"To promote the health of people in developing countries by the development, introduction and use of new and improved vaccines"

- From: Constitution of IVI (1996)

Rational and sustainable vaccine introduction

- Disease surveillance
- Technology transfer
- Demonstration projects
- Clinical trials for licensure
- Vaccine development
- Advocacy

RATIONAL AND SUSTAINED VACCINE INTRODUCTION

REDUCED DISEASE BURDEN
Vaccines (Developed World Markets)

- World Vaccine Market expected to be worth US$ 40 Billion by 2015

- Four companies (GSK, Sanofi-Aventis, Wyeth and Merck) together control 71% of the vaccine market worldwide.

- Faster growing vaccines
  - Malaria Vaccine (US$ 400 million by 2025)
  - Prevnar a Pfizer product is expected to become the first vaccine to cross US$ 5 Billion mark by 2015
  - Influeza vaccine market globally is forecasted at 7 Billion US$ by 2015

www.researchandmarkets.org
Vaccines (Developing Countries Markets)

- **Markets**
  - Emerging markets growing at 16-17%.
  - China expected to become #2 market after North America by 2020

- **Growth Drivers**
  - Large population, unmet vaccination needs and low vaccination rates
  - Increasing governments focus on prevention/childhood rates

- **Future Growth Expectation**
  - Combination vaccines, Influenza, Traveler vaccines, Neglected Tropical Disease

www.researchandmarkets.org
## Contrast between Vaccines and Pharmaceuticals

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Pharmaceuticals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus on prevention</td>
<td>Focus on treatment</td>
</tr>
<tr>
<td>– not patients, but healthy people</td>
<td>– patient is generally sick</td>
</tr>
<tr>
<td>Key role for the government agencies</td>
<td>Key role for the doctors and pharmacists</td>
</tr>
<tr>
<td>Very low acceptance of side effects</td>
<td>Acceptance of side effects varies with severity of disease</td>
</tr>
<tr>
<td>Large clinical trials</td>
<td>Less demanding clinical trials</td>
</tr>
<tr>
<td>5,000 to 10,000 subjects before registration</td>
<td>2000 to 3000 subjects before registration</td>
</tr>
<tr>
<td>(67,000 for Wyeth’s Rotavirus vaccine)</td>
<td></td>
</tr>
</tbody>
</table>
## Contrast between Vaccines and Pharmaceuticals

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Pharmaceuticals</th>
</tr>
</thead>
<tbody>
<tr>
<td>High manufacturing complexity</td>
<td>Medium manufacturing complexity</td>
</tr>
<tr>
<td>Biological processes are difficult to control</td>
<td>Easier to manage chemical synthesis in most cases</td>
</tr>
<tr>
<td>Supply chain complexity generally require storage at or below 4°C</td>
<td>Supply chain less complex, many drugs stored at room temperature</td>
</tr>
<tr>
<td>Very few generic products (Due to manufacturing complexity)</td>
<td>Increasing generic threat</td>
</tr>
</tbody>
</table>

![Vaccines manufacturing](image1.png)

![Pharmaceuticals manufacturing](image2.png)
Milestone Technologies in Vaccine Development

- **No understanding of mechanism of action**
- **Genome-based approaches**
- **Therapeutic vaccines**
- **Recombinant DNA technology**
- **Microbial origin of infectious diseases:**
  - Live-attenuated and killed vaccines
- **Chemical inactivation of toxins**
- **Subunit vaccines**

- **Glycoconjugate chemistry**
- **21st century**
- **20th century**
- **19th century**
- **18th century**
- **17th century**
- **16th century**
- **15th century**
- **14th century**
- **13th century**

Key Milestones:
- 1721: Smallpox introduction to Europe from Variolation from Asia
- 1796: 1st Smallpox Vaccine
- 1885: 1st Live-attenuated Vaccine
- 1886: Killed Vaccines
- 1900’s: Toxoid Vaccines
- 1948: 1st Combination Vaccines
- 1950’s: In vitro Cell culture
- 1970’s: Polysaccharide Vaccines
- 1980’s: Glycoconjugate Vaccines
- 1981: 1st Recombinant Antigen Vaccines
- 2013: Reverse Vaccinology
- 2010: 1st Therapeutic Vaccines

Prostate cancer: HEV

HEV: Hepatitis E virus
HIV: Human immunodeficiency virus

Vaccines approved in last five years have created new markets. These leading products in 2008 totaled to US$10 billion.

<table>
<thead>
<tr>
<th>Product</th>
<th>Company</th>
<th>Sales US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevnar</td>
<td>Wyeth</td>
<td>2.7 billion</td>
</tr>
<tr>
<td>Gardasil</td>
<td>Merck</td>
<td>1.4 billion</td>
</tr>
<tr>
<td>Proquad/Varivax</td>
<td>Merck</td>
<td>1.3 billion</td>
</tr>
<tr>
<td>Infanrix</td>
<td>GSK</td>
<td>1.3 billion</td>
</tr>
<tr>
<td>Polio/whooping cough/Hib vaccines</td>
<td>Sanofi Pasteur</td>
<td>1.1 billion</td>
</tr>
<tr>
<td>Influenza</td>
<td>Sanofi Pasteur</td>
<td>1.1 billion</td>
</tr>
<tr>
<td>Hepatitis Vaccines</td>
<td>GSK</td>
<td>1.2 billion</td>
</tr>
</tbody>
</table>
Types of Licensed Vaccines

There is no generic technology for making vaccines, all vaccines are different, even the same vaccine produced by a different manufacturer can be different.

- Inactivated toxins
- Inactivated whole bacteria or viruses
- Live attenuated bacteria or viruses
- Subunit vaccines
- Genetically engineered proteins
- Polysaccharide vaccines
- Conjugate vaccines
Increased emphasis on safety

Oral Live attenuated polio vaccine (OPV) has been replaced by the inactivated polio (IPV) in industrialized countries. Issues with reversion to virulence with one of the three strains in the OPV

Whole cell pertussis (wP) which is reactogenic has been replaced with acellular pertussis (aP) in industrialized countries. Currently aP is too expensive for routine use in developing countries.

Single use auto-disable syringes so that syringes cannot be reused.

Preservatives such as thiomersal being excluded from formulations (particularly single dose presentations)
cGMP compliance

Vaccine manufacturers must comply with current Good Manufacturing Practice (GMP), keeping track of the latest guidelines is time consuming and difficult.

cGMP is a part of the quality system used in the manufacturing, testing and development of vaccines

Companies who fail inspections can expect to face penalties. Fines and product bans are common but often most damaging is the loss of consumer confidence in the product.
Manufacturing

Stronger emphasis on Validation

Use of single use technologies has simplified validation.

Single use technologies are easier to install and facilitate earlier time to market than conventional equipment.
Manufacturing

Technologies used for production and purification

Increasing use of disposable fermentation equipment.

The workhorses of purification are still remain various forms of chromatography and tangential flow filtration. We see more and more single use technologies being developed.
Storage of vaccines is costly, generally require refrigeration

**Experience with monovalent H1N1 vaccine for the 2009/2010 season.**

**Significant losses due to expiry of product.**
- US: 71 out of 162 million doses.
- Australia: 9.7 out of 19 million doses.

**Vaccine recall due to inadequate stability.**
- 13 lots of live attenuated H1N1 influenza.
- One lot split H1N1 pediatric vaccine (800,000 doses).

Substantial amount of work being done on developing more stable formulations to reduce product loss and reduce the dependence on the cold chain
Vaccine development focus

**Antigen discovery**

**Protein**
Various new genetic techniques such as reverse vaccinology to identify new protein candidates. Targeting antigens likely to be expressed on the surface of pathogens.

**Polysaccharide**
Traditionally capsular polysaccharides have been targeted as vaccine antigens. The O specific polysaccharide component of lipopolysaccharide of some gram negative bacteria is targeted for some diseases.
- Non Typhoidal Salmonella
- Cholera
Vaccine Process Development at IVI

- Inactivated Cholera Vaccine
- Typhoid Conjugated Vaccine
- Paratyphoid Conjugated Vaccine
- Pneumonia Vaccine
Process

Oral inactivated Cholera vaccine

Seed bank

Expansion of Cell numbers in Shaker Flask Culture

Seed Fermentation

Production Fermentation

Inactivation

Concentration Diafiltration

Bulk monovalent concentrates

Formulation

Fill and Finish

Manufacturing method and facility meets WHO standards for cGMP

Testing is compliant with WHO recommendations for Oral Inactivated Cholera Vaccine

New QC assays LPS (Antigen) and Toxin
IVI transferred its technology for oral cholera vaccine to Shantha which was subsequently licensed in India and WHO prequalified and is now saving lives in India, Bangladesh, Haiti and Africa.
Technology Transfer

- Eubiologics, Korea

- Incepta Vaccine Ltd.
Vi conjugate vaccine development at IVI

First step: develop a high yielding fermentation system.

High density bacterial culture
- Fed batch culture increased OD$_{600}$ four fold

Optimal chemical concentrations for the biosynthesis and polymerization of Vi
- High concentrations of glucose and high pH inhibited Vi biosynthesis

![High density bacterial culture - Fed batch culture increased OD$_{600}$ four fold](image)

Optimizing Vi yield

![Optimizing Vi yield](image)

Cell growth (optical density)

![Cell growth (optical density)](image)

Vi polysaccharide production

![Vi polysaccharide production](image)
Downstream purification of Vi polysaccharide

Second step: removal of contaminants, high recovery of Vi.

Clarification
Cells
Crude Vi
Crude Vi concentrate

Cetavlon precipitated Vi
Ethanol precipitated Vi
Vi dissolved in water
Purified bulk Vi
Downstream purification of Vi polysaccharide

IVI method is unique in the way the precipitate is handled

No centrifugation
No chromatography
Single use disposable sterilizing grade filters

Transferrable to developing country manufacturers with minimal capital equipment expenditure

Scalable and GMP compatible
Conjugate vaccines

**Vi polysaccharide only vaccines** (T-cell independent response)
- Poor anti-Vi antibody responses in older children and adults
- No response in infants (< 2 years of age)
- No memory response
- Short lived immunity

**Vi conjugate vaccine** (T-cell dependent response)
- Higher antibody responses in all age groups
- Induction of response in infants
- Induction of memory
- Duration of immunity much longer
Scanning Electron microscopy of Vi and Vi-DT

Vi Polysaccharide

Catalytic domain
Inactivated during toxoiding

Vi-DT Conjugate 2

Translocation domain

Receptor domain

Diphtheria Toxoid
Immune responses to conjugates

The more DT bound the higher the anti Vi response.
A critical concentration of DT required to induce optimal T-cell help?
Technology Transfer

- Shantha Biotech, India
- SK Chemicals, Korea
- Incepta Vaccine Ltd., Bangladesh
- PT biofarma, Indonesia
Salmonella paratyphi A OSP purification

- High cell density fermentation/Inactivation of culture broth
- Washing of the cells
- Purification of crude LPS
- Detoxification of the LPS
- Contaminating Protein precipitation using detergent
- Removal of insoluble impurities
- Concentration/Diafiltration & Sterile filtration
- Purified bulk OSP ready for conjugation

<table>
<thead>
<tr>
<th></th>
<th>Protein %</th>
<th>Nucleic acid %</th>
<th>Endotoxin EU/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO Specification for Polysaccharide vaccines</td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>25</td>
</tr>
<tr>
<td>Consistency Run 1</td>
<td>0.34</td>
<td>0.36</td>
<td>0.4</td>
</tr>
<tr>
<td>Consistency Run 2</td>
<td>0.52</td>
<td>0.60</td>
<td>0.17</td>
</tr>
<tr>
<td>Consistency Run 3</td>
<td>0.68</td>
<td>0.34</td>
<td>18.72</td>
</tr>
</tbody>
</table>

Salmonella paratyphi A OSP purification
Immune responses to OSP conjugates

4, 8, 10 weeks IgG GM Titer of OSP-TT
(Inoculation 0 day, 4 and 8 weeks)
(Bleeding 4, 8, 10 weeks 1 day prior to immunization)

IgG GM Titer

Antigen Groups

OT1 Group 1
OT2 Group 2
OT3 Group 3
OT4 Group 4
OT5 Group 5
OT6 Group 6
OSP Group 7
TT Group 8
PBS Group 9

4 weeks
8 weeks
10 weeks
Comparison of different manufacturer production process for Vi and OSP

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Vaccine Technology Developer</th>
<th>Purification Steps</th>
<th>Purified Antigen yield gm/l of fermentation batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vi Polysaccharide</td>
<td>Novartis Vaccine, Italy</td>
<td>Centrifugation</td>
<td>0.3g/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Filtration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solubilization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVI, South Korea</td>
<td>Ultrafiltration</td>
<td>0.3g/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Filtration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solubilization</td>
<td></td>
</tr>
<tr>
<td>OSP Polysaccharide</td>
<td>Novartis Vaccine, Italy</td>
<td>Chromatography</td>
<td>0.15g/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Centrifugation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Precipitation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LIBP, China</td>
<td>Phenol</td>
<td>0.003g/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultracentrifugation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Centrifugation</td>
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<td></td>
<td>IVI, South Korea</td>
<td>Ultrafiltration</td>
<td>0.8g/l</td>
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<td></td>
<td></td>
<td>Filtration</td>
<td></td>
</tr>
</tbody>
</table>
PspA – common pneumococcal protein antigen

- Fed batch fermentation
- Soluble protein purified by membrane absorber filters
- Protein yield: 380mg/litre of fermentation broth

Conjugation of Vi to PspA-1 and PspA-2

Conjugation is a two-step process:

1. Derivatization of Protein
   - PspA protein
   - Adipic acid dihydrazide (ADH)
   - Carbodiimide
   - pH 5 - 6
   - Result: PspA-ADH

2. Conjugation of PspA-ADH with Vi
   - Vi
   - PspA-ADH
   - Carbodiimide
   - pH 5 - 6
   - Result: PspA-ADH-Vi
The response to the best PspA family 1 conjugate was 51 times higher than un-conjugated PspA.
Immunogenicity of Vi-PsA-2 conjugates

- The response to the best PspA family 2 conjugate was 5 times higher than un-conjugated PspA family 2
These results indicate that conjugates of both proteins need to be added in a vaccine formulation to provide broad protection against *Streptococcus pneumoniae*.
Challenge studies with Bivalent vaccine

- 90% of mice vaccinated with bivalent were protected
# Future

## Phase III vaccines in development

<table>
<thead>
<tr>
<th>Disease</th>
<th>Company</th>
<th>Drug Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking addiction</td>
<td>Nabi Biopharmaceuticals</td>
<td>NicVAX</td>
</tr>
<tr>
<td>Grass Allergy</td>
<td>ALB Abello</td>
<td>GRAZAX</td>
</tr>
<tr>
<td>Grass Allergy</td>
<td>Allergy Therapeutics</td>
<td>Pollinex Quattro Grasses</td>
</tr>
<tr>
<td>Ragweed Allergy</td>
<td>Allergy Therapeutics</td>
<td>Pollinex Quattro Ragweed</td>
</tr>
<tr>
<td>Grass Allergy</td>
<td>Fornix Biosciences</td>
<td>Oralgen Grass Pollen</td>
</tr>
<tr>
<td>Grass Allergy</td>
<td>Greer Labs</td>
<td>Sublingual-oral immuno-therapy</td>
</tr>
<tr>
<td>Grass Allergy</td>
<td>Paladin Labs</td>
<td>Oralair Grasses</td>
</tr>
<tr>
<td>Pollen Allergy</td>
<td>Schering-Plough/Merck</td>
<td>Allergy Immunotherapy Tablet</td>
</tr>
<tr>
<td>Dengue</td>
<td>Sanofi Pasteur</td>
<td>ChimeriVax</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Diamyd Medical</td>
<td>Diamyd</td>
</tr>
<tr>
<td>ETEC infection</td>
<td>Intercell</td>
<td>Traveler's Diarrhea vaccine patch</td>
</tr>
<tr>
<td>Herpes virus</td>
<td>GlaxoSmithKline</td>
<td>Simplirix</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>Tehran University of Medical Sciences</td>
<td>Alum-ALM</td>
</tr>
<tr>
<td>Malaria</td>
<td>GlaxoSmithKline</td>
<td>Mosquirix</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>NICHHD, NIH</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Other interesting disease targets

**Vaccines currently in phase II trials**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical development phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer's</td>
<td>Phase II</td>
</tr>
<tr>
<td>Ebola</td>
<td>Phase II</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Phase II</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Phase II</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Various phases</td>
</tr>
<tr>
<td>MRSA</td>
<td>Phase II</td>
</tr>
<tr>
<td>(Methicillin-resistant <em>Staphylococcus aureus</em>)</td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>Phase II</td>
</tr>
<tr>
<td>Obesity</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Cancer</td>
<td>Many in various stages of development</td>
</tr>
</tbody>
</table>
Thank you