delivery	Multiple patient doses --separate mouthpiece or mask --space or reservoir
Single dose on demand	Single dose on demand

Field trials of the Mexican Classic Jet nebulizer, conducted by the National Institute of Public Health of Mexico (INSP) show the device to be safe and immunogenic. It costs less than syringes, prevents risks of parenteral administration, and is generally accepted by children/mothers.

Age	Immunogenicity
Infants < 9 months old	>80%
>9 months old and school-aged children	86%-100%
School-aged children	Mucosal and cellular

THE REGULATORY PERSPECTIVE: Fast Track Approval for a Vaccine

The main reasons for accelerated approval of a vaccine include emerging and re-emerging diseases such as SARS, bioterrorism agents such as smallpox and anthrax, the rise in vaccine shortages such as PVC-7 and influenza, and the need for getting new vaccines of public health importance, such as HPV and HIV.

Early and frequent consultation between a vaccine sponsor and the FDA improves quality and efficiency of the drug's review and reduces misunderstandings and the potential for multiple cycles of review, according to the FDA's Dr. Egan.

The vaccine development process includes:

- Pre-IND
  - Development of rationale based on disease pathogenesis
  - Immunogen identification
  - Development of manufacturing process
  - Non-clinical studies
- IND, including clinical studies, additional non-clinical work, scale-up
- Meetings with the FDA
  - Pre-IND meeting
  - Manufacturing product lot
  - Release animal safety and immunogenicity Phase I protocol
  - End of Phase II meeting
  - Efficacy trial protocols
  - Phase I and II data
- Pre-BLA (Biologics License Application)
- Clinical Data Summary – S&D
- Update: product, etc.
- Outline of BLA

Considerations for Vaccine Type

Type of vaccine	Questions
Live attenuated	Is it sufficiently attenuated? Is there potential for reversion? Are there markers for reversion? Is there potential for transmission? What are the consequences of potential transmission?
Inactivated	What is the adequacy of the inactivation process (assays)? Are critical protective antigens/epitopes preserved (assays)? Are potentially deleterious neo-antigens created?
Subunit or recombinant	Have critical protective antigens been included and presented in a manner that induces protective immunity?
Nucleic acid-based	What is the distribution, integration, and persistence of the vector?

Some issues dealing with production of viral vaccines include:

- Source and quality of starting materials
- Characterization of cell substrate
- Characterization of viral seed
- Validation of manufacturing process for removal or inactivation of viruses
- In-process testing
- Release testing of bulk and final products for purity, potency, and safety

Expediting the Review Process

The formal mechanisms for expediting the FDA's review process of vaccines are the same as for most drugs, and include:

- **Fast Track.** This is designed for new drugs intended to treat serious or life threatening conditions or that demonstrate the potential to address unmet medical needs. It is incorporated at the end of the Phase I meeting and allows for a priority review, a rolling review, and an accelerated approval of the product. The FDA review the parts as they become available; the company doesn't have to wait until everything is completed and submitted as an entire package. Instead, the sponsor submits sections: CMC, statistical, pharmatopic, and clinical.

- **Priority Review.** This is a six-month review of the entire BLA. The review clock does not begin until the company has informed the FDA that a complete BLA has been submitted.

- **Accelerated Approval.** This allows the FDA to approve an agent on surrogate endpoints. Phase IV studies are required.

The threat of bioterrorism has resulted in legislation passed in the House and Senate that provides authorization for the use of products not yet approved by the FDA in a public health emergency. In this case, Egan said, "The potential benefits must outweigh the known and potential risks."

Review of IND Clinical Holds

A company developing a vaccine for clinical study in the U.S. must submit an IND to the FDA. The prime objective of the FDA in reviewing an IND is to assure the safety and rights of the subjects, as well as the quality of the proposed clinical investigations. At any time during product development, review of information submitted regarding the product or study conduct may prompt a **clinical hold**. Usually subjects cannot be administered investigational products until the hold is removed.

The FDA presented a poster with the results of a study of clinical hold letters issued by DVRPA during a two-year period for original INDs unrelated to counter-bioterrorism. They found 92% of hold letters stated the IND was placed on clinical hold, in whole or in part, because the IND did not contain sufficient information required to assess the risks to subjects of the proposed studies. In addition, 46% of hold letters stated the IND was placed on clinical hold, in whole or in part, because the subjects would be exposed to an unreasonable and significant risk of illness or injury. Besides insufficient information, the most often cited reasons for clinical hold included risk, design flow, and misleading brochures.

FDA Study Clinical Hold Letters

Issue	Letters cited
Reasons for clinical holds	
Insufficient information	47.0%
Both insufficient information and unreasonable risk	38.0%
Unreasonable risk	3.0%
Both design flow and insufficient information	3.0%
Misleading brochure, insufficient information, and unreasonable risk	3.0%
Design flow	1.5%
Misleading informational brochure	1.5%
Both design flow and unreasonable risk	1.5%
Both misleading brochure and insufficient information	1.5%
Hold letter comment topics	
Clinical	31.8%
Chemical, manufacturing, and controls (CMC)	18.2%
Pre-clinical	6.1%
Pre-clinical and CMC	39.4%
Both pre-clinical and clinical	1.5%
CMC, pre-clinical, and clinical	3.0%

Examples of clinical deficiencies identified in Clinical Hold letters:

- Stopping rule for individuals, cohorts, and the whole study was not approved
- Safety monitoring, including use of a diary card and plans for follow-up care was not described
- Inclusion/exclusion criteria not adequate
- Justification for dose levels not provided
- Endpoints not appropriate for specified Phase or didn't meet objectives
- Toxicity grading scale not provided or some values inappropriate

Having a pre-IND meeting with the FDA helped avoid a hold letter, according to the study results.

Status	Pre-IND meeting	Post-IND meeting
Hold	20%	60%
No hold	80%	40%

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