The Intradermal Immunization meeting organized by Fondation Mérieux was held at “Les Pensieres” Conference Center from April 7 to the 9, 2008 in Veyrier du Lac, France. The meeting brought together foremost international experts from North America & Europe, scientific personalities that have performed private and public research investigation on the subject.

The following report summarizes the information provided during the Intradermal Immunization Meeting based on abstracts and speaker’s lectures, procedure specifics for the research investigation are not detailed in this report.

Meeting reporter Valentina Picot
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Important Notice of Confidentiality: Lectures briefings title and author red color coded in this report are confidential, and cannot be released to any source.
I. Preface

INTRADERMAL IMMUNIZATION: An Alternative Route for Vaccine Administration

The skin is a physical barrier that protects from potential invaders of the external environment such as pathogenic microorganisms. Structured in layers, the skin is divided in the epithelium stratum corneum, the epidermis, and the dermis containing specialized tissues, hair follicles, nerves, blood & lymphatic vessels. Besides other functions the skin is an active immune organ, cells of the innate and adaptive immune system are found in the layers of the skin, such as the antigen presenting Langerhans cells, macrophages and other immunity cells that liberate different types of cytokines such as: interleukins, growth factors, and interferon. The presence of these cell components makes of the skin an excellent immunogenic organ.

Immunization studies have shown that the intradermal route (ID) for vaccine delivery can produce the same or more vigorous immune responses with lower doses of antigens when compared to the intramuscular and subcutaneous routes, and this could have an important impact in the use of vaccination resources. Moreover, in terms of immunogenecity the intradermal route seems to be especially beneficial in the elderly and in persons with weakened immune systems.

Many different technologies have been developed to accurately aim vaccination doses intradermally; these techniques include bifurcated needles, fine-gauge needles, microneedle arrays; as well as, various types of free needle devices as jet injectors, and patches. Novel technologies for ID delivery may simplify the logistics of vaccine administration, avoid needle dangers and overcome other drawbacks facilitating vaccination mass campaigns.

The increased attention for the intradermal route for vaccine administration is also due to the possible advantages over the more common intramuscular (IM) or subcutaneous (SC) routes regarding its dose-sparing ability; a reduced volume of antigen could produce immune responses equivalent to full doses in other tissues. The ID route has been used with great success for Smallpox, Rabies, Tuberculosis, and Yellow Fever vaccines. Other licensed and investigational vaccines usually administered IM or SC have been studied, but with varying degrees of success, including Hepatitis A and B, HIV/AIDS, seasonal and avian Influenza, Measles, Polio, Rabies, and many others.

The Intradermal Immunization meeting organized by Foundation Mérieux welcomed experts from Europe, and North America; divided in four sessions the meeting addressed the topic as follows:
Session I: Basic aspects: Anatomy, historical and clinical aspects, challenges, and immunology.
Session II: Different vaccine delivery technologies for intradermal immunization.
Session III: Proven or promising antigens to be administered by intradermal route.
Session IV: Intradermal vaccination from the perspectives of manufacturing, R&D philanthropy and regulation.

The meeting provided a platform for exchanging information regarding the impact of the intradermal route and of new delivery technologies in vaccination immunity and public health strategies, this by understanding the skin immunology, reviewing clinical trial data and program & regulatory strategies. The meeting’s objectives aimed to: Disseminate information and debated questions on research evaluating current knowledge on ID immunization, foster dialogue among different actors of the scientific and decisional communities involved in vaccinology, and focus the discussions on research to obtain the best vaccine delivery route in view of reducing the volume, the dosage, the number of doses, and its potential impact on production capacity, and the cost.

Fondation Mérieux is grateful to all participants, speakers and to the scientific committee for their cooperation and input in making of this meeting a successful gathering for knowledge sharing with regards of intradermal immunization.
II. Summary of Scientific Agenda Lecture Presentations

1. Session I: Basic Aspects: Anatomy, historical and clinical aspects, challenges and immunology

ARTICLE SESSION I

Through the years vaccine delivery has faced many challenges that range from the vaccine components, to the delivery route & methods, to the logistics & economical related aspects that influence the availability and costs of vaccines in a given population. To face some of these challenges new vaccine delivery routes and methods have been tested through various clinical trials and preclinical studies. It's been claimed that intradermal (ID) delivery may help overcome some of these challenges as the ID route implies, for certain vaccines, smaller dose volumes which reduces vaccine weight, storage capacity, logistics & cold-chain burden, and facilitates transport.

More than 200 articles have been published on transcutaneous immunization. The increased interest in the use of the ID route is based on the fact that the skin is an important immune organ, thus has the potential to enhance vaccine efficacy. Rich in dendritic cells (DC), the skin seems to be a better place than the subcutaneous or muscular tissue to trigger protective immunity with lower doses to produce T-cell responses. If a fraction of the dose can be given via ID and achieve same or higher protective immunity, this could increase supply to a larger number of people and reduce costs.

DCs control primary T cell responses through specific signals to naïve Th cells that determine specific immunity pathways. The skin holds two types of DCs, one is the Langerhans cell and the other is the Dermal dendritic cell (DDC). Each cell type carries a different Toll Like Receptor (TLR) expression, thus have different functions in response to a given antigen. For instance, both cells respond in similar manner to viruses’ antigens; however, in response to whole bacteria the production of cytokines is lower from Langerhans than that of the Dermal DCs. Understanding these cells phenotypes and immunological functions can help develop better technologies to enhance the efficacy of ID vaccination delivery by improving the immune response obtained. Some proposed ways to accomplish this are: The manipulation of the cells TLRs to speed up the activation of the T cell response and / or of the cytokine profiles that affect the DCs maturation, migration and polarization; the aiming of antigens to specific DC surface receptors.

ID vaccination has exhibited different immunization pattern outcomes, for instance excellent to good immunization results have been obtained for Rabies, Influenza and Polio vaccines and middle to poor for Hepatitis B and Measles.

In summary, some known advantages of intradermal vaccination delivery are: Its dose-sparing capacity and direct impact in vaccine logistics and costs (this is specially pertinent for expensive vaccines such as Rabies); the minimal invasiveness causing less systemic adverse events than that of other routes; the possibility of using needle free technologies eliminating the risk of needle stick injuries and reducing the management of biohazard disposal, among other. Some disadvantages of the ID route may include the potential of producing stronger local site reactions than other routes; the unknowns regarding the optimal dose; and that the classical ID injection requires training and skills.
LECTURES BRIEFINGS SESSION I

1.1. **Keynote Lecture: The Potential of Intradermal Delivery of Vaccines to Improve Immunization in Developing Countries.**

*Martin Friede, WHO – Geneve, Switzerland*

The challenges in vaccine delivery go from the factory until the vaccine reaches the dendritic cells; this includes the means of transport which in many countries still very primitive, until vaccine is finally administered to the people at the right time and at the right place to trigger protection immunity.

When looking at the top ten causes of death in children under the age of 5, Measles is just behind Malaria, this besides the fact that for Measles a vaccine exist for a least 40 years with a minimal cost of about 10 cents / dose. This portrait the clear challenge of getting existing vaccines to the people.

**The challenges of global immunization include:**
* Improve the delivery of the vaccines we have: Improve access meaning ease of administration and logistics, safety of administration, cost and capacity.
* Make the vaccines we don’t have: Developing effective vaccines against Malaria, HIV, TB, Hep C, Pandemic flue, Leishmania, etc.

The ultimate ID delivery example was the Smallpox eradication, although the procedure used at the time will not have been allowed nowadays.

How ID delivery can tackle modern immunization problems? Intradermal delivery may simplify transport and storage of vaccines, ID delivery implies small volumes (0.1 ml) which could reduce cold-chain storage volume and weight.

New methods for intradermal delivery may simplify administration, making administration safer even in the hands of health care workers with limited training, for this a reliable device is required such a patch, micro-needles or jet-injectors, this also requires that a safe method for device filling or pre-filled devices.

New methods for intradermal delivery may also simplify safe disposal, some of these devices cannot result in needle stick injury and cannot be reused, so they are risk free disposal; some other have microneedles which require safe disposal procedures; others are patches which may have toxins and require the evaluation of risk if disposal is done in a non biohazard manner.

Intradermal delivery may increase vaccine supply and reduce costs, if a reduce dose can be given and reach good immunity this could increase supply to a larger number of people, and diminish the costs of vaccine which is a major incentive to countries especially for expensive vaccines as Rabies.

Some issues appear though as, whether dose reduction is really due to ID delivery or not?, and the off label use of the vaccines via ID, this unless the manufacturer changes officially the vaccine labeling. Other challenges are related to end-user filling with mono-dose or multi-dose vials; multi-dose vials represent less volume/dose in the cold chain, and are more cost effective for cheaper vaccines; however, the device must be reliable to load 0.1ml into the delivery device. Other concern is how long a multi-dose vial can be opened to avoid wasting vaccine.

Regarding the vaccines we don’t have, it’s believed that intradermal delivery may enable new vaccines taking into account that the skin is rich in dendritic cells. It’s been claimed that the skin is a better site to induce immunity than the muscle because is easier to produce T -cell responses and cross-talk with mucosal surfaces.

This delivery site is especially good for vaccines that produce T -cell immunity as TB, HIV, malaria, among others; ID delivery may achieve this with minimal adjuvant. It has also been suggested that ID delivery could overcome the maternal antibody inhibition allowing earlier immunization to children.
The challenges of the intradermal delivery method is that the method for delivery has an impact on the type of immune response produce, so the delivery method for one vaccine cannot be standard for another one, even if the aim is to administer via ID. The vaccine is closely tied to the delivery system. Also is necessary to take into consideration that most vaccines use as adjuvant alum, however, alum is likely inappropriate for ID delivery, this means there is a need to reformulate existing vaccines in order to make them viable for appropriate ID delivery. When comparing ID delivery to other simplified routes as follows, is easy to observe that ID delivery has many advantages. *Nasal delivery: With adjuvant non-live vaccines can produce adverse neurological events (Bell’s Palsy), for live attenuated influenza: increased hospitalization in <1yr olds>. *Oral delivery: Does not work well with recombinant antigens, much lower immunogenicity. *Aerosol (lung) delivery: Can produce Inflammation at lung site.

Today there are many known unknowns regarding ID delivery and its effect in immunization as:
- Effect of ID delivery method (depth / surface /reliability)
- Tolerability (reactogenicity, role of adjuvants)
- acceptability (scar, pigmentation)
- Duration of immunity
- Immune bias
- Need for reformulation to achieve optimal immunity
- Other factors (skin age, skin type, UV exposure, hydration,...)

Conclusions
ID delivery of vaccines has the potential to be...
- Easier to administer (with ID specific devices).
- Safer to administer (with ID specific devices).
- Cheaper to administer (with reduced doses of vaccine).
- Induce immune responses that are not readily achieved with IM/SC.

Research required on...
- Immunity induced by ID route.
- Challenges to introduction of different ID delivery devices and formats.

1.2. Historical and Clinical Background on Cutaneous Vaccination

Bruce G. Weniger, MD, MPH, Centers for Disease Control and Prevention, Atlanta, USA (Lecture as per CDC disclaimer)

Some of the proven and theoretical advantages of current and future cutaneous vaccination are:
*Minimal invasiveness
Less serious unanticipated adverse events than other routes, some examples are:
Oral - e.g., intussusception (Rotashield®, Wyeth)
Intranasal - e.g., Bell’s palsy (Nasalflu®, Berna)
Intramuscular/Subcutaneous injection - abscess, nerve injury, hematoma

Local adverse reactions easier to monitor and treat.
Less dependent on patient cooperation to administer, this especially applicable when handling children.

*Relatively sure and certain delivery
Exception: improper Mantoux method for classical intradermal (ID) injection.

*Needle-free delivery
Reduce risks and costs of sharps in medical waste disposal stream.
Exception: Mantoux method for classical ID.

*Dose-sparing ability (documented for classical ID)
Enhanced or equivalent immune response for many antigens compared to IM and SC. Protect larger populations with scarce or expensive vaccines.

*Large surface area for simultaneous vaccination of competing antigens.*

Some disadvantages to take into consideration are:
- Difficult to perform Mantoux method of classical ID injection.
- Local reactions from irritating vaccine components, (e.g., some adjuvants).
- High cost of newer patented technologies.

First, a review of the various terms used for delivering antigen into or into the skin. The suggested terminology is as follows:

**Adjectives**
- "**Cutaneous**" – All processes that target any part of the skin for delivery of antigen.
  - Excludes needles or jets passing through to deposit into fat (SC) or muscle (IM)
- "**Intradermal**" (aka "Classical Intradermal") – A type of cutaneous vaccination in which a bolus of liquid is deposited into the dermis to raise a visible bleb.
  - Includes the Mantoux needle method and newer techniques that achieve a similar result.

**Nouns**
- "**Vaccination**" (per Dr. Pasteur to honor Dr. Jenner), the mechanical process of introducing foreign substances into the body to stimulate an immune response
- "**Immunization**" The broad field of manipulating the immune system to confer disease protection, including related programs and policies.

Regarding the Mantoux method it was a simultaneous invention in 1908 by Felix Mendel in Germany and Charles Mantoux in France. It was originally created for TB skin testing and vaccination with a fine-gauge needle, bevel up parallel into skin, the fluid bolus stay below the basement membrane.

The advantages with this method is that uses existing off the shelf vaccines, enhances the immune response and permits dose-sparing; the disadvantages is that requires training and skills, time, represent needle dangers, also can produce local reactions from irritating ingredients.

Regarding jet injectors, this is a method that was once used, was abandoned and then again reconsidered for ID immunization. Jet injectors pressurize liquid via tiny orifices that squirts paths into tissues. This technology was invented in 1860 in France, in 1940 was resurrected to be used for single-use devices for insulin and other drugs, and in 1950 was adapted for high speed vaccination mass campaigns.

For about three decades many institutions have addressed the safety concerns over multi-use-nozzle jet injectors (MUNJs).

There are many new methods in development phases for ID vaccination and example is the Soluvia micro-delivery system, which consist of a 30 gauge needle, OD=0.305mm, projects 1.5 mm, and like this other methods are being developed.

**In relation to the literature ID vaccination demonstrated immunization patterns as follows:**

**Excellent results**
- Rabies (~117, already widely used ID in developing world)

**Good results worth pursuing**
- Influenza (~2 dozen)
- Polio (IPV) (~16)

**Poor to mixed results**
- Hepatitis B (~90)
- Measles (~15)

**No data**
- Polysaccharide vaccines (MEN, PNU, HIB)

Exception: Gotschlich 1972 – good results for MENps-A
Key research questions with regards of the following aspects in relation to ID vaccine delivery are:

**Reactogenicity**
* Alum: irritating aluminum salts (hydroxide, phosphate), used as an adjuvant to boost the action of vaccines.
* Currently in killed and subunit vaccines DTPa, DTPw, DT, HAV, HBV, MENcn-ACYW135, PNUcn-7 and Td vaccines.
* Alum being added to H5N1 INF vaccines for dose-sparing booster effect.
* Many early studies do not meet current standards for assessing safety.
* Will local skin reactions to existing and future adjuvants be tolerable?
* Will these two major dose-sparing strategies – ID route and adjuvantation – be synergistic or antagonistic?
* How tolerable in the skin will be Novartis’ MF-59 and GSK’s “AS” adjuvant family (RTS,S, AS02A, etc.)?

**Immunogenicity**
* Current vaccine formulations differ from antigens previously studied; must repeat studies using current trial standards
* How will current formulations fare when used in targeted populations?

**Polysaccharide vaccines**
* These vaccines are expensive, and are urgently-needed in the developing world.
* Can any be delivered ID in economical reduced doses?

**Study design**
* Many ID studies lack a reduced-dose IM or SC control arm, in addition to the full-dose control.
* Must establish that the ID route, not a flat dose-response curve, made the difference.
* Is the intradermal route really dose sparing?
* Would a reduced dose into the traditional IM or SC compartment work as well as ID?

**Promising methods for cutaneous delivery include different devices that can be classified into:**
- Passive diffusion with or without enhancers
- Mechanical disruption of stratum corneum
- Coated microtines
- Hollow and dissolving microneedle arrays
- Electromagnetic energy
- Sound energy
- Gas mediated kinetic deposition

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**1.3. Intradermal Delivery: The Challenges, The Pros and Cons**
__Ru-Chien Chi, University of Washington, Seattle, USA__

The skin being such an important immune organ has the potential to improve vaccine efficacy. Some of the potential advantages of the ID route are dose sparing during shortage for example with seasonal influenza vaccine, reduce costs as the case of rabies vaccine, and overcome poor-response as in the case of elderly or immunocompromised.

Some disadvantages of intradermal delivery include the limited volume that the skin holds, the optimal dose is not known, the ID injection technique requires skill and time, the risk of needle dangers, and the injection site reactions such as swelling, itching, discoloration.
Following the results of a study performed to compare the effects of the ID versus the IM route *A Randomized, Open-label, Phase II Clinical Trial Comparing Safety Reactogenicity & Immunogenicity of Trivalent Influenza Vaccine by ID or IM Vaccination Among Healthy Elderly*. 

The objectives of this study were to compare the efficacy of influenza vaccine given by intradermal (ID) and intramuscular (IM) route in healthy elderly, evaluate reactogenicity and safety of influenza vaccine given by intradermal (ID) route, at volumes up to 0.3 ml, compare differences in priming by intradermal (ID) and intramuscular (IM) routes.

**The study methods:**
Design: Single center (Seattle VA Hospital), phase II, randomized, open-label clinical trial.
Participants: 258 healthy veterans/partners aged ≥65 yrs.
Intervention: Full dose IM or 60% IM or ID vaccination with trivalent inactivated influenza vaccine (TIV).
Data collection: Pre- and post-vaccination blood specimens and safety diary.
Measurements: HAI antibody titers and adverse event scores.

The study had four groups total, the first group was the control group with an IM dose of 15µg and the volume 0.5 ml, the rest of the groups can be observed in the table as follows.

<table>
<thead>
<tr>
<th>Route</th>
<th>Dosage</th>
<th>Volume</th>
<th>September/ October Visit 1</th>
<th>October/ November Visit 2</th>
<th>November/ December Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM</td>
<td>15 µg</td>
<td>0.5 ml</td>
<td>Blood draw, vaccination</td>
<td>Blood draw, exit study</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>9 µg</td>
<td>0.3 ml</td>
<td>Blood draw, vaccination</td>
<td>Blood draw, standard flu shot IM</td>
<td>Blood draw, exit study</td>
</tr>
<tr>
<td>ID</td>
<td>9 µg</td>
<td>0.3 ml</td>
<td>Blood draw, vaccination</td>
<td>Blood draw, standard flu shot IM</td>
<td>Blood draw, exit study</td>
</tr>
<tr>
<td>ID</td>
<td>4.5 µg twice</td>
<td>0.15 ml</td>
<td>Blood draw, vaccination</td>
<td>Blood draw, standard flu shot IM</td>
<td>Blood draw, exit study</td>
</tr>
</tbody>
</table>

The participants of the study mean age was 70 with about 10% of 85 years or older, mostly caucasian men, the number of participants with chronic conditions such as heart disease, lung disease or diabetes was quite high, however, those participants with unstable conditions were excluded such as for the insulin dependant diabetes patients. Most patients had previously received the flu shot vaccination.
In terms of the severity of adverse events (AE), these were classified in Grade I, II and III, being III the most severe.

See table as follows:

<table>
<thead>
<tr>
<th>Adverse Event AE</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redness</td>
<td>≤8 cm</td>
<td>&gt;8 cm = 15 cm</td>
<td>&gt;15 cm to whole arm</td>
</tr>
<tr>
<td>Swelling</td>
<td>≤8 cm</td>
<td>&gt;8 cm = 15 cm</td>
<td>&gt;15 cm to whole arm</td>
</tr>
<tr>
<td>Arm motion limitation</td>
<td>Easily tolerated</td>
<td>Interferes with normal activities</td>
<td>Interferes with any arm motion</td>
</tr>
<tr>
<td>Fatigue, myalgia, itching, pain</td>
<td>Easily tolerated</td>
<td>Interferes with normal activities</td>
<td>Severe, incapacitating</td>
</tr>
<tr>
<td>Fever</td>
<td>Oral T = 38.0°C &lt; 39°C</td>
<td>Oral T = 39.0°C &lt; 40°C</td>
<td>Oral T = 40.0°C</td>
</tr>
</tbody>
</table>

Intradermal influenza vaccination: Local symptoms:

<table>
<thead>
<tr>
<th></th>
<th>0.5 ml IM, % (n=65)</th>
<th>0.3 ml IM, % (n=64)</th>
<th>0.3 ml ID, % (n=63)</th>
<th>2 X 0.15 ml ID, % (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>14.1</td>
<td>10.9</td>
<td>71.4*</td>
<td>80.0*</td>
</tr>
<tr>
<td>Grade II-III</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Swelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>20.3</td>
<td>6.3</td>
<td>58.7*</td>
<td>67.7*</td>
</tr>
<tr>
<td>Grade II-III</td>
<td>0</td>
<td>0</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Itching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>6.3</td>
<td>7.9</td>
<td>23.8†</td>
<td>29.2*</td>
</tr>
<tr>
<td>Grade II-III</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Local and systemic symptoms of the Intradermal influenza vaccination:

<table>
<thead>
<tr>
<th></th>
<th>0.5 ml IM, % (n=65)</th>
<th>0.3 ml IM, % (n=64)</th>
<th>0.3 ml ID, % (n=63)</th>
<th>2 X 0.15 ml ID, % (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local pain</td>
<td>10.9</td>
<td>17.2</td>
<td>11.1</td>
<td>21.5</td>
</tr>
<tr>
<td>Arm motion limitation</td>
<td>1.6</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>6.1</td>
</tr>
<tr>
<td>Chills</td>
<td>3.2</td>
<td>3.2</td>
<td>1.6</td>
<td>10.7</td>
</tr>
<tr>
<td>Myalgia</td>
<td>11.0</td>
<td>10.9</td>
<td>8.0</td>
<td>23.1</td>
</tr>
<tr>
<td>Headache</td>
<td>15.6</td>
<td>9.4</td>
<td>6.4</td>
<td>21.6</td>
</tr>
<tr>
<td>Nausea</td>
<td>8.3</td>
<td>4.7</td>
<td>3.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Regarding the Titers obtained per group with two different virus strains of the influenza virus. Following a proportion of subjects achieving serum HAI antibody titer =40 against A/Soloman Islands/3/2006 (H1N1)

Proportion of subjects achieving serum HAI antibody titer =40 against A/Wisconsin/67/2005 (H3N2):

In conclusion some of the challenges to ID vaccination

It is important to have studies where the dose of the vaccine is controlled to determine optimal dose, volume, and what the vaccination schedule should be.

There is also need to refine the intradermal technique, and further understanding of the immunology involve in ID vaccination.

1.4. Intradermal Vaccination: Mechanism of Action

Marcel B.M. Teunissen, University of Amsterdam, The Netherlands

The adaptive immune response in the skin, the skin often is exposed to different types of dangers as bugs, chemical agents, etc, the purpose of the dendritic cells (DC) found in the skin is to take up all those antigens to digest them and transport them to the local lymph node to present them to the T cells. Then T cells proliferate and activate to perform their antigen-specific effector’s function.

It's important to take into consideration that the migration of dendritic cells also takes place during homeostasis (in healthy skin), in this case the DC take up autologous antigens (as apoptotic cells). In this situation the DCs are immature; they transport these antigens to the lymph node and activate suppressor T cells for autoregulation.

The situation is different when infection at the skin is present, the antigens are taken by the DCs and in this case they are fully mature, this means that they upregulate all kinds of costimulatory molecules, cytokine production, and when they arrive at the lymph node they activate all kinds of T cells, for example Th1 cells to make Interferon gamma, Th2 cells, Th 17 cells, T citotoxic cells and T regs to shoot down the immune response once danger has disapareed.
DC control primary T cell responses by instructing naïve Th cells through different signals that can be antigenic, activating, or polarizing signals.

**Signal 1:** Ag specific stimulation, **Signal 2:** Co-stimulation such for molecules B7-CD28, **Signal 3:** Polarization (Th1/Th2/Th17), **Signal 4:** Homing directions.

The nature and strength of these signals are determined by the condition of the skin if in homeostasis, infection, or other.

Type 1 immunogenic factors such as viruses, protozoa, and tissue factors (IFN gamma or beta, IL-18) that are presented by mature DC to the Th naïve cells release IL-12, IL-27, IFN beta y gamma, ICAM-1 to activate them into Th1 cells to release IFN gamma.

Type 17 immunogenic factors are bacteria and tissue factors that are presented by mature DC to Th cells to release IL-23, IL-1, IL-6 to activate Th17 to release IL-17.

Type 2 immunogenic factors such as helminthes, and tissue factors that are presented by mature DC to Th naïve cells to activate Th2 cells to release IL-5, IL-13, IL-4.

Regarding the DCs themselves, we can observe two types in the skin, one is the Langerhans cell and the other is the dermal DC. They are two different cells, Langerhans cells have a specific molecule called langerin (CD207) that is specific for the production of the birbeck granules and has a CD1 molecule marker. The dermal DC has CD1b marker and have DC-sign (CD209).

They both have different functions, this is because there is different Toll like Receptor (TLR) expression between Langerhans cells and Dermal DC cells, the different expression of TLRs in each cell make them react different to the antigen in question. Langerhans cells show an impaired production of cytokines in response to whole bacteria compared to Dermal DCs, in the case of viruses both cells respond in relatively equally manner. Although Langerhans cells do not upregulate well in the presence of bacteria they can still phagocyte bacteria, the same as Dermal DCs, also both cells can present antigens to T cells but Langherhans cells are much less efficient than the Dermal DCs, these among other differences.

**From the immunological point of view some ways to enhance the response obtained from the ID vaccination can be achieved by:**

- Enhancement of DC maturation by TLR triggering improving T cell activation.
- Manipulation of DC cytokine profile which is important for polarization.
- Manipulation of skin tissue response cytokines, important for DC migration and maturation and polarization of T cell response.
- Targeting the antigen to DC specific cell surface receptors (improvement of uptake enabling dose reduction / selection of DC subset).

TLR ligands may potential vaccination, the antigen presenting capacity of Langerhans and Dermal DCs can be enhanced when TLR7 or TLR8 ligands are used as adjuvant, this is not the case when using CPG because none of these two cells express TLR9 that is necessary for the recognition of CpG. In the case of CpG a plasmacytoid DC that express TLR9 can be placed for the recognition of the CpG, but this plasmacytoid is not normally found in human skin.

**In summary,**

* DC control the sensitization (the danger signal and tissue derived factors are translated by DC into activation of the appropriate type of effector T cell).
* Epidermis and dermis harbor distinct types of DCs, Langherhans and Dermal DC, which have different phenotypes and functions.
* Manipulation of DC function can improve vaccination (enhancement of maturation / steering polarization). Superiority of either Langerhans or Dermal DC in generation of an immune response is still unknown.
1.5. Immunological Basis of the Efficacy of Intradermal Vaccination
Dominique Kaiserlian, INSERM, Lyon, France

The gold standard for an ideal vaccine will be one that is immunogenic after a single immunization, that has prophylactic and therapeutic efficacy, is suitable for healthy humans (autoimmunity, allergy, neurologic disorders), and has not tolerance on vaccine challenge. The question is how to achieve this?

As is well known epithelial surfaces of mucosa and skin are a common entry route for pathogens and environmental antigens.

In the skin even without the presence of danger there is constant immune tolerance in the presence of autologous antigens (gut flora, dietary antigens, etc.) to vaccinate is necessary to break this tolerance, tolerance that is mediated by regulatory T cells which can prevent the efficacy of vaccination by preventing the priming of protective immunity T cells in the presence of antigens (virus, bacteria, etc).

In the skin what is crucial is the dynamic of dendritic cells (DC) of which there are different populations, Langerhans cells (LC) and Dermal DCs (DDC).

Some types of DC migrate to the lymph node to produce tolerance against self antigens during homeostasis, other mobilized from blood to tissue upon inflammation to produce immune response.

So DCs are known to play an important role in tolerance or immunity, whether a DC plays a role in one of the other seem to depend on the distinct DC subsets, the role of the tissue microenvironment, the adjuvants, and of the route of immunization.

In an effort to understand whether DCs are the right cells to target for immunization, studies have been performed to understand the function of the different subtypes of DCs.

Langerhans cells are not responsible for CD8 T cell priming in vivo, Ag induces LN migration of dermal DC first and much later of LC.

It has also been observed that DDC and LC occupy distinct regions in the lymph node; but more importantly is that when an inflammatory signal is placed in the skin, the LC comes 4 days later than the DDC, meaning that is unlikely the LC primes the T cells.

Using transgenic mouse model of conditional ablation of Langerine+ cells, we found that Langerhans cells (LC), the prototype of immature DC, capture antigen in the epidermis but migrate to the draining lymph node long after dermal DC, colonize distinct areas and are not required for in vivo priming of CD8+ T cell-mediating delayed-type hypersensitivity responses (Kissenpfennig et al. Immunity 2005). Moreover, LC are responsible for CD8+ T cell tolerance to Ags delivered epicutaneously (Gomez et al in preparation). Along these lines, we found that transcutaneous vaccination of human with the live-attenuated measles vaccine ROUVAX using a patch, is unable to activate MV-specific T cells responses, or raise the level of neutralizing MV-specific IgG in serum, but induces MV-specific IgA in saliva (Etchart et al. Vaccine, 2007). This underscores the intriguing possibility that preferential vaccine uptake by LC can induce a negative regulation of systemic T and B cell immunity that favors induction of a mucosal immune response.

The second important finding pertains to the nature of DC that can induce cross-priming of CD8 CTL responses, after intradermal vaccination with a protein antigen delivered via the buccal mucosa or skin. Contrary to the dogma, we found that it is not the resident DC (either LC or dermal DC) but the DC that are recruited at the dermo-epidermal junction that induce the priming of specific CD8+ cytotoxic T cells. These DC originate from blood Gr1+ monocytes attracted at the site of immunization via the CCR6/CCL20 pathway of migration and are responsible for direct cross-presentation of the protein antigen to CD8+ T cells.

Remarkably, components including Toll-like receptor ligands derived from bacteria, virus as well as certain haptens, by increasing CCL20 production in epithelia, promote CCR6-dependent DC recruitment, and are adjuvants for cross-primed CD8 CTL (Leborgne et al. Immunity 2006; Leborgne et al. Medecine Sciences 2007).

These advances in the dynamic and function of mucosal DC provide a rationale for the design of novel mucosal and skin vaccines.
References


2. Session II: Different vaccine delivery technologies for intradermal immunization

ARTICLE SESSION II

Several intradermal vaccine delivery technologies have been developed to overcome the constraints of the classical methods as the Mantoux technique & hypodermic needles, and to improve the extrinsic and intrinsic aspects in the entire scope of vaccination delivery. Technologies range from needle improved technologies as microneedles arrays and microneedle syringes which can be or not prefilled devices; needle free technologies as the transcutaneous patches & related skin permeation enhancers, and various types of jet injectors operated by kinetic gas or air. It is important to keep in mind that the vaccine delivery system has an important impact in the type of immune response triggered, implying that the delivery system used cannot be universal for all vaccines but tailored according to the vaccine that will be administered.

Regarding needle improve technologies, studies for the development of Microneedle technologies showed this system to be advantageous for intradermal vaccine delivery. Coated microneedles carrying antigen enter the skin epithelium to reach the antigen presenting immune cells to set off the pathways for protective immunity; microneedles have proved to trigger intense humoral and cellular immune responses. Some advantages with this system is that is painless, low cost, safe, possible to be self administered, and it’s believed to enhance vaccine efficacy. The coated microneedles have shown to release substances into the skin with ease; a study with fluorescent calcein coated microneedles demonstrated a rapid release of this molecule into the skin after one minute. These results support the use of microneedles for the delivery of vaccines at good dose concentrations. Microneedles are made of stainless steal or silicon; other materials for microneedle making are under study, these are re-absorbable polymers which can dissolve and then release the antigen into the skin. The use of this kind of technology could have an important impact in vaccine delivery safety, and biohazard disposal, among other benefits.

Regarding needle free technologies several studies have been done to evaluate the safety and efficacy of these technologies for ID delivery. In Brazil a phase I clinical trial (Brito et al) was performed to evaluate via 3D ultrasound imaging the impact and efficacy of Jet injectors ID delivery. Two jet injector devices were assessed the Pharmajet and the Antares by positioning the devices in the arm lateral side and ultrasound in the opposite side, imaging was registered at the time of injection. The 3D ultrasound system proved to be a good media to evaluate the devices performance. Taking into account that a good injection inoculation must be produced in the dermal layer, this study results showed that the devices did execute inoculation as expected, with almost inexistent local reactions.

A study done in Australia (Kendall et al), tested the needle free injection Gene Gun (PowderJect), this device technology is based on the ballistic delivery of micro-particles. The device allows micro-particles to enter the skin at 1500 miles per hour, possibly producing collateral cell death. A concern with this technology exists regarding the impact that cell death can have in the skin immunogenic response, to answer this the skin biological differences and the environmental factors must be taken into consideration.

Other novel needle free technologies are the transcutaneous patches; the patches contain a dry formulation of the vaccine antigen, the adjuvant or of the vaccine and adjuvant mix. As with other skin immunization technologies, the patch intents to sparkle immunity by stimulating dendritic cells; vigorous immune responses have been observed with this system. For instance, a phase 2 field study (Glenn et al) of a diarrhea patch demonstrated that this device was immunogenic and provided protection against travelers diarrhea. To enhance the patch functioning, skin permeation enhancers have been created to lightly disrupt the epithelium and facilitate the entrance of the patch formulation into the skin, at the
same time this promotes the hydration of the formulation by skin cells water lose. Some advantages observed with the patches is that are safe, thermostable between 5°C and 25°C eliminating cold chain, could be self-administered, and not serious adverse events related to the vaccine have been noted.

LECTURES BRIEFINGS SESSION II

2.1. Ultrasound Image Analysis of Needle-free Jet Injector Tissue Delivery

Sergio Kodaira, Institute of Radiology of School Hospital Sao Paulo State University, Brazil

Glacus Brito S, Clinical Immunology Department, Sao Paulo State University, Brazil

Brazil is a highly populated country of about 200 million people and a worldwide reference on immunization mass strategies such as Polio, Measles and Rubella. In the past great success was achieved with the MUNJI devices PED-O-JET used in several vaccination campaigns, as to eliminate the Measles in Sao Paulo by vaccinating 10 million children from 1 to 10 years old.

Studies have been developed since 1987 on needle free safety that are WHO referenced. Also studies are being developed for the Disposable syringe Jet Injector (DSJI) technology. A phase I clinical trial was done on ultrasound images of Jet tissue delivery, additional cadaver studies on children under two years old and adults are on the way.

Phase II clinical trials for serological evaluation and adverse events for Chicken pox, MMR, Yellow fever are in progress. All projects are followed by the scientific committee on immunization and the ethics committee.

Two devices are being under study the Pharmajet (left image), and the Antares (right image).

In the study the differences found in technology among the devices are as follows:

Antares Pharma
- Same equipment using different volumes for the Study – 0.2 ml for Intradermal delivery and 0.5 ml for SC delivery.

PharmaJet
- One device with specific spring power for 0.2 ml ID delivery and another device with different spring power for 0.5 ml SC delivery.

A General Electric L-9, with 14 MHZ of frequency was used for this trial and three dimensional techniques were used to evaluate superficial structures; Freehand Technique, Multiplan Reconstruction, Layer by Layer erosion. The two DSJI equipments performance were assessed Antares Pharma and Pharmajet, and a volume of 0.2 ml of saline solution was administered in the right arm for ID delivery and 0.5 ml in the left arm for SC.
A group of 20 health care volunteers were chosen to participate in the study under specific selection criteria, such as: sex, age group, skin color or race, and Body Mass Index Rate (BMI).

The injectors were located in the lateral side of the arm and the ultrasound probe was at the opposite to acquire a real time scanning image of the injection, after the injection another ultrasound image seeing superficial skin was performed.

3D Ultrasound method proved to be very appropriate for the needs of the performance evaluation stage.

The 3D images show the devices inoculation structure on the epidermal layer, on the dermal layer, and in the subcutaneous layer where the performance of the devices was tested.

In the dermal layer is possible to observe a spot of microbubbles

A good inoculation produces distribution only in the dermal layer,

A SC inoculation produces a more spread pattern of echo in the subcutaneous conjunctive stripes, as observed in the following ultrasound image:
Regarding ultrasound images evaluation, it’s possible to conclude that it’s a good method to assess the accuracy of the ID inoculation performance of the different devices, it is fast, inexpensive, provides real time evaluation, it’s harmless, and world wide accessible.

Statistical analyses were applied and showed the comparability of the selection criteria for both device samples. The study showed significant direct correlation on the linear regression of BMI and Dermal thickness p<0,041. Also a significant difference between dermal thickness and the age group was found to be similar between teenagers and adults, from 2.0 to 2.5 mm in teenagers and 1.8 to 2.3 mm in adults, and from 0.17 to 1.2 mm in elderly people. Also according to color skin, white people had significantly more dermal thickness than Asian and or blacks.

Considering local Adverse Events for local trauma evaluation, for Intradermal only one person had little pain at the moment of the injection with the Pharmajet Device. No one had pain at 10 minutes and 24 hours after injection. For erythema and swelling only one person had these signs in a very small spot after 30 minutes of the injection, non relevant effects. For subcutaneous 8/20 (40%) for Antares, and 5/20(25%), only one person had only little pain at the moment of the injection. When asked, all volunteers preferred rather to receive needle free that conventional needles, the needle free injection was very well accepted.

The observed distribution for Intradermal, Pharmajet had 13/20 of 100% of ID distribution, 16/20 for 75% and more and 17/20 (85%) of 50% and more distribution of ID. Antares had 2/20 for 100% distribution, 6/20 for 75% and more and finally 10/20 (50%) of 50% and more of ID delivery. For SC distribution, both devices had 14/20 70% of 50% of distribution and more. Considering that they have received 0.2 ml for ID administration, and Pharmajet had 85% and Antares 50% of 50% and more of ID delivery. From this is considered that both devices have appropriate performance authorizing the next step for a Needle Free serological evaluation for attenuated viral vaccines.

Summary Findings
*Both devices performed consistently according to their intended design.
*3D ultrasound imaging enabled us to capture all relevant information of DSJI tissue delivery.
*The scientific immunization committee adopted this ultrasound technique as a standard method to perform evaluation of new devices.

2.2. Needle Free Injection
Professor Mark Kendall, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia

Some of the disadvantages of using needle devices are the high risk of needle injuries and importantly the issue that many vaccines do not work appropriately with needle devices.

Even though the immunotherapeutic approach of Langerhans cells still a debate, dendritic cells are proven to be important actors in the triggering of immunity acting as antigen presenting cells.

Physical Targeting Methods- Ballistic delivery of micro-particles and micro-nanoprojection array:
Some of the physical targeting methods can be a patch, the needle free jet injectors, micro-needles and the Gene Gun (PowderJect/PowderMed).

In a case study to test the ballistic delivery of micro-particles, the device used was the Powder Ject. The device allows micro-particle flow to hit the skin to allow the particles inside, having the vaccine in microparticle formulation. The particles enter at 1500 miles per hour, this process produces different distribution of cell death, and thus the question that arises is: What is the impact of cell death in the expected immunogenic response of the skin? The answer to this still in research; it is also important to take into consideration the biological variability of the skin (thickness, etc) and that of the environment (temperature humidity etc) which have a strong impact in ballistic penetration.
Regarding the micro-nanoprojection patches, the arrays of projections—on a patch—accurately, efficiently and safely deliver biomolecules not just to specific skin cells, but also to organelles within them. Conceptually, the delivery device is a set of needles (of microscale length with nanoscale tips), coated with a drug substance and applied to the skin as a small patch. The patch is pain-free and needle-free, do not required refrigeration thus eliminating the cold-chain, it is applicable to developing world vaccinations. Besides the nanoprojection array attributes efficiently target cells, small scale projection, delivers biomolecules large and small. The nano projection array method is been proven in-vitro and demonstrated in early clinical trial, one of the challenges found with this method is getting the vaccine dry-coating right and release.

2.3. Evaluation of Microneedles for Intradermal Vaccine Delivery
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1Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, 313 Ferst Drive, Atlanta, GA 30332; 2Division of Clinical Virology, Karolinska Institute at Huddinge University Hospital, Stockholm, Sweden; 3School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, GA 30332

The base of this study is to demonstrate that microneedles for ID vaccine delivery is at least as good as IM delivery, or better. The concept of using coated microneedles is to deliver with this system antigens through the skin epithelium, reaching the underlying cells including the immune cells present in the skin.

Different types of microneedle designs have been used with generally similar dimensions of around 700 micrometers in length.

Some of the potential advantages of using this system of microneedle patch vaccines are: Painless, Safe disposal, Lower vaccine dose, Improve vaccine efficacy, Increase stability according to the formulation used, Low cost, Rapid distribution, Possible self-administration.

For the Metal microneedle fabrication, the material being used is stainless steel sheets cut by infrared laser, and de-burring by electropolishing. The design must commonly used is the so called “Washington monument”. Another type of microneedle is the silicon microneedles fabricated by reactive ion etching.
Silicon Microneedles  Metal Microneedles

The coating of the metal microneedles with the antigen solution (vaccine) encountered initially a problem in the capillarity of the device; when dip coating the microneedles in the solution the Ag solution was being deposited in the needles but also in the underline substrate of the structure.

A redesign of the microneedle patch was done to design a chamber in which the microneedle can be dipped precisely so that the Ag solution goes to the needle accurately, producing a uniform coating on the microneedles only. See bottom picture.

With the chamber system it is possible to achieve reproducible and stable solutions in which Ags or other substances can be coated in the microneedles.
The concentration dependence for the coating and number of microneedles can be observed in the following graphs.

**Drug concentration**

It is observed that the amount coated per needle is essentially independent of the number of needles in an array, as shown in the above graph.

Another important criteria is the ability of the microneedles to release Ags rapidly into the skin, this possibility was demonstrated in the study using fluorescent calcein small molecule to coat the microneedles and after searching into the skin. After one minute the microneedles were empty and the substance observed around the site of the insertion. See bottom picture.
Initial studies to quantify the amount of material inserted into the skin, the efficiency of delivery after insertion into the skin, have been done taking to account the initial loading into the needle and what is left after delivery. Results of this experiment as follows:

Amount deposited inside the skin: 91%
Amount left on the skin surface: 2%
Amount left on the microneedle: 7%

In terms of immune responses, the results obtained in some initial studies with regards of immune responses after immunization with HA DNA-Coated arrays (A) and (B) or OVALBUMIN (C) are as in the following graphs.

For the case of the A panel a single dose may be sufficient for protection, and a subsequent booster results in a 6 fold rise of protection of the immunoglobulin, similar results for panel B. For panel C priming with the OVA result in the 10 fold or more increase and a further increase upon subsequent boosting.

Also it is believed that the physical insertion of the needle into the skin might generate increase in the immune response.

Notably, these immune responses were observed in the absence of any adjuvant. Microneedle delivery also induced binding as well as functional antibodies to viral protein antigens after one or two immunizations of mice. To assess the ability to induce CTL activity by microneedle based immunizations, groups of 4-8 week old female C57BL/6 mice were either immunized via gene gun at a dose of 4 micrograms/mouse or via microneedles at a dose of 8 micrograms. A third non-immunized group of naïve mice served as the negative control.

DNA-coated microneedle rows were manually inserted into trimmed abdominal or back skin and held for 1 min to allow dissolution of coated DNA into the skin. For gene gun-based immunizations, plasmid DNA was linked to 1μm diameter gold particles and used for immunizations. Strong CTL responses to a DNA vaccine encoding the NS4a protein of hepatitis C virus were induced by microneedle delivery, which were comparable to those obtained using gene gun immunization. These results demonstrate that effective concentrations of vaccines can
be delivered to the skin using microneedles, which represent a painless alternative to traditional immunization using hypodermic needles.

When comparing the induction of cellular immune responses using different devices, and the hepatitis C virus, it is observed that relatively similar response was obtained with the Gene gun and the microneedle.

Another approach being tested is the idea of a rapidly dissolving polymer microneedle, a formulation in which the antigen itself is contained within the needle and so when this one dissolves it releases the Ag.

Some advantages of this approach is improved safety thus once the needles are dissolved they do not represent further danger. Some obstacles are: that polymerization is achievable at low temperatures, that the antigen is actually preserved, and that the needles effectively penetrate and dissolve in the skin.

In the following picture is possible to observe how the needle dissolves leaves a space where it was inserted and the substance is around the surrounded tissue.

There are different kinds of polymers that can be used to create this type of bio-microneedle arrays, as the PLGA (poly-glycolic acid).
Some current directions of this project are:

- Determine the protective efficacy of immunization.
- Focus on seasonal and pandemic influenza viruses.
- Determine the breadth of immunity induced by microneedle vaccine delivery.
- Adjuvants to enhance immune responses to microneedle delivery.

Conclusions

- Solid metal microneedles can be effectively coated with alternative vaccine formulations.
- Intradermal delivery of vaccines occurs with high efficiency.
- Microneedle immunization results in strong humoral as well as cellular immune responses.
- Antigen containing microneedles can also be constructed using resorbable polymers.

2.4. Putting Skin Immunization into Practice to Prevent Mucosal Disease.

Gregory M. Glenn, M.D., lecture given by Robert Said, IOMA Corp, Gaithersburg, USA

Transcutaneous immunization done by a relatively new method for vaccine administration which consists of a patch that contains in a dry format an antigen, an adjuvant or a combination of both. The patch’s aim is to target the skin immune system, the immune cells (Langerhans cells) that are found in the epidermis, so that these cells can act as antigen presenting cells in the lymph nodes to trigger the Th1, Th2, etc immune response pathways.

It is important to take into consideration that there is a difference between transcutaneous (TC) and transdermal (TD) immunization; there are more differences than similarities. The main difference is related to the targeting area, in the TC you deliver in the Langerhans cells in the skin epidermis, in the TD in the vascularized dermis. Also there is a misconception that you cannot deliver big molecules through the skin, via TC you can deliver proteins up to 1.5 M daltons, whole virus particles (~200M), via TD more small molecules of 100 to 800 daltons.

In terms of delivery duration, the patch was placed for delivery for about 6 hours via TC, likely it will need less time, TD is likely used for sustained delivery, some patches can be used a whole week. Some factors that could enhance delivery via TC, it is the performing of a mild disruption of the stratum corneum that produces hydration through the process of TEWL (trans-epithelium water lose), this water lose can be used to hydrate the patch formulation. For TD more complex methods such as permeation enhancers have been used.

Regarding the evidence of delivery, for TC it is measure the antibody levels in blood searching for clinical protection; for TD it is measure the levels of drug in the blood and the clinical response in patients.

The skin has really become an attractive, non-invasive route of vaccine products, within the last decades it’s been probably more than 200 scientific papers published on transcutaneous immunization. There are number of Ag bacterial products as the protein toxoids, the glyconjugates, etc; as well viral Ag products as the synthetic peptides conjugates for foot and mouth disease, etc.

At IOMAI focus has been placed on two main transcutaneous technology applications:
Needle free Vaccine patch which is an adjuvant and antigen patch applied alone, and the Immunestimulant (IS) patch which is an adjuvant patch applied after the injectable vaccine.

The adjuvant being used is the LT (heat labile enterotoxin from E. coli) which is known to:
-Activate skin immune cells.
-Enhance immune response to vaccines.
-Demonstrated clinical activity as adjuvant on skin.
-Can be safely delivered via skin; studies have been done in >3200 humans.

In addition to developing the adjuvant, the IOMAI Patch system also includes the following components, to enhance immune response:
-Skin preparation system: It is a device to gently disrupt the stratum corneum, this is a non-event for the patient.
-IOMAI Patch: which can contain the adjuvant only, the antigen only or both mixed; these components are stabilized to very hard conditions of shipping, shelf storage to enhance delivery.

**The comparison of a dry patch vs. lyophilization vaccine presentations:**

<table>
<thead>
<tr>
<th></th>
<th>Lyophilization</th>
<th>IOMAI Patch Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation Additives</strong></td>
<td>Protein stabilizers: Buffer salts, other excipients.</td>
<td>Proprietary formulations.</td>
</tr>
<tr>
<td><strong>Fill container and typical volume</strong></td>
<td>Glass vial (3 ml size), 0.5ml</td>
<td>Patch Matrix (3cm cubic)</td>
</tr>
<tr>
<td><strong>Process Steps</strong></td>
<td>Freezing 1st drying (below Tg of formulation) 2nd drying (shelf temp) Cycle time: 1 to 4 cl</td>
<td>No freezing Moderate drying conditions Cycle time= hours</td>
</tr>
<tr>
<td><strong>Production Mode</strong></td>
<td>Batchwise</td>
<td>Continuous High Throughput, easily scalable.</td>
</tr>
<tr>
<td><strong>Operation environment</strong></td>
<td>Class A</td>
<td>Class C</td>
</tr>
<tr>
<td><strong>Moisture levels of final product</strong></td>
<td>3%</td>
<td>Higher levels, tolerable</td>
</tr>
<tr>
<td><strong>Sterility</strong></td>
<td>Sterile (injectable)</td>
<td>Low bioburden</td>
</tr>
<tr>
<td><strong>Packaging</strong></td>
<td>Vials in cartons</td>
<td>Patchwise in foil packet</td>
</tr>
<tr>
<td><strong>Reconstitution and administration</strong></td>
<td>Diluent addition Mixing by hand Solution withdrawal Injection with needle</td>
<td>Patch applied after skin pre-treatment Hydration by TEWL Patch removal after 6 h</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>Major capital cost of equipment</td>
<td>Low capital cost of equipment</td>
</tr>
</tbody>
</table>

**Regarding some of the applications of the patch, the experience of travelers diarrhea vaccine patch:**

Over the past two years, extensive clinical testing and optimization has allowed the transcutaneous patch to evolve to a late-stage product for ETEC with Phase 2 efficacy. Enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of diarrhea in travelers to endemic areas and in young children in developing countries. Each year, diarrhea afflicts approximately 27 million travelers and 210 million children, causing 380,000 pediatric deaths.

In a recent trial, the feasibility of a travelers’ diarrhea vaccine patch containing heat labile enterotoxin from *E. coli* (LT) was evaluated in 201 travelers to Mexico and Guatemala. Subjects were vaccinated prior to travel with 2 patches 2-3 weeks apart with either 37.5µg of LT or placebo patch. The vaccine was safe and immunogenic and vaccinees (n=59) were protected against moderate/severe (PE 76%, p=0.007) and severe diarrhea (PE=84%, p=0.03). Vaccinees who became ill had shorter diarrheal episodes (0.45 vs. 2.1 days, p<0.001) with fewer loose stools (3.7 vs. 10.5, p<0.001). The combination of LT, a highly immunogenic antigen and a key pathogenic factor from ETEC, and the delivery of this antigen to the dendritic cells in the skin, may explain the robust immunity and protective efficacy seen in this trial. Additionally, both preclinical and clinical investigations have shown that TCI results in mucosal immunity to LT, which may contribute to protection. In addition to the biological rationale for delivering antigen to the skin, the transcutaneous patch lends itself to simple, needle-free application, use of the vaccine outside the cold chain, and has been designed to be suitable for both travelers and use for children in the developing world.

**Summary of Transcutaneous Immunization and Patch Formulation Experience:**

- Delivery of antigen to the skin epidermis can induce robust immune responses.
- Minor disruption of stratum corneum improves efficiency of antigen penetration and skin immunity.
- LT delivered to the skin markedly enhances immune responses to antigens. Fewer doses and dose-sparing are possible.
- LT patches proven safe; >3200 human subjects with no vaccine related SAE.
- Biological products, such as LT and trivalent influenza vaccines have been formulated via Iomai's proprietary dry formulation technology.
- Final patch products exhibited acceptable 5°C and 25°C stability profiles and prove thermostable to temperature excursions. This allows the elimination of cold chain and distribution by mail is possible for self-administration.
- Phase 1 / 2 interim data showed a single 45μg H5N1 vaccine dose coupled with an LT patch achieved greater than 70% seroproteccion level.
- Phase 2 field study showed that dry LT patch was safe and immunogenic and protected travelers against moderate and severe diarrhea from any cause.

2.5. Intradermal Injection Systems for Improving Vaccine Delivery
Philippe E. Laurent, BD Medical Pharmacelutical Systems, Le Pont de Claix, France

In terms of technology classification we can classify microneedle based technology as follows:

Microneedle based

1.5 mm length

Prefilled system product

BD Soluvia™

Filled at time of use product

BD ID µneedle

0.2 to 1 mm length

Filled at time of use products

Nano PASS

Debiotec

Others

The main difference between the two microneedle systems is that with the 1.5 mm length prefilled system exists and this provides many benefits in terms of logistic aspects which will be seen later in this document.

The second family of technologies is transdermal, which has two classifications as seen in the following flow chart, the permeation enhancers and the passive patch.

Transdermal

Permeation enhancers

Electricity based Products

Electroporation

Ultrasound

RF channels

Iontophoresis

Skin disruption Products

Abrader - BD

Macroflux

Sozana

Biovalve

Passive patch

Prefilled Products

IOMAI

In fact the IOMAI product actually also uses the skin disruption system and can be classified under the permeation enhancers category as well.
The third family is the Advance Jet-injectors targeting intradermal, classified as follows:

In summary delivery of vaccines via transcutaneous and intradermal routes using microneedles will decrease the risk of post delivery blood-born diseases and may eliminate the need for trained personnel and management of multi-components medical wastes. Currently various techniques involve the matrix patch, combined patch (IOMAI) with skin abrader or microneedle arrays (Microflux- Alza) and prefilled microinjection system (BD Soluvia®). These delivery systems are prefilled with the vaccine solution, in a ready to use format which contribute in reducing the logistic burden of immunization procedure. In contrast, Jet injectors (Biojector 2000-Bioject; Pharmajet) must be filled with vaccine solution at time of use. Because transdermal delivery through topical application to the intact skin surface requires formulation excipients and epidermis permeation enhancers several methods breaking the skin barriers are under investigation. Alongside these methods three main families are under preclinical and clinical development: i)- electrically-based (iontophoresis, ultrasound, photomechanical waves, electroporation); ii)- velocity-based (Optimized jet injector, pulsed microjets); iii) - others (transfersomes, medical tattoos, skin abrasion, laser radiation, magnetophoresis). For obvious reasons these emerging technologies will not improve the logistic burden associated with vaccine delivery.

In the following table a comparison regarding the previous categorized technologies is made taking into consideration the criteria as follows:

<table>
<thead>
<tr>
<th>Yes = 1</th>
<th>BD Soluvia™ Transdermal passive</th>
<th>Transdermal with SSM</th>
<th>Jet injector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven Clinical Efficacy</td>
<td>3/4</td>
<td>2/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Phase 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Phase 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marketed</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Safety</td>
<td>4/5</td>
<td>4/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Sharp less</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prevention NSI</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No reuse</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prevent dosing error</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prevent counterfeit</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Performance</td>
<td>2/2</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Dose accuracy</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tissue delivery consistency</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
As noted in the above table, the scoring is simple Yes=1 or No=0, for example regarding the clinical aspects when the technology has a successful Phase 1, 2 and 3 trials is a 3 points, when the technology has covered all aspects is 4 points, and so on. Some deductions from analyzing the table: The only system that can prevent dosing error is the prefilled systems, or the importance of the ergonomic features of the devices so that are friendly to use by the end user.

The table key message is that the comparative multifactorial risks/benefits analysis of existing intradermal and transdermal delivery methods favors prefilled microneedle system. The most critical differentiation factors are:

- Dose accuracy, and consistency intradermal delivery
- Ergonomic
- Regulatory pathways
- Manufacturing capacity/capability
- Cost reduction

All these technology and techniques are different in terms of immune response, and biological impacts, in these three aspects can be considered: Tissue injury as a mechanical factor (the physical insertion of the needle does account for the triggering of immune events in the skin), The chronology of immune-competent cells for antigen processing, and the overall biological impacts.
It is important to recall that the skin besides being an immune organ is a mechanical barrier, a sensorial organ, and importantly skin is a microvascular organ. Based on this concept the following categorization diagram:

When comparing the different vaccine delivery processes, the kinetics of each given process have different outcomes, in the Transdermal delivery system the antigen moves from the skin surface towards the boarder membrane, and the antigen crosses the membrane and filtrates in some way the dermis; in this process there is absence of biomechanical tissue stress. The kinetic of this is very important as the cells in contact with the antigen are keratocytes and langerhans cells, and the immune cells as soon as they are priming they influence the generation of the immune dialog.

In the Intradermal delivery (a needle delivery system) the first area touched by the antigen is the area surrounding the capillaries, and the first cells touched by the antigen are dermal dendritic cells that will trigger also the immune responses, in this process there is substantial biomechanical tissue stress.

With the Jet-injector you have the possibility to touch simultaneously both langerhans cells and dermal dendritic cells, and there is substantial biomechanical tissue stress.

In terms of the biological impact on the Neurogenic response of the vaccine delivery methods, we can consider:

- Cutaneous injury causes the release of epidermal factors, mast cell degranulation and depolarization of sensory nerves
- Nerve impulse transmits a nociceptive message from nerve receptors and ending in the dermis to the spinal cord where sensory neuropeptides are released both locally (in dermis) and centrally.
- These neuropeptides plus locally released peptides act upon post-capillary veinules which increase vascular permeability and leads to plasma extravasion and chemotoxis for leukocytes.

Taking the above into account, a deregulation of nerve endings produces inflammatory processes that can have an impact in the outcome of the vaccine delivery method used, and in the immune response triggered.

In summary some key learning to take into consideration regarding the technology delivery used is:

- Chronology of immune cells involvement play a role in forming adaptive immune response
- Tissue injury related to delivery method plays a role through innate immune response and associated pro-inflammatory effects.
• The vaccine delivery methods have an obvious impact on the immunity outcomes.

**Conclusions**
*Comparative multifactorial risks/benefits analysis of existing intradermal and transdermal delivery methods favors prefilled microneedle system.*
*Delivery method and system to achieved antigen delivery in skin directly impact the early phase and deployment of innate and adaptive immune response.*
3. Session III: Proven or Promising Antigens to be Administered by Intradermal Route

ARTICLE SESSION III

The research for promising antigens for intradermal delivery is been a long time quest, special attention has been driven to the development of a HIV vaccine as it represents the main control strategy for this disease epidemics. More than 30 HIV potential vaccines have been evaluated, none eliciting neutralizing antibodies. New research studies are focusing in vaccines that could trigger T-cell immune responses under parameters such as: Recombinant live vectors, DNA vaccines, adjuvanted proteins and peptides, heterologous prime/boost. Intradermal vaccine delivery has been taken into consideration to determine if it can set off T cells CD4 and CD8 immunity. A study published in 2007, entitle “Cellular Immune Responses Induced with Dose-Sparing Intradermal Administration of HIV Vaccine to HIV Uninfected Volunteers in the ANRS VAC16 Trial” (Launay O., et al) assessed the immune responses obtained from a lipopeptide HIV vaccine delivered via ID and via IM routes; no significant adverse events were noted with the ID vaccination, only minor local reactions when compared to the IM route. The study results showed that one fifth of the intradermal route dose induced higher CD8 T cells response than did the IM route, at its turn higher results for the CD4 T cells were induced by the IM route.

Clinical research has also focused in improving the immunity response obtained by currently used vaccines. In the case of Hepatitis B Virus (HBV) infection, which continues to be a concern in dialysis units in developed countries but importantly in developing countries, HBV vaccine trials have been done to test the ID route as a potential way of enhancing immunogenicity in hemodialysis (HD) patients. HD Patients remain at risk of acquiring HBV infection as dialysis is a procedure done by extracorporeal circulation; several preventive strategies are part of the dyalisis protocol to avoid blood-born contamination, the HBV vaccine is one of them. A concern with the HBV vaccine is that patients under long term dialysis treatment trigger low immune responses. Multiple strategies have been studied to face the vaccine immune response drawback, as the administration via IM of double doses, different adjuvants (zinc, levamisole, gamma-interferon, etc), and as mentioned earlier the utilization of the intradermal route. A group of 12 control clinical trials (Frabrizi F., et al) were performed to compare the ID vs. IM routes for HBV vaccine delivery in HD patients. In terms of seroprotection, the results at completion of the vaccine schedule were of 81.5% ID vs. 63.2% IM, and over follow up (data only from 6 out of the 12 trials) of 57.9% ID vs. 62.2% IM; in terms of vaccine doses, higher volumes were administered via IM than via ID (840 mcg ID vs. 1500 mcg IM) making the ID route more cost-effective. Minimal adverse events such as itching, headache were noted with the ID route. The results indicate that the ID route could trigger better immune responses; this could also be linked to the number of vaccine shots administered; however, in general is observed that HD patients respond to the vaccine with low seroprotection titers.

A need for improved seasonal influenza vaccines to face the immune response limitations that the current vaccines encounter in the elderly and adults has lead to the testing of new vaccine dose formulations and new methods for delivery. Using a novel intradermal microinjection delivery system, two influenza randomized controlled phase 2 trials were performed (Saville, et al), one in elderly 60 – 85 years old, and other in healthy adults 18- 58 years old, different vaccine dose presentations were tested in each trial. The trials were done to compare the immunogenicity response obtained from vaccine administration via ID microinjection vs. IM. Each trial main objective respectively was to prove that the immune response elicited via the ID route was higher than that of the IM for the elderly, and to prove that the immune response elicited via ID was not less than that of the IM for the adults. Results demonstrated that both trials complied with the EMEA criteria for immunogenicity regarding seroconversion rate, geometric mean of individual titer rations GMTR, and seroprotection rate, the
adults showed higher responses compared to the elderly in reference to this criteria. The trial results in the elderly indicate that the intradermal route can be more immunogenic than the intramuscular route; the adult's trial showed the intradermal route to be as immunogenic as the intramuscular route. Both trials exhibit only minor local reactions with almost all vaccines.

A successful story of a proven antigen administered via ID is the prophylactic Rabies vaccine. Rabies disease is mainly present in the developing world, primarily in Asia and Africa where accounts for more than 55000 annual deaths, often affected populations live in poor areas and have very low income per capita. Deaths from this disease are unlikely due to vaccine inefficacy but to reduce vaccine availability and high cost; the cost of this vaccine can range from 40 to 50 US. The IM route used to be the vaccine delivery standard for the prophylactic post-exposure (PEP) regimens, but the new regimes via ID have reduced up to 60% to 80% vaccine dose volumes and costs. Rabies vaccine ID delivery has proved to trigger same or more vigorous immunogenicity than the IM, also in immunosuppress patients; furthermore, it’s not inhibited by the Rabies immunoglobulin, produces minor side effects, and potentially reduces the number of medical visits required due to the new ID regimes.

LECTURES BRIEFINGS SESSION III

3.1. Promising Antigens to be Administered by Intradermal Route: HIV
Odile Launay, Cochin-Pasteur Clinical Research Center Institute “CIC de Vaccinologie Cochin-Pasteur”, Cochin Hospital, Assistance Publique-Hopitaux de Paris INSERM – Paris Descartes University

To begin, following some general data regarding HIV global estimates for adult and children 2007 (WHO, UNAIDS).

* People living with HIV ________ 33.2 million [30.5 – 36.1 million]
* New HIV infections in 2007 ______ 2.5 million [1.8 – 4.1 million]
* Deaths due to AIDS in 2007 ______ 2.1 million [1.9 – 2.4 million]

The number of people living with HIV globally is continuously growing, and is expected to join one of the main three causes of death by 2030.
The antiretroviral therapy has reduced suffering and extended the life span on HIV patients, but needs to be taken for life which implies side effects. The control of HIV transmission remains one of the most important public health priorities for the 21st century. The vaccine seems to be the only real prophylactic measure to control the HIV epidemic.

One of the greatest challenges in HIV/AIDS remains to develop a prophylactic vaccine that can prevent transmission. Since phase I vaccine trials, started in 1987, more than 30 vaccine candidates have been evaluated in clinical trials. First generation of trials evaluated envelope-based vaccine such as gp 120, and gp 160 vaccines with the aim to induce neutralizing antibodies. In 2003 the results form the Vaxgen trial of gp 120 recombinant protein was unable to protect against HIV / AIDS.
Many of the vaccine candidates were highly immunogenic but unable to produce neutralizing antibodies.

Second generations of vaccines capable of eliciting protective T cells immunity, T-cell vaccines, are being researched; for this four major approaches have been used:
* Recombinant live vectors
* DNA vaccines
* Adjuvanted proteins and peptides.
*Heterologous prime/boost.

The majority of vaccines candidates have been administered using the IM route; however, the ID route has been studied in both humans and animals to evaluate if the ID route could be better than the IM to stimulate specific HIV immunity, and if the ID route may elicit both CD4 and CD8 T cells for durable protective immunity.


In an animal model using intradermal administration of SIV lipopeptides where multispecific and sustained SIV-specific T-cell responses was triggered in rhesus macaques (Coutsinos et al 2005, FEMS Immunol Med Microbiol 43: 357-366).

Based on the results obtained in the animal model a study named “Cellular Immune Responses Induced with Dose-Sparing Intradermal Administration of HIV Vaccine to HIV Uninfected Volunteers in the ANRS VAC16 Trial” was done to evaluate the safety and cellular immunogenicity for a lipopeptide candidate HIV vaccine. The study results were published in 2007.

The trial objective was to determine whether intradermal versus intramuscular LIPO-4 vaccine injection resulted in significantly different percentages of participants experiencing any grade 2 or higher adverse events, possibly to certainly related to the vaccination, and to evaluate T-cell immune responses.

**The trial methods included:** 68 HIV-negative healthy adult volunteers randomized to receive at weeks 0, 4, and 12 the vaccine to be delivered either: 3 IM doses of 0.5 ml of LIPO-4 vaccine containing 500µg of each peptide (n=35), or 3 ID (Mantoux method) doses of 0.1 ml, containing 100µg of each peptide (n=33). The participants were seen 3 days during two weeks after each vaccination for safety assessment; immune responses were also assessed at week 0 before injection, and then two weeks after each vaccination, then at weeks 24 and 48.

As the trial was held-up by the French authorities, only 44 volunteers received the third vaccine injection.
Regarding the vaccine composition:

Construction of 4 lipopeptides containing HIV-1 peptides convalently linked to Tetanus Toxoid 830-843 as a universal human T-helper peptide.

**Regarding the vaccine composition:**

68 subjects were enrolled in one of the 6 clinical trial locations in France, and were randomly assigned to one of the two treatments arms, 35 received IM injections and 33 ID injections. Their median age was 47 years, all of the 68 subjects received the first two vaccinations; and in 44 only, 22 per arm, received the third immunization. Vaccinations were stopped for 23 volunteers because of severe adverse events occurring in a vaccine trial using another HIV lipopeptide in the USA that resulted in one death casualty, these SAE lead French authorities to stop temporarily the trials using these HIV lipopeptides.

Meta-analysis of lipopeptides trials during that period concluded that lipopeptides vaccine safety was acceptable.

Regarding ID local reactions showed that the ID immunization was well tolerated, not Grade III or IV adverse events were reported, and injections pain was significantly less frequent after ID injection, 27 in ID group vs 80 in the IM group. However, local reactions were more frequent in the ID group than in the IM, all local reactions resolved without sequel. No differences were noted for systemic reactions between the ID or IM groups.

**Immunogenicity Assessment of the Vaccine**

Immune responses were assessed at week 0 before injection, at week 2, 6, 14, 24 and 48. The responses were assessed by the following techniques:

*ELISPOT IFN-γ assay*

12 HIV peptides overlapping the vaccine sequences, known as optimal CD8 T lymphocyte epitopes, were tested in cultured ELISPOT (one step stimulation strategy amplifying the responses). A cell line is assessable if one pool of viral peptides (CMV, EBV, FLU) or PHA is positive. The positive cut-off was > 100 SFC/106 PBMC and 3-fold higher than the background.

*Proliferation assays:* Tetanus Toxin peptide TT830-846 was used in quadruplicates. Stimulation Indexes (SI = 3) were considered as positive.

In the following graph the results of cumulative responses to at least one HIV peptide:
CD8+ T-cell responses (ELISPOT IFN-γ) to HIV peptides

In the results we can observe that the fifth of the dose administered by ID route was at least as immunogenic as the entire group dosed via IM.

In the above graph and in contrary to the results described before, with the Tetanus Toxin higher CD4 T cells results are achieved with the IM than with the ID route.

Conclusions
- Intradermal LIPO-4 administration was well tolerated, requiring 1/5ème of the intramuscular dose to induce similar HIV-specific CD8+ T-cell responses.
- The immunization route influenced which antigen-specific T-cells (CD4+ or CD8+) were induced.
- The intradermal route would be a valid dose-sparing strategy for an AIDS vaccine to induce CD8 T cells.
3.2. Intradermal Vaccination against Hepatitis B Virus (HBV) in Dialysis Population: Recent Evidence

Fabrizio Fabrizi,¹² MD, and Paul Martin,² MD. ¹Division of Nephrology and Dialysis, Maggiore Hospital, IRCCS Foundation, Milano, Italy; ²Center for Liver Diseases, School of Medicine, Miami, Florida.

The spread control of hepatitis B virus (HBV) infection within dialysis units has been one of major success in the management of end-stage renal disease (ESRD). In the last 10 years recent epidemiological surveys have demonstrated a low but not negligible frequency of HBV infection in dialysis facilities of developed countries; however, prevalence and incidence rates of HBV infection remain high within dialysis units of the developing world.

Outbreaks of HBV infection continue to be reported within dialysis units in industrialized world. Some epidemiological surveys that have been done during the last 10 years to determine the prevalence of chronic HBsAg seropositive rate in dialysis patients in the developing world has shown a frequency of 9% in India, 10% in Brazil, 22% in Eastern Europe, among other countries. Some relevant survey clinical trials done in the developed world has shown an important range of results as 0 in United Kingdom, 3% in United States, 5% in France, 7% in Italy, among other countries.

Regarding data about the most important outbreaks of HBV infection on hemodialysis patients have happened in Brazil with 27 patients infected, Japan with 5, and USA with 6.

What this comes down to is that hemodialysis (HD) patients remain at risk of developing blood-borne infections including hepatitis B virus (HBV) as hemodialysis is made by extracorporeal circulation.

The most important procedures to avoid this type of infection for the control of transmission of HBV within dialysis units have been made by several procedures including:

- Screening of blood for HBV
- Standard precautions
- Routine HD precautions
- Isolation of HBsAg positive patients (by staff, rooms, and machines)
- Hepatitis B vaccine

When considering dialysis and the HBV vaccine, there are two important issues in the use of this vaccine in HD patients: Patients on long-term dialysis have a suboptimal response to HBV vaccine:

* The seroprotection rate (i.e., the frequency of responder patients) is lower in dialysis than in general population 50-60% versus >90%.
* After completion of vaccine schedule, the titers of protective antibodies are low and decline logarithmically over time.

In the literature various approaches have been tried in order to increase the responsiveness of dialysis patients to recombinant HBV vaccine:

- The intramuscular administration of double doses.
- The intramuscular administration of multiple doses.
- The intramuscular administration of HBV vaccine plus adjuvants.

An alternative approach is to start vaccination in the early stages of a renal disease, when one could anticipate that the primary immune response still sufficient.
Following the most important agents used in order to improve vaccine response:
The most important adjuvants are: Zinc supplements, Thymopentine, Levamisole, Granulocyte-macrophage colony stimulator factor (GM-CSF), Interleukin-2, Gamma-Interferon.
The recommendation from the CDC (Centers for Disease Control and Prevention) in the USA is the use of 40 mcg recombinant vaccine by intramuscular route at months 0, 1 and 6.

With regards of the use of the ID route for the delivery of HBV vaccine, some authors have administered recombinant vaccine against HBV by intradermal route with encouraging results. Several of the studies were control clinical trials (CCTs) and cohort studies made with recombinant HBV vaccine to dialysis patients, allowing the comparison of the results of ID and the IM routes.

Studies on ID vs IM vaccination against HBV in patients with chronic kidney disease: Meta-analysis of clinical, controlled trials were performed (Fabrizi F., et al. Aliment Pharmacol Ther 2006; 24: 497-506), a total of 12 CCTs were included involving 640 unique patients. 11 of 12 studies were RCTs (prospective, randomized, controlled trials).

Eight (67%) of 12 trials included only chronic HD (hemodialysis) patients, four (33%) of 12 trials included CRF (chronic renal failure) patients, patients on PD (peritoneal dialysis), and HD patients.

Regarding the studies results
Numbers of patients in the studies mentioned above included in the meta-analysis:

<table>
<thead>
<tr>
<th>Patients, n (ID)</th>
<th>Patients, n (IM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang PC</td>
<td>10</td>
</tr>
<tr>
<td>Mettang T</td>
<td>18</td>
</tr>
<tr>
<td>Terribile M</td>
<td>15</td>
</tr>
<tr>
<td>Fabrizi F</td>
<td>25</td>
</tr>
<tr>
<td>Vincent L</td>
<td>20</td>
</tr>
<tr>
<td>Propst T</td>
<td>27</td>
</tr>
<tr>
<td>Vlassopoulos D</td>
<td>13</td>
</tr>
<tr>
<td>Milkowski A</td>
<td>40</td>
</tr>
<tr>
<td>Charest AF</td>
<td>41</td>
</tr>
<tr>
<td>Somboonsilp W</td>
<td>21</td>
</tr>
<tr>
<td>Chau K</td>
<td>27</td>
</tr>
<tr>
<td>Roozbeh J</td>
<td>35</td>
</tr>
<tr>
<td>Overall</td>
<td>292</td>
</tr>
</tbody>
</table>

Cumulative vaccine dose by ID or IM route administered:

<table>
<thead>
<tr>
<th></th>
<th>ID (mcg)</th>
<th>IM (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang PC</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Mettang T</td>
<td>60</td>
<td>160</td>
</tr>
<tr>
<td>Terribile M</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>Fabrizi F</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Vincent L</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Propst T</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>Vlassopoulos D</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Milkowski A</td>
<td>30</td>
<td>160</td>
</tr>
<tr>
<td>Charest AF</td>
<td>60</td>
<td>160</td>
</tr>
<tr>
<td>Somboonsilp W</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>Chau K</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Roozbeh J</td>
<td>60</td>
<td>120</td>
</tr>
</tbody>
</table>
Following the results of the meta-analysis:
For each clinical trial a calculation was done of the OR (Odds Ratio) the risk of failure to respond to HB vaccine by the vaccine route (ID vs. IM). One month after vaccine schedule:

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang PC</td>
<td>0.22</td>
<td>0.04; 1.3</td>
</tr>
<tr>
<td>Mettang T</td>
<td>1.14</td>
<td>0.28; 4.7</td>
</tr>
<tr>
<td>Terribile M</td>
<td>0.25</td>
<td>0.04; 1.6</td>
</tr>
<tr>
<td>Fabrizi F</td>
<td>0.08</td>
<td>0.02; 0.26</td>
</tr>
<tr>
<td>Vincent L</td>
<td>0.24</td>
<td>0.07; 0.80</td>
</tr>
<tr>
<td>Propst T</td>
<td>0.27</td>
<td>0.07; 1.1</td>
</tr>
<tr>
<td>Vlassopoulas D</td>
<td>1.0</td>
<td>1.0; 1.0</td>
</tr>
<tr>
<td>Milkowski A</td>
<td>0.21</td>
<td>0.08; 0.51</td>
</tr>
<tr>
<td>Charest AF</td>
<td>0.29</td>
<td>0.05; 1.8</td>
</tr>
<tr>
<td>Somboonsilp W</td>
<td>1.4</td>
<td>0.22; 8.9</td>
</tr>
<tr>
<td>Chau K</td>
<td>0.39</td>
<td>0.12; 1.2</td>
</tr>
<tr>
<td>Roozbeh J</td>
<td>1.1</td>
<td>0.39; 2.9</td>
</tr>
<tr>
<td>Pooled OR</td>
<td>0.36</td>
<td>0.21; 0.62</td>
</tr>
</tbody>
</table>

At the end of the table is noted the pooled ratio of 0.36 with 95% confidence intervals of 0.2 to 0.6 very small, showing statistical association.

(Odds Ratio) for failure to respond to HB vaccine by vaccine route (ID vs. IM), over follow-up:

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabrizi F</td>
<td>0.16</td>
<td>0.02; 1.5</td>
</tr>
<tr>
<td>Propst T</td>
<td>2.8</td>
<td>0.97; 8.2</td>
</tr>
<tr>
<td>Vlassopoulas D</td>
<td>11.7</td>
<td>0.22; 634</td>
</tr>
<tr>
<td>Charest AF</td>
<td>0.31</td>
<td>0.09; 1.1</td>
</tr>
<tr>
<td>Chau K</td>
<td>1.0</td>
<td>0.32; 3.2</td>
</tr>
<tr>
<td>Roozbeh J</td>
<td>1.6</td>
<td>0.32; 3.2</td>
</tr>
<tr>
<td>Pooled OR</td>
<td>1.1</td>
<td>0.47; 2.5</td>
</tr>
</tbody>
</table>

As observed the pooled OR was 1.1 with 95% confidence intervals of 0.47 to 2.5 showing not statistical association. For the follow up not all the clinical trials gave information, only 6 trials provided data.

In summary the results presented in terms of seroprotection rate after hepatitis B vaccine were as follows:

<table>
<thead>
<tr>
<th></th>
<th>ID vaccine</th>
<th>IM vaccine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>At completion of vaccine schedule</td>
<td>81.5% (238/292)</td>
<td>63.2% (184/291)</td>
<td>0.03</td>
</tr>
<tr>
<td>Over follow-up</td>
<td>57.9% (80/138)</td>
<td>62.2% (76/122)</td>
<td>NS</td>
</tr>
</tbody>
</table>
In the whole group of clinical trials, a significant association was observed between the seroprotection rate and the number of vaccine shots $r=0.59$, $df=22$, $P=0.002$. Moreover, there was no association between seroprotection rate and cumulative vaccine dose $r=0.10$, $df=22$, $P=NS$, this in any of the clinical trials performed. The cumulative HB vaccine dose was greater in patients who received vaccine by IM route: 840 mcg versus 1500 mcg.

No major side-effects were observed in any of the patients included in this analysis. Minor local (soreness, itching) and systemic (arthralgia, general pruritus, headache, nausea, low-grade fever) side-effects were found after ID administration of HB vaccine. These side-effects were not found to be unacceptable by any of the participants.

Our results suggest that the higher efficacy of ID vaccine is related to the larger number of vaccine shots but the ID presentation of HB vaccine is likely per se more immunogenic than other routes of active immunization. The results of our meta-analysis suggest that intradermal vaccine is cost effective compared with IM vaccination.

**Conclusions**

- Dialysis patients remain at risk of HBV infection.
- The seroprotection rate after HBV vaccine is low in dialysis population

### 3.3. Economical Intradermal Rabies Vaccine Application

**F.X Meslin and M. Warrell, WHO, Geneva, Switzerland**

Rabies is a disease that kills people mainly in Asia and Africa, more than 55 000 deaths due to dog Rabies. Even though, the death cases of Rabies is not as high as other diseases such as HIV, Malaria or Tuberculosis, nevertheless, Rabies ranks 10 in the list of communicable disease killers.

Rabies is a viral zoonosis and many carnivores and bat species are hosts of the Rabies virus in nature. Globally, in terms of human disease the dog represent the most important reservoir and transmitter of the virus. Infection of humans usually follows bites by rabid animals and is almost invariably fatal once signs of disease occur. More than 3.3 billion people live in regions where there is risk of rabies.

Rabies has especially significant presentation of cases in India with about 19000 deaths annually. In general the disease affects people that live in rural poor areas in the developing world.

98% of human Rabies deaths come from bites of rabid dogs, many are children in average 40%. Rabies in Africa and Asia accounts for one person dying every 10 minutes.

As an example, the number of rabies prophylactic post-exposure regimens (PEP) administered is estimated at more than 12 million a year in Asia alone, following some numbers per country:

- 8 million in China (600/100000)
- 2.5 million in India (250/100000)
- 700.000 in Vietnam (1000/100000)
- 350.000 in Thailand (550/100000)
- 200.000 in Sri Lanka (1000/100000)
- 80.000 in Bangladesh (60/100000)
- 115.000 in Iran (180/100000)

These regimens where mostly provided by the Intramuscular route (IM).

The vast majority of people dying of rabies have no received prophylactic post-exposure regimens, so deaths are not due to failure of the vaccine or weakness of the treatment, the reason is that people have not access to the vaccine. It is important to recall that Rabies vaccine is used as a therapeutic vaccine.

The reason for the limited access to the Rabies vaccine is that the availability is reduced, specially where the most affected populations are located, this combine with the cost of the vaccine which is many cases is far beyond what people in this regions can afford. For example the cost of the vaccine alone excluding the
Immunoglobulin is about 50 US in Asia and 40 US in Africa which represent about 6% of the per capita gross national income in those areas of the world. The intradermal (ID) Rabies vaccine application has contributed to overcome these limitations, thus the use of this route decreases costs of the PEP prophylaxis. For example the experiences in Vietnam with the PEP showed that the vaccine used IM cost 45 US dollars vs. 20 US dollars when administered ID. The very same vaccine that is used IM is the one used ID.

ID regimens requiring considerably less vaccine than IM regimens are particularly appropriate where vaccine and / or money are in short supply. ID reduces modern vaccine regime and cost by 60% to 80%.

The second contribution of ID delivery besides costs is to help the discontinuation of the production of Rabies vaccine in brain tissue, (sheep or goat brain, among other species).

Other reasons for the use of classical IM Rabies vaccine via the ID route are:
* Equal or higher immunogenicity that IM.
* Strong immune stimulus for immune-compromised persons such HIV patients.
* Reduces the number of visits to the medical center due to the immunogenicity triggered by the vaccine, which indirectly also reduces costs for the patients in transportation etc.
* It is not suppressed by the Rabies immunoglobulin.
* Has minimal side effects.

Many clinical trials have been performed and proved that the ID regimens is as immunogenic as the IM route, trials have been performed since the 1980s. Many trials have been done for dose optimization and for the efficacy of a given PEP regime.

There are different PEP regimens that are now recommended by the WHO, the regime is applied in 8 specific body sites to mobilize the immunological system as much as possible, this regimen known as 804011 goes on a number of weeks. It is 1ml the first day in 8 body sites, 0.4ml in day 7 on 4 sites, 0.1ml in day 28 in 1 site, and 0.1ml in day 90 in 1 site.

Another PEP regime is the 22202 which is distributed 0.2 ml on the first day in 2 sites, the same for the 3 day, for the 7 day and for the 28 day.

WHO encourages carefully designed studies on the feasibility and impact of incorporating modern rabies vaccines to be used via the intra-dermal route in the routine immunization program's of infants and children in communities where rabies is a major health problem.

WHO recommends the use of ID route for PEP Rabies vaccines but not for all vaccines, the approved vaccines must have:
* Potency of at least 2.5 IU per single immunization dose (IM).
* Shown to be immunogenic and efficacious in 0.1 ml injection per ID site against a known reference vaccine using one of the WHO recommended regimens described above.

A list of vaccines that comply with the above are: PVRV (Vrorab, Immovax), HDCV (Rabivac), and PCECV (Rabipur).

ID immunization is getting more widely used in developing countries, Thailand with 50 to 70 thousand PEP per year, Sri Lanka with 100 thousand PEP per year, Philippines with 100 thousand PEP per year and 25 thousand preventive immunizations, also some significant numbers for Vietnam and India.

Although ID immunization is spreading, expansion is not as fast as expected; perhaps because the lack of confidence in low dose regimens, confusing regimens and dosages, need for specific training, vaccines are not in multidose vials (off label use), the technique can be used only in large clinics.
3.4. Influenza Vaccination by Classical Intradermal (ID) Route

Bruce G. Weniger, MD, MPH, Centers for Disease Control and Prevention, Atlanta

Influenza vaccination by classical intradermal (ID) route has gone through strong development since 1937 to nowadays. Many pandemic preparedness research agendas that considered ID delivery for influenza vaccination have been issued by organizations such as the WHO with the “Global pandemic influenza plan to increase vaccine supply” guidelines, an equivalent document was issued by the US Department of Health and Human Services, and also in mass vaccination campaigns.

Regarding the classical intradermal injections (deposit of liquid bolus under basement membrane, creating a visible wheal or bleb) two main methods exist:

* Mantoux method by needle syringe

![Mantoux method](image)

*Jet injectors with intradermal nozzles or spacers. Mutl-use-nozzle jet injectors (MUNJIs).

![Jet injectors](image)

Regarding the future classical ID delivery methods can be found:

*Jet Injector with investigational spacer from Biojector:
Investigational prefilled intradermal syringe from Becton, Dickinson and Co.

Many clinical trials since 1930 were performed on ID delivery of influenza vaccination by needle, and comparing the immunogenicity of delivering the vaccine in various routes such as SC, IM in relation to ID route. Many trials showed the advantages of ID delivery. Some of these trials references are:


A specific example of these trials is that of Halpering W, et al, (AJPH, 1979;69(12):1247-1251) who reported in 1979 about a bivalent swine flu vaccine where different antigens and antigen quantity were compared 0.1 ml ID, and 0.5 ml SC, in a population of 18-24 years, 1 dose. The results can be observed in the following table:

<table>
<thead>
<tr>
<th>Antigen Route and dose</th>
<th>4-fold rise †</th>
<th>HAI ≥40 †</th>
<th>HAI GMT-1 †</th>
<th>Local reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/New Jersey/8/76</td>
<td>ID 0.1 mL</td>
<td>nr</td>
<td>13/64 (20%)*</td>
<td>22.71*</td>
</tr>
<tr>
<td></td>
<td>SC 0.5 mL</td>
<td>nr</td>
<td>12/60 (20%)*</td>
<td>25.19*</td>
</tr>
<tr>
<td>Hsw1Nsw1</td>
<td>A/New Jersey/8/76</td>
<td>ID 0.1 mL</td>
<td>17/31 (55%)*</td>
<td>18/33 (55%)*</td>
</tr>
<tr>
<td></td>
<td>SC 0.5 mL</td>
<td>11/28 (39%)*</td>
<td>11/33 (33%)*</td>
<td>39.12* ditto</td>
</tr>
</tbody>
</table>

* No significant differences between ID and SC / † nr = not reported / † Analyses limited to subjects with pre-vaccine titers <10

In terms of reactogenIcity, it is observed in this study that the ID route has a higher % of local reactions 41% vs. 23% via SC.

Another example of trials for influenza vaccination by needle was the one performed by Phillips et al, who reported in 1970 (JID, 1970;122:26-32) his study on the Lilly 1968 season monovalent: A2/Aichi2/68 strain. In the study he compared ID vs. SC vs. Intranasal (IN) routes with different antigen quantity ID and SC.
Following the results:

<table>
<thead>
<tr>
<th>Route and dose</th>
<th>n</th>
<th>4-fold rise</th>
<th>HAI ≥ 40</th>
<th>HAI GMT-1</th>
<th><em>Significant</em> local reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID 0.1 mL 80 CCA</td>
<td>34</td>
<td>27 (79%)*</td>
<td>18 (53%)</td>
<td>26.7</td>
<td>7/35 (20%)</td>
</tr>
<tr>
<td>SC 0.5 mL 400 CCA</td>
<td>71</td>
<td>63 (89%)*</td>
<td>53 (75%)</td>
<td>46.7</td>
<td>15/80 (19%)</td>
</tr>
<tr>
<td>IN 0.5 mL 400 CCA</td>
<td>33</td>
<td>24 (73%)*</td>
<td>13 (39%)</td>
<td>18.8</td>
<td>1/34 (3%)</td>
</tr>
</tbody>
</table>

*No significant differences

As seen in the above table results, the ID and SC with different antigen quantities resulted in almost similar fold rise of titers (79% and 89% respectively), but the frequency of reaching a titer 40 or higher was not as high with the ID route, and also the GMTs were lower.

Other trials aimed to compare the ID route with the same use for the SC, to understand whether the ID route was really dose-sparing vs. the SC. Following a briefing of some of these trials:

- Adult GMTs: ID 0.1 mL > SC 1.0 mL
- Child GMTs: ID 0.2 mL > SC 0.2 mL

- Adults: “small doses” ID equivalent to “small doses” SC

- Dose ranging, equal ID vs. SC: small antigen mass - ID > SC
- large antigen mass - SC > ID

- 2m-5y children, same dose ID vs. SC: no difference seroconversion or GMTs

A trial performed by Jet injector was done by Davies JW, et al reported in 1969 (Canadian J Public Health 1969:60:104-108) study a Monovalent: A2/Australia/54 (600 CCA/mL) strain, the same antigen quantity compared ID vs. SC, via Jet injection and via needle syringe. The Conclusion of the study is that intradermal is superior to subcutaneous at same dosage. Following the results:

<table>
<thead>
<tr>
<th>Method</th>
<th>Route and dose (n=)</th>
<th>HAI titer 4-fold rise †</th>
<th>Post HAI GMT-1 (post/pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jet Injection</td>
<td>ID 0.1 mL 60 CCA (64)</td>
<td>37 (58%)</td>
<td>114.0 ‡ (3.3x)</td>
</tr>
<tr>
<td>SC 0.1 mL 60 CCA (91)</td>
<td>29 (32%)</td>
<td>77.6 (2.3x)</td>
<td></td>
</tr>
<tr>
<td>SC 0.5 mL 300 CCA (85)</td>
<td>50 (59%) ‡</td>
<td>140.4 ‡ (4.0x)</td>
<td></td>
</tr>
<tr>
<td>Needle-syringe</td>
<td>SC 0.5 mL 300 CCA (77)</td>
<td>31 (40%) ‡</td>
<td>75.8 ‡ (2.5x)</td>
</tr>
</tbody>
</table>

† Serologic results shown are for vaccine strain
‡ p<0.05 for Jet injection vs. needle-syringe
Following a list which account for a minority of studies where the ID was shown to be less immunogenic than SC or uncertain:

- **Boger W, et al, JAMA 1957;165:1687-1689**
- **Sigel M, et al, JAMA 1975;165:1860-1861**

About a quarter of the old trials literature does not support the ID route as being better than SC.

**In terms of the recent clinical trials of ID influenza vaccination by needle:** nothing was much published in the 1980s, main recent studies started to appear in 2000.

**Following a briefing of some of these trials:**

  - Trivalent Fluvirin® (Evans/Chiron), 2003-04 season
  - N=100 patients, age: 19-41 years, 1 dose - 0.1 mL ID vs. 0.5 mL IM
  - Seroprotection was measured by HAI-1 ≥40, among all 3 strains:
    - no difference ID vs. IM
  - A/New Caledonia/20/99 (H1N1) strain results:
    - HAI titer, fold increase, s.c. rate: no difference ID vs. IM
  - A/Panama/2007/99 (H3N2) strain results:
    - HAI titer, fold increase: ID > IM (p<0.001)
  - B/Shangdong/7/97 (like B/Hong Kong) strain results:
    - HAI titer, fold increase: ID > IM (p<0.04)
  - Local reactions: erythema, pruritis, swelling, induration
    - ID > IM (p<0.05)

  - Trivalent, investigational, GSK “AS”-adjuvanted 6 µg/strain (60% sparing)
    - Using the 1.5mm, 30-gauge, BD intradermal syringe (not stated in text)
  - Control: IM full-dose trivalent: Fluzone® 2001-2002 (Aventis), 15 µg/strain
  - N=238, 18-81 years, 0.1 mL ID vs. 0.5 mL IM
  - Subjects 18-60 years
    - All strains, HAI GMT, seroprotection rate ≥40, fold increases
      - no difference ID vs. IM
  - Subjects 61-91 years
    - Generally decreased GMT, all 3 strains, compared to younger group
      - A/Panama/2007/99 (H3N2)
      - GMT HAI titer, seroconversion rate: ID less than IM (p=0.02)
      - Seroprotection rate: no difference ID vs. IM
    - A/New Caledonia/20/99 (H1N1); B strains (Johannesburg, Victoria)
      - GMT HAI titer, sero-conversion/-protection: no difference ID vs. IM
  - Local reactions
    - Induration, redness, swelling: ID > IM
    - Grade 2-3 pain: IM > ID

  - Fluarix® (GSK), 2005-06
    - 0.1 mL ID vs. 0.5 mL IM
    - Children 3-17 years, 1 dose
  - Immunogenicity
    - ID equivalent to IM
  - Reactogenicity
Mild induration ID (25%) > IM (5.4%)
Mild erythema ID (57%) > IM (3.6%)

*NIAID-Sponsored phase I trial
- VTEU at Baylor College of Medicine, S. Patel et al (2005-2006).
- 18-49 year old healthy adults
- Intervals: 0, 1, then 8 months
- Doses 1+2: No of patients=100
- Dose 3: n=77
- H5N1 recombinant A/Vietnam strain
  - ID: 3 µg or 9 µg in 0.1 mL
  - IM: 15 µg or 45 µg in 0.5 mL
- Reactogenicity ID > IM, but both routes “safe and well tolerated”
- Immunogenicity Results:
  * Reduced-dose ID (3 or 9 µg) as poor as routine dose quantity (15 µg) IM.
  * All doses <45 µg not immunogenic, even after 3rd dose at 8 months.

*Auewarakul, et al., Vaccine 2007;25:659-663
- 2006 Intradermal Seasonal INF Study, Thailand
- No of patients=500 (100 IM, 400 ID)
- 20-50 year old healthy adults
- 1 dose during January-March, 2006
- Trivalent, inactivated INF vaccine from Govt. Pharm. Org.
  - Bulk-supplied antigen from Mérieux Biological Products Co., Ltd.
  - 3 µg per strain via ID (0.1 mL); 15 µg per strain via (0.5 mL) control
  - H1N1: A/New Caledonia/20/99-like strain
  - H3N2: A/Wellington/1/2004-like strain
  - B/Shanghai/361/2002-like strain
- Local reactions: ID>IM, but in all cases “mild and transient”
  - Erythema: ID 92% IM 2% (p=0.001)
  - Induration: ID 68% IM 4% (p=0.001)
  - Pruritis: ID 49% IM 6% (p=0.001)
- Systemic reactions: no differences ID vs. IM except:
  - Generalized itchiness: ID 16% IM 5% (p=0.005)
  - Malaise: ID 13% IM 24% (p=0.006)
  - Myalgia: ID 18% IM 30% (p=0.008)
- Immunogenicity results at day 28
  ID < IM in both naive and non-naive subjects. Satisfies CPMP registration requirements.

Recent study trials comparing Equal Doses by ID and IM Routes:
- Adults 18-49 years of age, 3-year-retrospective naives
- Equal doses of Fluzone® INF (2006-2007) by both routes
  - ID: 3 µg, 6 µg (0.1 mL x 2), 9 µg (0.1 mL x 3)
  - IM: 3 µg, 6 µg, 9 µg, 15 µg (full)
- Immune response: ID >= IM at all doses.
- Local reactions: ID > IM

A Jet-injected Intradermal Influenza Vaccination Trial, is being developed at the Dominican Republic, Phase 1 has already been finished and Phase II is starting. Results will be officially published later.
Information on this trial can be found at http://clinicaltrials.gov/ct2/show/NCT00386542.
Some more information on Clinical trials in this field can be found at: WHO International Clinical Trials Registry Platform Search Portal.
3.5. Seasonal Influenza Vaccination by Intradermal Microinjection
Melanie Saville, Sanofi Pasteur, Marcy l’Etoile, France

Each year trivalent inactivated influenza vaccines protect hundreds of millions of individuals worldwide; however, improved vaccines are needed for the elderly to overcome immunosenescence, in healthy younger adults, vaccine uptake remains low.

Widespread annual vaccination of adults needs the use of a vaccine that is convenient, rapid, reliable and safe to administer.
Sanofi Pasteur has developed an intradermal influenza vaccine using BD’s Microinjection system to target the skin’s immune system, this vaccine has two dose presentations: ≥60 years: 15 µg haemagglutinin / dose and the <60 years: 9 µg haemagglutinin / dose. The European marketing authorization application process has been filed for these two formulations.

This article will present data from two randomised controlled phase 2 trials performed on this vaccine.
*The first trial was done on the elderly under the following criteria:
The trial was done on medically-stable elderly in Australia & New Zealand, 60–85 years old, vaccinated ID or IM in 2006 with 15 µg H.A/strain/dose.
Primary Objective: To demonstrate whether immunogenicity of ID vaccination was superior to IM, for this it was looked that for each strain non-inferiority was reached, then, if ID was non-inferior to IM, superiority was tested.

*Second trial was performed in healthy adults as follows:
The trial was done in healthy adults from 18–58 years of Belgium, Germany, Switzerland, vaccinated ID or IM twice: 2005 & 2006 with 9 µg HA/strain/dose.
Primary Objective: To demonstrate whether immunogenicity of ID vaccination was non-inferior to IM.

*In terms of endpoints:
Immunogenicity was assessed using the haemaglutination inhibition (HI) which is the common assay to be used for influenza; taking blood samples on day 0 and day 21 after vaccination.
The primary end point was to look at the geometric mean titre (GMT) by day 21 post-vaccination.
The secondary endpoint was to also evaluate on day 21 the Seroprotection rate, on days 0-21 Seroconversion rate & on days 0-21 the GMTR (Geometric Mean Titre Ratio).
In terms of safety in both clinical trials were looked in days 0-7 for solicited injection site and systemic reactions; and in days 0-21 for unsolicited Adverse Events (AE) and for Serious Adverse Events (SAE) for the duration of the trial.
For both trials the control vaccine used was the Vaxigrip® (15µg HA/strain/dose).
Following just a brief recall regarding the immunogenicity criteria defined by EMEA:

<table>
<thead>
<tr>
<th>Immunogenicity criteria defined by EMEA</th>
<th>Required result for adults &lt;60 years</th>
<th>Required result for adults &gt;60 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of seroconversion or significant titer increase (% with D0 titer &lt;10 and D21 titer =40 dil-1 or with D0 titer =10 dil-1 and =4-fold titer increase on D21)</td>
<td>&gt;40%</td>
<td>&gt;30%</td>
</tr>
<tr>
<td>GMTR: Geometric mean of individual titer ratios (D21/D0)</td>
<td>&gt;2.5</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Seroprotection rate (% with titer =40 dil-1 on D21)</td>
<td>&gt;70%</td>
<td>&gt;60%</td>
</tr>
</tbody>
</table>

As we can observe the criteria for acceptance in adults is higher than for the elderly. For vaccines they need to meet at least one of these three EMEA criteria to have the file accepted.

Regarding the trial methods, the microinjection system developed from BD (Becton Dickinson; Franklin Lakes, N.J., USA) is a pre-filled ready to use syringe featuring:

An integral 30G micro-needle protrudes 1.5mm from the proximal end of the glass syringe. A glass syringe as proprietary vaccine container which corresponds to skin anatomical requirements for fluid delivery in papillary and reticular dermis, and a needle shielding system that conveniently protects the needle after injection; reducing the risk of inadvertent needle-stick injury. This system provides safe, reliable, rapid and reproducible injection of 100 µl into the dermis.

Trial Results:
Participants Flow Chart

In the elderly trial the enrolled subjects were randomized in three groups, for the purpose of this article focus will be placed in the ID15µg: 370 and the IM15µg: 368.
For the adult trial it’s observe that for the second year further randomization took place, subjects receive either ID or IM, subjects not only repeat vaccination but also inter-changeability between IM and ID vaccination.

**Immunogenicity Results: Elderly 15µg ID**
Significantly higher GMTs for the ID vaccination compared to the IM against all strains, meeting the primary objective of the study. The three EMEA criteria (seroprotection, seroconversion and GMTR), earlier described in this document was met; the higher immune responses were in terms of EMEA criteria for all three strains.

**Immunogenicity Results: Adults Trial <60 (9µgID)**
Immune responses satisfied all EMEA criteria for each strain. The GMTs obtained via ID were equivalent (non-inferior) to IM.
When looking at the two trials it is observed that the response obtained for the adults is much higher than that of the elderly.

In general, for the ID vaccination all of the three previously described EMEA criteria (Seroprotection, Seroconversion, GMTR) were met, for each of the strains; this criteria was met for the elderly and adult trials.

Assessing Safety
ID and IM vaccines had comparable safety profiles in both adults and elderly adults:
* Systemic reactogenicity was comparable between ID and IM groups: Reactions were mainly mild, started within 3 days of vaccination, lasted for 3 days or less, and were most commonly headache, malaise, and myalgia. Injection site pain was comparable between ID and IM two groups; other injection site reaction was mainly erythema, more frequently observed with ID than with IM.

In adults, after the second annual vaccination in 2006: No difference in reactogenicity was observed in 2006 vs 2005; also the first ID vaccination in 2005 did not result in higher reactogenicity after either IM or ID vaccination in 2006.

Conclusions
Phase 2 trial (Elderly & Adults) results showed that:
* Intradermal vaccination can be used to elicit higher immune responses against seasonal influenza in the elderly, at a dosage of 15 µg HA/strain.
* Intradermal vaccination provides an alternative to intramuscular vaccination, at a dosage of 9 µg HA/strain.
* The safety profile of intradermal influenza vaccination is comparable with that of conventional intramuscular vaccination. After two annual vaccinations in adults & after one vaccination in the elderly
* Almost all vaccines display minor transient reactions at the point of injection.

Using a simple and reliable intradermal microinjection system, Sanofi pasteur has developed a new seasonal influenza vaccine, targeting:
* Superior immunogenicity for the elderly, which is expected to translate to improved protection against influenza.
* An alternative to intramuscular vaccination to favour increased uptake among healthy adults.

3.6. Intradermal Administration of Fractional IPV Doses
Roland Sutter, World Health Organization, Geneva, Switzerland

Since the World Health Assembly resolved in 1988 to eradicate poliomyelitis by the year 2000, substantial progress towards this goal has been made. The number of polio-endemic countries decreased from >125 to 4, and the number of reported polio cases declined by >99%. During 2007, the progress has further accelerated in the most difficult area of Northern India, where the wild type 1 poliovirus incidence has reached a historical low.

The objective of the polio post-eradication planning strategy of the WHO is to minimize the risk of paralytic disease due to poliovirus among current and future generations of children, taking into consideration that there are no risk-free options.

Some of the original options for longterm polio immunization policy are: To stop all polio immunization, to introduce new polio vaccines, to stop all OPV and limited use of IPV, and to replace all OPV with IPV. The last two options are the ones currently pursue.
The following table shows the risks of polio vaccine after eradication with the continued use of OPV (Oral Poliovirus Vaccine):

<table>
<thead>
<tr>
<th>Risk</th>
<th>Frequency to date</th>
<th>Annual burden</th>
<th>Evolution over time</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAPP</td>
<td>2-4/m birth cohort</td>
<td>250-500</td>
<td>stable</td>
</tr>
<tr>
<td>iVDPV</td>
<td>39 identified (since 1962)</td>
<td>~1</td>
<td>decreases</td>
</tr>
<tr>
<td>cVDPV</td>
<td>0-3* per year</td>
<td>~20</td>
<td>increases</td>
</tr>
</tbody>
</table>

| IPV sites    | 1 accident (1990s) | <1           | decreases           |
| Lab accident | 1 investigation    | NK           | decreases           |
| Deliberate   | 0                  | NK           | unknown             |

*based on current understanding

VAPP: Vaccine Associated Paralytic Poliomyelitis.
IVDPV: Chronic Excreters of Polio Virus among immune deficient persons.
cVDPV: Circulating vaccine-derived polioviruses.

After the eradication of wild poliovirus the use of oral poliovirus vaccine (OPV) must be discontinued because of the well-characterized risk of vaccine-associated paralytic poliomyelitis (VAPP) and the likely emergence of circulating vaccine-derived polioviruses (cVDPVs). We will never be saved from Polio as long as the use of the oral life virus vaccine continues.

After interruption of wild poliovirus, continue use of OPV would compromise the goal of a polio-free world conclusion stated at the Expert Consultation on Vaccine-derived Polioviruses (VDPVs), Sept 2003, Geneva.

The Advisory Committee on Polio Eradication (ACPE) in 2004 created the following Policy Directive: After interruption of wild type poliovirus, continued OPV use would be incompatible with eradication. Regarding Timing it states: Cessation of routine OPV must occur while population immunity & surveillance sensitivity are high.

Pre-requisites for OPV cessation:
*Wild virus certification & containment.
*Global surveillance & notification.
*mOPv stockpile & response.
*Affordable IPV & use in PV-retaining areas.
*Synchronization of OPV cessation.
*Containment of Sabin virus.

In countries that retain polio virus after polio eradication need to have an IPV policy and achieve high coverage.

In terms of the WHO Program of Work
The program is based on the safe for production in developing countries and affordable IPV, for this the program is working on:

Containment Guidelines that will include: Temporary modification of 2nd safeguards for developing countries for the production of S-IPV not the wild type production. (NPEV, sewage, IPV coverage).

Schedules for routine for IPV use in tropical areas that will appropriate and optimal for developing countries, these studies have been done in countries where there is not OPV virus circulating at the time, as in Puerto Rico(1), in Cuba(2) OPV only appears twice a year. (1 J Infect Dis 2007;195:12-20; 2 N Engl J Med 2007;356:1536-1544).

Dose Reduction: Based on two studies that where performed in Cuba & Oman which showed dose reduction by the ID administration of 1/5 fractional IPV dose.
**Schedule & Dose Reduction**: The program expects that the initial studies data will provide the bases to go on schedule and dose reduction studies; 2-dose fractional schedule (DTP3 & measles) – planned for 2009 if fractional dose studies are successful.

**Adjuvants**
Evaluate adjuvants for antigen reduction (S-IPV project, FDA, others).

**Alternate seed strain for IPV production** (Sabin & others)
May permit production of IPV in developing countries after OPV cessation (S-IPV project, NIBSC & CDC).

**Needle-free device to administer IPV intradermally**
Easier administration of fractional doses & can be used by volunteers (IVR, manufacturer of device).

**Optimize production processes**
Increase cell density in bioreactors; suspended cells (e.g. Vero cells), and alternative inactivation agents (β-propiolactone, etc.).

---

**The Intradermal Administration of IPV Program of Work**

**The objective of this program**: It’s to provide the option for IPV use to decision makers in lower middle and low income countries.

**The rationale:**
For routine use: To make IPV potentially affordable to lower-middle and low income countries. It is important to realize that the combination of IPV vaccines is expensive.
For campaign use: ID delivery could offer important advantages as stretching limited supplies of IPV, lower the expenses, and facilitate the administration of IPV by volunteers in large-scale campaigns.

The lines of interest for these studies are to reduce schedule and fractional doses.

**Progress Up to date from clinical trials with ID delivery of IPV**
The following is very preliminary data on these studies:

*Study overview of the clinical trials with fractional IPV doses*: Both studies used very similar protocols with the following criteria summary.

- **Cuba**
  - Mothers are contacted during the last antenatal visit.
  - IPV at 6, 10, 14 weeks.
  - No concurrent vaccines.
  - Blood collected at birth, 6, 10, 14 and 18 weeks (5 specimens of blood).
  - Results end of April 2008.
  - Project in collaboration with the Ministries of Health, PKI, Camaguey Province, SSI, Bioject & WHO.

- **Oman**
  - Mothers are contacted during the last antenatal visit.
  - IPV at 2, 4, and 6 months.
  - Concurrent vaccine: Penta (DTP-Hib-Hep B) by GSK left tight
  - Blood collected at birth, 2, 4, 6 and 7 months (5 specimens of blood)
  - mOPV1 challenge at 7 months.
  - Stool at 7 months and 7 months +7 days.
  - Results mid-2008.
  - Project in collaboration with the Ministries of Health, CDC, RIVM, PATH, Bioject, WHO.

The tool being used for the administration of the intradermal vaccines is the Biojector® B2000, licensed in USA and EU for intramuscular and subcutaneous injection (but not for intradermal administration).
*Doses: Efficacy Endpoints of the clinical trials with fractional IPV doses*

- Cuba
  - Efficacy endpoints:
    - Seroconversion between birth to 18 weeks.
    - Seroconversion after each dose.
  - Laboratory testing:
    - Neutralization
  - Laboratories:
    - IPK in Havana

- Oman
  - Efficacy endpoints:
    - Seroconversion between birth to 7 months.
    - Seroconversion after each dose.
    - Reduction in excretion of poliovirus type 1 after mOPV1 challenge.
  - Laboratory testing:
    - Neutralization, IgA and IgE ELISA
    - Viral isolation.
  - Laboratories:
    - CDC in Atlanta; RIVM in Netherlands.

*Adverse Event Monitoring of the clinical trials with fractional IPV doses*

- Cuba
  - According to the protocol, 30 minute observation after each IPV dose and a home visit after 24 hours, however, in Cuba it is required a 60 minute observation time and home visits at 24, 48, 72 hours, and at 7 days
  - There is no concurrent medication.

- Oman
  - According to the protocol, 30 minute observation after each IPV dose and a home visit after 24 hours.
  - Concurrent medication: Paracetamol 50 mg/8 hours/3 days.

*Doses: Parental preference of the clinical trials with fractional IPV doses*

- Cuba & Oman
  - Parents demanded that their child be enrolled in study because of ID delivery.
  - Questionnaire of ID vaccinated subjects: Parents expressed overwhelming preference for ID delivery.
  - Reason: "baby does not cry".

Following some results preliminary data on the Cuba & Oman studies

*Trial Cuba*

Two-steps randomised controlled open label clinical trial:
Step I: Safety assessment of intradermal administration of IPV in infants included during the first two weeks of the trial.
Step II: After step I results, inclusion of all the planned volunteers for the trial.

In terms of Safety Results
Step I: 63 out 72 eligible subjects were enrolled in the first two weeks. No differences between both groups were observed in terms of adverse events.

**Overall results**
1215 doses were administrated about half were given ID.
No serious adverse events, no severe adverse events related to study procedures were reported.

**Trial Oman, 2007**

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<th>Salalah</th>
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<th>Sur</th>
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<tbody>
<tr>
<td>Enrolment target</td>
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<td>105</td>
<td>65</td>
<td>85</td>
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<td>6</td>
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<td>Completed study</td>
<td>142</td>
<td>105</td>
<td>59</td>
<td>82</td>
<td>388 (97%)</td>
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</tbody>
</table>

**Conclusions**
Two clinical studies one in the laboratory analysis phase (Oman), the other in the data analysis phase (Cuba).

**Preliminary conclusions:**
*ID delivery of fractional IPV with Biojector®2000 in very young infants (from 6 weeks of age) appears to be safe (~1,200 doses administered ID).
*No moderate or serious adverse events were reported. 
*Parents by questionnaire (health providers anecdotally) expressed strong preference for ID delivery.
*Immunogenicity results are pending
Session IV: Intradermal Vaccination from the Perspectives of Manufacturing, R&D Philanthropy and Regulation

ARTICLE SESSION IV

The different players in vaccine development and vaccination implementation such as industry, government, regulatory authorities, and other related organizations are continuously in the look for better approaches to respond to vaccination needs, and to punctually address all aspects that regulate the vaccine environment.

R&D is constantly adapting to comply with higher criteria standards elicited by local & international regulatory authorities, besides the inherent challenges of research. This is indeed the case in the development of new intradermal vaccine delivery technologies. An efficient, secure and reproducible system for intradermal immunization needs to comply with narrow criteria to accurately respond to the ID vaccination delivery needs. Featuring one of these technologies is the microinjection pre-filled system developed by Sanofi Pasteur and Becton Dickinson which consists of a glass syringe with a 30G micro-needle that protrudes 1.5mm from its proximal end, and a needle protection system post-injection to avoid needle stick injuries, this technology enables the consistent application into the dermis of 100 µl. The development of this technology needed to overcome various industrial challenges such all the aspects in the precise manufacturing of a syringe with such small needle criteria, and the evaluation of small volumes filling technologies understanding the vaccine density, viscosity, foam formation, etc.

Another example of intradermal delivery research projects are the ones undertaken by the PATH organization, their portfolio include several projects to evaluate intradermal delivery vaccines and technologies as the: Elderly influenza intradermal study, microneedle feasibility project, Rabies intradermal study and needle free injector project. In general the projects aim to determine and evaluate the efficacy, safety and viability of a given technology, the vaccination response and its applicability for immunizations in the developing world. For instance the needle free injector (NFI) project, is a 4 year project with target date of 2011 that intents to unveil the question: Are NFIs appropriate technology for developing countries immunization programs? To answer it, the project activities include the performing of clinical trials and the harmonizing of regulatory guidelines for the entire field of NFI devices. NFI project will be guided by an expert’s scientific advisory group to provide tuition and feedback in the different scientific, regulatory and technical aspects of the project. A pilot introduction study will take place in Brazil and the projects expect to achieve the WHO pre-qualification.

Although the movement towards the use of vaccines via the intradermal route is constantly growing, regulatory constraints need to be face to legitimize the use of current and new vaccines this via. It is important to keep in mind that the new application via ID of a current vaccine needs to be approved by competent regulatory agencies to avoid off label use, only Rabies and Tuberculosis vaccines have been officially approved for ID delivery. New ID regimens of current vaccines make of them a new vaccine, as indicated by Dr. Nakayama regulatory affairs expert during the intradermal immunization meeting. Some challenges facing intradermal delivery is that most vaccines do not have this route as standard application, meaning that all potential vaccines that are not currently applied via ID need to go through the entire scope of regulations requirements to be on the market for the ID use. Other drawbacks are that ID delivery still lacks on sufficient clinical evidence data to answer many unknowns as what is the optimal dose, the adverse event profile to ensure safety guidelines, among other. In general, the utilization of the ID route for immunization delivery must take into account the regulatory and legal requirements regarding clinical evidence and product approval processes, the ethical considerations regarding the patient’s rights during clinical trials, beside other considerations.
4.1. New System for Intradermal Vaccination (Industrial Challenges)

Antoine Alarcon, Sanofi Pasteur, Lyon, France

A new system for intradermal vaccination should be developed to meet the following requirements:
- A ready to use system.
- To inject a vaccine in the intradermal layer of the skin - the right place.
- With a very low and accurate volume (0.1ml) – the right dose.
- A convenient, easy to use and safe system which avoids needle stick injuries after injection.

Taking the above criteria into consideration a simple but sophisticated Micro-injection system was designed jointly between Sanofi Pasteur and Becton, Dickinson. The system is fairly simple; a glass syringe with a special tip with a small needle, all is well covered by a needle shielding system. This system allows performing the ID delivery without the Mantoux technique.

To achieve the above describe Microinjection system, a development mapping was follow:
First, the functional and technical agreement was followed without having a clear idea of what the future system will be like; then marketing surveys, design studies; characterization of the system (materials, assembly etc); characterization of the ID syringe; drug characterization; end user instructions for use; clinical trials, tolerance of the material, and all other aspects for the device development were performed to comply upon health and regulatory authorities.

The development of this system represented some industrial challenges and constraints for both developing companies:
For Becton, Dickinson:
The challenge of manufacturing a new glass syringe (a new tip, the assembly of a microneedle with a very high accuracy, and a very low dead volume <10µl), etc.

For Sanofi Pasteur:
The use of new technologies as: New filling technologies and working methods for low volumes (high accuracy at large scale with high speed of 24000 strokes/hour), new electronic inspection technology developed for small volumes, dedicated automatic modules for the assembly of the syringe and the system and new packaging lines. The time to market still the same regardless of additional operations in the bulk and F&P processes.

Examples of some of the investigations performed to face different challenges in the development process:
* The investigation and assessment on vaccine behavior depends on: Density, viscosity, surface tension, and foam formation, to avoid problems of such in the production of the device. These criteria needed to be analyzed to ensure consistency in the large scale manufacturing.

* Investigation on various filling technologies for a consistent accuracy of the industrial filling equipment at high speed / large scale, the filling process also generated new working methods.

Conclusions
Addressing all these challenges allowed the better understanding of the process required for the production of this system and the limits of the current processes. Then to perform improvements of the current processes, to better know the behavior of the product, and to have an overview of the maturity of the available technologies.
4.2. PATH’s Intradermal Delivery Research Projects

Darin Zehrung, PATH, Seattle, USA

The mission of PATH is to improve the health of people around the world by advancing technologies, strengthening health systems and encouraging healthy behaviours, with programs in 65 countries. The PATH’s Program areas are: Maternal and Child Health and Nutrition, Reproductive Health, Health Technologies, Emerging and Epidemic Diseases, Vaccines and Immunizations.

In terms of Intradermal Delivery the following projects have been undertaken:

* Elderly Influenza Intradermal Study - (Seattle VA Hospital – Dr. Ru-Chien Chi).
* Microneedle Feasibility Project.
* Rabies Intradermal Study.
* Needle-Free Injector Project.

-In terms of the Microneedle feasibility project: This is a 2 year project (2008 – 2009) from the PATH’s Health Innovation Portfolio, this program portfolio intent to catalyze research from within PATH or other partner institutions on technologies that could be applied in developing countries. This portfolio has placed special interest in ID delivery technologies and how they can be beneficial in developing countries. The projects is built upon the 2007 PATH Microneedle Landscape Analysis.

The project objectives:

* Determine initial technical feasibility of microneedle delivery for certain vaccines.
* Evolve the economic value proposition for microneedles in developing country immunization contexts.
* Identify the areas for which basic research needs to be further pursued.

Some of the microneedle feasibility project researches have been done on:

* Studies that have compared the two different types of aluminium adjuvant vaccine (aluminium hydroxide and phosphate) through a hollow microneedle.
* Studies looking at a plasmid DNA vaccine (HSV 2) with a coated microneedle.
* Studies on a Malaria sporozoite vaccine with a hollow microneedle and jet injector.

The current status of the microneedle feasibility projects is:

* Drafting study designs and protocols.
* Evaluating microneedle technology platforms.
* Connecting with developers to ascertain interest.
* Establishing contracts with research groups and collaborators.
* Value the proposition of work initiated, to make these technologies available in developing countries.

-In terms of the Rabies Intradermal study

This is a 2 year project (2008 – 2009) from the ID delivery projects that belong to the Health Innovation Portfolio. Objectives:

* Identify and qualify appropriate microneedle delivery devices or other technologies to facilitate intradermal delivery of rabies vaccine.
* Generate necessary pre-clinical data, including immunogenicity data for identified devices.
* Facilitate the manufacture of clinical grade prototypes for use in a Phase I clinical trial (to be conducted in Brazil).
* Quantify the value proposition for public health buyers, technology suppliers, and vaccine manufacturers.
* Prepare the groundwork for commercialization, policy, and procurement mechanisms needed for developing a functional market.

The current status of the Rabies intradermal study:

* Preparing technology evaluation protocols and agreements with collaborators.
* Connecting with developers to ascertain interest in project participation.
* Value the proposition of work initiated.
In terms of the Needle Free Injector (NFI) Project

This is a 4 year project (2007 – 2011) from the Health Innovation Portfolio, funded by the Bill and Melinda Gates Foundation within the PATH Technology Solutions program, and the ID delivery project. NFI is the same as a disposable cartridge or syringe jet injector technology.

The benefits of a NFI system is that: Needle-free provides safety thus eliminates the risk of needle injuries, it is compatible with current vaccine vial formats (single or multi dose), does not require vaccine reformulation, it is able to deliver ID, SC and IM injections and its ease to use.

The NFI project objectives

Key Question: Are NFIs appropriate and suitable for developing-country immunization programs? The goal of the project is to answer that question, and to do so some objectives have to be followed:

- Generate clinical, regulatory, and program-use data critical to advance not only a particular NFI design but the entire field of NFI technologies.
- Evaluate the clinical and program feasibility of intradermal delivery of select vaccines of importance to global public health programs in developing countries.
- Advance the potential of NFIs appropriate for developing country use by assisting developers in optimizing their product designs, obtaining regulatory approval, planning for scalable production, and facilitating early-adopter markets.

The NFI project activities are as follows:

- Conduct EPI vaccine bridging studies.
- Conduct ID clinical trials.
- Device regulatory approval in the USA, Europe and Brazil.
- Pilot introduction study in Brazil.
- WHO pre-qualification.

One key aspect of the NFI project is to assemble a scientific advisory group that will be comprised of experts who will provide technical, scientific, program and regulatory advice and recommendations for NFI project. The advisory group will play a crucial role in the review of:

- NFI design information (pre-clinical, clinical, technical performance).
- Vaccine study designs, protocols and data (ID, SC, IM).
- Confirmation of regulatory strategy.

Regarding the EPI Vaccine studies it is planned to work in cooperation with the Brazilian National Program for Immunization, to demonstrate target immune response – comparison with needle and syringe delivery, with a EPI ‘set’ of vaccines focusing on SC and IM.

For the Intradermal Delivery trials, the following activities have been undertaken:

- Systematic literature review and analysis of previous ID vaccine studies.
- Support the completion of the WHO- Oman IPV study and the CDC Dominican Republic influenza studies.
- Current candidate vaccines (final list is pending): IPV, Hepatitis A, Yellow Fever, and BCG.

In terms of the NFI Device Regulatory Approval, is expected to obtain the device regulatory approval for ID, SC, and IM through the regulatory agencies in the USA the FDA, in Europe the EMEA, and in Brazil the ANVISA. To obtain a device clearance for is intended use and general indication for use.

Pilot Introduction Study in Brazil

- Cooperation with Brazil NPI
- Immunization clinic pilot use
  - Deliver immunization program vaccines
  - Multiple sites / locations
  - Cost modeling – verify assumptions / variables
WHO Pre-Qualification

- Support WHO PQS standard development for NFIIs (Performance, Quality, and Safety)
- Device third party testing (WHO PQS requirement)
- Data submission to WHO TLAC (Technology and Logistics Advisory Committee) for policy recommendation

The current status of the NFI project is:
Working to finalize timelines and research agenda
- Project partners, agreements, vaccine selection, study designs
- Drafting global regulatory strategy
- Establishing Scientific Advisory Group

4.3. How does Intradermal Delivery Modify the Vaccine Environment: Challenges to Intradermal Delivery
Von Nakayama, Maryland, USA

The regulatory world tends to operated under three basic rules: First “simple is not easy”, second “obvious is not proof”, and third “data is what counts and not opinions”.

Today’s vaccine environment is characterized by the following drivers and constraints:
Drivers: Desire for profits by industry, immunization initiatives, new markets, availability of new processes, funding for new vaccine development, and social responsibility.
Constraints: regulations, costs, and anti-vaccine concerns.

From all the current vaccines delivered via ID, only Tuberculosis and Rabies are labeled for ID use.

The challenges facing the adaptation and implementation of ID reduce dose implementation of vaccines have been group in the following four categories: The usual and customary medical practices for vaccination, the regulatory and legal requirements, the ethical considerations and the economic costs of new vaccine development.

It is important to remember that the intradermal reduce dose version of an existing vaccine makes of it a new vaccine.

Medical issues regarding ID vaccine delivery are:
ID is not the standard of care for the majority of vaccines: Due to this off label use occurs (this is permitted for the treatment of an specific case on an individual patient but is not institutionally accepted, and is not promoted by the industry for that use), ID is a new route of administration and the reduce ID dose leads to a new treatment regimen.

Unproven safety and effectiveness: The clinical evidence does not come from adequate control studies, the conditions for use have not been characterized, and importantly the patient population on clinical trials tends not to represent the patient population that is intended to be used in. This is a major requirement in the USA to demonstrate safety and effectiveness in the population targeted and therefore the study population must have the same characteristics of the study population. Moreover, the adverse events profile for ID use is unknown, this unable to write adequate warnings to the patient or practitioner.

Other issues for the ID reduce dose is that it must be accepted by the patients, requires the training of users, and the development of new devices for delivery.
Regulatory & Legal requirements for ID vaccine delivery are:
Adequate support for product claims: The clinical effectiveness of the reduce dose, the identification of adverse events, the specifications for the conditions of use that might affect the safety and effectiveness of the new treatment regimen.
The clinical evidence: This evidence for product claims must come from well controlled studies, studies in the appropriate population, and its risk and benefits.
Product approval: This is one of the most complex processes to get a product in the market. Regarding control labeling, it is the manufacturer of the vaccine that does control this labeling, including the cross-labeling when to be used with a specific device, such as the different types of delivery devices.

Ethical considerations for ID vaccine delivery are:
Experiment vs. treatment: If the ID delivery is experimental all the document compliance for a clinical trial has to be provided such as informed consent, study design, protocol etc.
Patient rights: patients must be treated with dignity, must be given proper choices for treatment and must be informed about available conventional therapy.
Priority of Interests: To ensure that provider’s intent goes in tune and are consistent with the patient needs and align with national goals.

Economic considerations for ID vaccine delivery are:
Costs: Regarding product development, facilities conversion when necessary, and the equilibrium in finding a return on investment.
Resource utilization: Regarding old, modified and new products, and also human resources as skill personnel etc. Incentives of pharmaceutical companies can yield from cooperate philosophy, humanitarian market entry strategies etc.
III. References: Speakers & Lectures as follows:

(Lectures from red color coded authors are confidential)

**Antoine Alarcon**, Sanofi Pasteur, Lyon, France
New System for Intradermal Vaccination (Industrial Challenges).

**Bruce G. Weniger**, MD, MPH, Centers for Disease Control and Prevention, Atlanta, USA
Historical and Clinical Background on Cutaneous Vaccination

Harvinder Gill¹,², Ioanna Skountzou¹, Jonas Söderholm³, Matti Sällberg³, Mark R. Prausnitz⁴, Richard W. Compans¹,¹ Emory University School of Medicine, 1510 Clifton Road, Atlanta, USA
Evaluation of Microneedles for Intradermal Vaccine Delivery

**Darin Zehrung**, PATH, Seattle, USA
PATH’s Intradermal Delivery Research Projects

**Dominique Kaiserlian**, INSERM, Lyon, France
Immunological Basis of the Efficacy of Intradermal Vaccination

Fabrizio Fabrizi,¹² MD, and Paul Martin,³ MD, ¹Division of Nephrology and Dialysis, Maggiore Hospital, IRCCS Foundation, Milano, Italy; ²Center for Liver Diseases, School of Medicine, Miami, Florida
Intradermal Vaccination against Hepatitis B Virus (HBV) in Dialysis Population: Recent Evidence

**F.X Meslin** and M. Warrell, WHO, Geneva, Switzerland
Economical Intradermal Rabies Vaccine Application

**Gregory M. Glenn**, M.D, lecture given by Robert Said, IOMA Corp, Gaithersburg, USA
Putting Skin Immunization into Practice to Prevent Mucosal Disease.

**Marcel B.M. Teunissen**, University of Amsterdam, The Netherlands
Intradermal Vaccination: Mechanism of Action

**Mark Kendall**, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia
Needle Free Injection

**Martin Friede**, WHO – Geneve, Switzerland
The Potential of Intradermal Delivery of Vaccines to Improve Immunization in Developing Countries.

**Melanie Saville**, Sanofi Pasteur, Marcy l’Etoile, France
Seasonal Influenza Vaccination by Intradermal Microinjection

**Odile Launay**, Cochin-Pasteur Clinical Research Center Institute “CIC de Vaccinologie Cochin-Pasteur”, Cochin Hospital, Assistance Publique-Hopitaux de Paris INSERM – Paris Descartes University
Promising Antigens to be Administered by Intradermal Route: HIV

**Philippe E. Laurent**, BD Medical Pharmaceuticul Systems, Le Pont de Claix, France
Intradermal Injection Systems for Improving Vaccine Delivery

**Roland Sutter**, World Health Organization, Geneva, Switzerland
Intradermal Administration of Fractional IPV Doses
Ru-Chien Chi, University of Washington, Seattle, USA
Intradermal Delivery: The Challenges, The Pros and Cons

Sergio Kodaira, Institute of Radiology of School Hospital Sao Paulo State University, Brazil
Glacus Brito S, Clinical Immunology Department, Sao Paulo State University, Brazil
Ultrasound Image Analysis of Needle-free Jet Injector Tissue Delivery

Von Nakayama, Maryland, USA
How does Intradermal Delivery Modify the Vaccine Environment: Challenges to Intradermal Delivery
### IV. Annexes

#### Annex 1: Meeting Agenda

**Monday, April 7, 2008**

| 17h30-18h30 | Registration |
| 18h30-18h45 | Welcome Address  
C. LONGUET |
| 18h45-19h15 | Keynote lecture  
The potential of intra-dermal delivery of vaccines to improve immunization in developing countries  
M. FRIEDE |
| 19h45 | Welcome Dinner |

**Tuesday, April 8, 2008**

#### SESSION I

**Basic aspects: anatomy, historical and clinical aspects, challenges, and immunology**  
Chair by: J.F. NICOLAS and S. SAELAND

| 08h30-09h10 | Historical and clinical background on cutaneous vaccination  
B. WENIGER |
| 09h10-09h30 | Discussion |
| 09h30-09h50 | Intra-dermal delivery: the challenges, the pros and cons  
R.C. CHI |
| 09h40-09h50 | Discussion |
| 09h50-10h10 | Intra-dermal vaccination: mechanism of action  
MBH. TEUNISSEN |
| 10h10-10h30 | Discussion |
| 10h30-11h00 | Coffee Break |
| 11h00-11h20 | Immunological basis of the efficacy of intradermal vaccination  
D. KAISERLIAN |
| 11h20-11h40 | Discussion |

#### SESSION II

**Different vaccine delivery technologies for Intra-dermal immunization**  
Chair by: D. ZEHRUNG and J. BIRCHALL

| 11h00-12h00 | Jet injectors for ID delivery  
R. STOUT |
| 12h00-12h20 | Discussion |
| 12h20-14h00 | Lunch |
| 14h00-14h20 | Preliminary findings from intradermal delivery using the PharmaJet and Antares Injectors  
G. de SOUZA BRITO A. KODEIRA |
| 14h20-14h40 | Discussion |
| 14h40-15h00 | Needle free injection  
M. KENDALL |
| 15h00-15h30 | Discussion |
| 15h30-15h50 | Coffee Break |
| 15h50-16h10 | Use of microneedles for cutaneous immunization  
R. COMPANS |
| 16h10-16h25 | Discussion |
| 16h25-16h45 | Patch technology  
G. GLENN |
### Wednesday, April 9, 2008

**Session III**

**Proven or promising antigens to be administered by intradermal route**

- **08h30-08h50**: HIV  
  Chaired by: O. LAUNAY
- **08h50-09h10**: Discussion
- **09h10-09h30**: HBV  
  Chaired by: F. FABRIZI
- **09h30-09h50**: Discussion
- **09h50-10h20**: Coffee Break
- **10h20-10h40**: Rabies  
  Chaired by: T. CARLTON
- **10h40-11h00**: Discussion
- **11h00-11h20**: Seasonal Flu  
  Chaired by: M. SAVILLE
- **11h20-11h40**: Influenza vaccination by classical ID route, 1937-2007  
  Chaired by: B. WENIGER
- **11h40-12h10**: Discussion
- **12h10-14h00**: Lunch
- **14h00-14h20**: IPV  
  Chaired by: R. SUTTER
- **14h20-14h35**: Discussion

**Session IV**

**Intra-dermal vaccination from the perspectives of manufacturing, R&D philanthropy and regulation**

- **14h35-14h55**: Industrial challenges  
  Chaired by: K. KOTLOFF
- **14h55-15h10**: Discussion
- **15h10-15h30**: The Gates Foundation/PATH R&D initiative  
  Chaired by: D. ZEHRUNG
- **15h30-15h45**: Discussion
- **15h45-16h05**: Regulatory aspects  
  Chaired by: V. NAKAYAMA
- **16h05-16h20**: Discussion
- **16h20**: End of the meeting
Annex 2: Disclaimer
Information on this report was obtained from the lectures and abstracts given by the speakers as per scientific agenda on the Intradermal Delivery Fondation Mérieux meeting held on April 2008 at “Les Pensieres” conference center in Veyrier du lac France. All graphs, flow charts and images were obtained from the speaker’s presentations to facilitate the comprehension on the subject. The information posted on this report, except for the lectures titles highlighted in red which are confidential, was authorized as per signed authorization form by the speakers in question. This report was created for meeting reporting information purposes for Fondation Mérieux; the different forms of vulgarization of this information might require further speakers authorization. The information provided does not constitute a manual or technical sheet on the subject, it might have omissions, and we cannot assure its completeness or accuracy, and should not be used for the diagnosis or treatment of disease. Commercial products and prototypes are named and illustrated for information only. No endorsement or recommendation by the Fondation Mérieux or that of the meeting reporter are implied or should be inferred. They do not necessarily represent the views of Fondation Mérieux or that of the meeting reporter and have not been formally disseminated and should not be construed to represent any agency determination or policy.