Vaccine Development

(Part 2)

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Vaccine
Clinical Development Programmes

With a fast-moving pandemic,
no one is safe, unless everyone is safe

General Consideration

- Consultation with regulatory authorities
  - Before starting the development programme of a vaccine it is necessary to have consultation with the NRA
    - This consultation should be done at regular intervals
    - This is required for agreement on content and extent of the application dossier
  - This consultation is more important in cases of:
    - The clinical programme proposes a novel approach to any aspect of development for which there is no precedent or guidance available

General Consideration

- The proposed programme conflicts with existing guidance to which the NRAs involved would usually refer when considering programme suitability
- Particular difficulties are foreseen in providing evidence to support an expectation of vaccine efficacy
  - This means that there is no immunological correlate of protection and a vaccine efficacy study is not feasible
- There are other special considerations for the total content of the pre-licensing programme
  - E.g. when different vaccine constructs are to be used for priming and boosting

General Consideration – Vaccine Constructs

- *Components of a vaccine*[9(a)]
  - *Vaccines include a variety of ingredients that include:*
    - *Antigen, stabilizers, adjuvants, antibiotics and preservatives*
    - *They may also contain residual by-products from the production process*
- *Knowing precisely what is in each vaccine can be helpful when investigating adverse events following immunization (AEFIs)*
  - *It may also help in choosing an alternative product if someone is allergic to a vaccine component*

General Consideration – Vaccine Constructs

- *About the main components of vaccines:*
  - *Antigens*
    - *They are derived from the structure of the disease causing organisms*
    - *Body recognises them as 'foreign'*
      - *Body's immune system triggers a protective immune response to the vaccine*
  - *Stabilizers*
    - *These are used to help the vaccine maintaining its effectiveness during storage*
    - *When cold chain is unreliable, stabilizers are very important*

General Consideration – Vaccine Constructs

- *Instability of the vaccine causes loss of antigenicity and decreased infectivity of Life Attenuated Vaccine (LAV)*
- *Factors that affect stability are:*
  - *Temperature and acidity or alkalinity of the vaccine (pH)*
- *Bacterial vaccine can become unstable due to hydrolysis and aggregation of protein and carbohydrate molecules*
- *Stabilizing agents include $MgCl_2$ (for Oral Polio Vaccine), $MgSO_4$ (for measles, lactose-sorbitol and sorbitol-gelatine*

- *Adjuvants*
  - *Adjuvants are added to vaccines to stimulate the production of antibodies against the vaccine to make it more effective*

General Consideration – Vaccine Constructs

- *Adjuvants have been used for a long time to improve the immune response*
  - *Most often this was done for inactivated vaccine*
- *In conventional vaccines, adjuvant is set to enhance accelerating and prolong the immune response to vaccine*
- *Some new vaccine which are purified subunit vaccine that used biosynthetic, , recombinant, and other modern technology are poor vaccine antigens and require adjuvants to provoke the desired immune response*
- *Adjuvants are highly heterogeneous chemical substances*

General Consideration – Vaccine Constructs

- *Antibiotics*
  - *In trace amounts antibiotics are used during the manufacturing phase to prevent bacterial contamination of the tissue culture cells in which viruses are grown*
  - *Example:*
    - *In MMR vaccine and IPV each contain less than 25 micrograms of neomycin*
- *Preservatives*
  - *They are added to multidose vaccines to prevent bacterial and fungal growth*
  - *Examples of preservatives:*
    - *Thiomersal, Formaldehyde, or Phenol derivatives*

General Consideration

In Immunological Correlate of Protection (ICP) is defined as a type and amount of immunological response that correlates with vaccine-induced protection against a clinically apparent infectious disease and that is predictive of clinical efficacy

- In India, Central Drug Standardisation and Control Organisation is the Regulatory Authority (NRA)
- Use of independent monitoring committee
  - The committee should not include persons who are employee of the sponsor of the clinical trial
- The responsibilities of the monitoring committee

General Consideration

- Ongoing review of safety data
- Oversight of planned interim analyses of safety and/or efficacy
- In case it is necessary to terminate the study, recommending to the sponsor that a trial is terminated early in accordance with predefined stopping rules
- Determination of the eligibility of individual subjects for inclusion in the primary analysis population or other analysis population(s), as defined in the protocol;
- Adjudication to determine whether cases of clinically apparent infections meet the predefined case definition for inclusion in analysis of efficacy

General Consideration

- Adjudication to determine whether reports of AEs meet the criteria for specified types of AEs and AESIs and/or to determine causality

- The same or different monitoring committees may be appointed to oversee one or more aspects of a clinical trial
  - Depending on their role(s), independent monitoring committees may be referred to by specific terms, such as:
    - Data Monitoring Committee
    - Safety Data Monitoring Committee
    - Independent data Adjudication Committee

Registering and Reporting Clinical Trial

- Clinical research
  - It is medical research involving people
  - Two types
    - Observational studies
    - Clinical trials
      - Phases of Clinical trials
        - There are four phases of clinical trials, Phase 1 to 4

Registering and Reporting Clinical Trial

- Clinical Trial[10]
  - **Clinical trials** are research studies performed in people that are aimed at evaluating a medical, surgical, or behavioral intervention.
    - A new drug, device or diet must be safe and effective in human being for treatment of disease.
    - Clinical trials are the main way these aspects can be found out
  - Sometimes, it is used to find out if a new treatment is more effective than the established treatment, or
    - There are less side effects

Registering and Reporting Clinical Trial

- How a clinical trial is conducted
  - Volunteer participants are recruited for a clinical trial
    - Study staff explain the trial in detail and gather more information about the participant
    - All questions of the participant are answered and all doubts are cleared
    - Then the participant signs an informed consent form.
  - Screening is done to ensure that the participant satisfies the pre-established criteria for inclusion in the study
  - If accepted into the trial, a first visit is scheduled (called the “baseline” visit).
    - The researchers conduct cognitive and/or physical tests during this visit.

Registering and Reporting Clinical Trial

- The participants are randomly assigned to a treatment or control group.
  - The participant and family members follow the trial procedures and report any issues or concerns to researchers.
- The participant may visit the research site at regularly scheduled times for new cognitive, physical, or other evaluations and discussions with staff.
  - At these visits, the research team collects information about effects of the intervention and information about safety and well-being.
- The participant continues to see your regular physician for usual health care throughout the study.

Registering and Reporting Clinical Trial

- Clinical Trial Registration
  - Before the trial is started it must be registered in the trial registry
  - The purpose is that the trial information is publicly available
    - It is a free access and can be searched
    - It should comply with the NRA requirements and should comply with the WHO internationally agreed standards
- In India, registration of clinical trials with ICMR has been made compulsory since 15th June 2009[11]

Pre-licensure Clinical Development Programmes

- Before the product is licensed:
  - The objective is to scientifically gather all the data that will support licensure
- The main components of the programme are:
  - To describe the interaction between the vaccine and the host immune response
  - To identify safe and effective dose regimens and schedules
  - To estimate vaccine efficacy and/or to provide evidence of vaccine efficacy based on immune responses

Pre-licensure Clinical Development Programmes

- To describe the safety profile
- To assess co-administration with other vaccines, if this is relevant

- Preliminary trials
  - To begin with, effects of different amounts of antigen(s) in each dose of candidate vaccine formulation are explored for safety
    - The formulation may be with or without adjuvant

- ***New Candidate Vaccine****: A new candidate vaccine is a vaccine that is taken in national regulations to be separate and distinct from other candidate and licensed vaccines*
- ***Antigen****: An antigen is a substance that can provoke an immune response. Typically antigens are substances not usually found in the body.*[12]

Preliminary Trials

- Usually in preliminary trials, immune responses to the antigenic components are also explored
- This part of the vaccine development programme is usually referred to as Phase 1 trial
  - This part of the trial is usually conducted on healthy individuals
    - These individuals should not have been previously exposed to the organism(s) against which the candidate vaccine is intended to protect
- In the next phase, i.e., Phase 2 of the trial, safety and immunogenicity trial are conducted
- These trials are conducted on subjects who are representative of the intended target population of the vaccine

Preliminary Trials

- For vaccines intended to protect a broad age it may not always be necessary to use an age de-escalation approach
- Age de-escalation approach means in a sequential manner:
  - First the antigen is administered to adults, then to adolescents and finally to children aged 6 to 12 years
  - This is followed by younger children, toddlers and finally infants.
  - For example,
    - If a vaccine has negligible potential benefit for older children it may be acceptable to directly proceed from trials in adults to trials in younger children, including infants and toddlers
- These trials are designed to provide sufficient safety and immunogenicity data

Pivotal Trials

- Pivotal trials are Phase 3 trials
  - These trials are intended to provide strong clinical evidence for regulatory authority to consider licencing
  - In exceptional circumstances only, licensure is based on strong conclusive statistical data of a Phase 2 trial
  - Pivotal trials may be designed to provide data on vaccine efficacy or the trial may provide immunogenicity data as an indication that the disease can be prevented
  - Sometimes, It may also be a primary or co-primary objective to assess the safety aspect of the vaccine

Post-licensure Clinical Evaluations

- Even after licensure, it is essential
  - To monitor vaccine safety
  - To address any safety concern that were identified earlier by conducting studies
  - To conduct studies specifically for determining vaccine effectiveness
- Situation may arise when
  - The use of the vaccine may be extended or modified
    - For this purpose additional trials may be required by the sponsor for revision of the prescribing information

Post-licensure Clinical Evaluations

- Some definitions:
  - Sponsor
    - *The individual, company, institution or organization that takes responsibility for the initiation, management and conduct of a clinical trial*
  - Vaccine Efficacy[13]
    - *Vaccine efficacy is an individual-level measure of vaccine effects defined as the proportionate reduction of the incidence of the target infection in vaccinated participants compared to controls*
    - *It is usually demonstrated in Phase 3 trial*
  - Vaccine Effectiveness[14]
    - *Vaccine effectiveness reflects direct (vaccine induced) and indirect (population related) protection during routine use*

Immunogenicity

- General consideration
  - In all the three phases of pre-licensure clinical evaluation studies of vaccine development immunogenicity studies are included
  - In post-licensure phase (Phase 4) also it may be required to carry out immunogenicity trials
  - Immune response evaluation is based on measurement of immune parameters relevant to the vaccine in samples drawn at appropriate time intervals
  - Immune response information is required to support relationship between immunogenicity and efficacy and identification of ICP

Immunogenicity

- Characterization of the immune response
  - Appropriate range of tests to be conducted should be discussed wit the NRA
    - Generally, if the vaccine contains microorganism that has not been used previously in any human vaccine then a thorough investigation needs to be done
    - These investigations should demonstrate the interaction of the candidate vaccine with the immune system
    - These investigation are to be conducted as part of overall vaccine development programme
    - In India, the CDSCO draft guideline on COVID-19 vaccine development requires assessment of both humoral and cell mediated immune response

Immunogenicity

- For known microorganisms, the range of parameters are chosen :
  - based on previous experience and
  - Whether there is an established Immune Correlate of Protection or not
- For organisms not previously included in any human vaccine, the parameters are based on
  - Natural immunity and *(Immunity developed after an infection)*
  - Immune response to infection in animal models
- In general, the clinical development programme should include a description of extent of immune response

Immunogenicity

- Also assessment of functional antibody to be included, if possible
- Functional antibody includes antibody:
  - That neutralises viruses or toxins, or
  - That mediates bacterial killing activity or opsonophagocytosis

Opsonins are proteins that bind to molecules on the surface of microbes and to specific receptors on phagocytes.

The binding of the opsonin to the phagocyte receptor activates phagocytosis.[15]

Immunogenicity

- Appropriate additional investigations may be required
- For this decision one should consider:
  - Immune response resulting from natural exposure
  - Whether this exposure provides partial or complete protection, and
  - Whether the protection is temporary or lifelong
- The decision for choosing the range of investigations should also take into account:
  - Characteristics of the infecting microorganism, and
  - Content of vaccine

Immunogenicity

- Depending on the case, the additional investigation that can be included are:
  - Assessment of T-cell dependent primary immune response
  - Assessment of the specificity of cross-reactivity of the immune response
  - Assessment of changes in antibody avidity with sequential doses
  - Evaluation of factors that could influence the immune response
    - Such as
      - Maternal antibody on the infant immune response to some antigens

Immunogenicity

- Measuring the immune response
  - Humoral immune responses
    - Immune responses to vaccination are commonly measured in serum
  - Cellular immune responses
    - These responses are measured in blood
  - Sometimes samples of other body fluids may also be examined
  - These body fluids depend on where the target microorganism infects and/or replicates
  - Pre-vaccination samples are collected for early preliminary immunogenicity trials
    - Later these may be omitted if it is found that there is no pre-existing immunity

By priming *Priming* The process of artificial induction of immunity, in order to protect against infectious disease. Priming the immune system involves sensitizing or stimulating an ir

Immunogenicity

- The timing of post-vaccination sample will depend on when the peak immune response occurs after the first and, if applicable, after the sequential doses
- For example:
  - Vaccines that elicit priming, rise in antibody is much more rapid after the booster dose

Priming

- *With an initial dose the immune system gets trained to recognise the pathogen*
- *By priming the immune system through vaccination, when the vaccinated individual is later exposed to the live pathogens in the environment , the immune system can destroy them before they can cause disease*

Immunological Parameters

- Immunological parameters measures either humoral or cell-mediated immune responses
- For licensure purposes, humoral responses are usually considered
- For known microorganisms or antigens in a candidate vaccine the choice depends upon previous experience and whether there is an established ICP
- For others, the selection is based on natural immunity
  - And also for some infections, the animal model data may provide the necessary information

Immunological Parameters

- Humoral Immune Response
  - This is assessed from:
    - Post-vaccination appearance of antibody directed at specific microorganism, or
    - Increase after vaccination in antibody against the specific microorganism or antigen in the vaccine
  - Subject to availability of data, most emphasis is placed on functional antibody responses
  - Examples:
    - Serum bactericidal antibody, toxin or virus neutralising antibody or opsonophagocytic antibody

Immunological Parameters

- Sometimes appropriate assay for measuring functional antibody may not be available, or may not be feasible to use the assay
- Alternatively, or in addition to assay of functional antibody, the immune response may be assayed by measuring total antibody
- For example
  - Total immunoglobulin G (IgG) may be measured by Enzyme-linked Immunosorbent Assay (ELISA) that binds to selected antigens
  - However, only a proportion of total antibody detected may be functional

Immunological Parameters

- Cell-mediated immune response
  - This is commonly assessed by detecting and quantifying sensitised T-cells in blood from trial subjects
  - These investigations may also help determining type of cytokines released and also differences in sensitization between T-cell subpopulation
  - Several methods are to be used
  - These are typically based on measuring the production of a range of cytokines following in vitro stimulation of T-cells with individual or pooled antigen

Immunological Parameters

- The results may provide useful comparison between treatment groups within any one study
  - For example:
    - They could describe if there is any effect of an adjuvant
- If there are marked discrepancies in the pattern of responses observed between cell mediated and humoral responses the findings should be carefully considered and discussed
  - Example:
    - If adding an adjuvant has a major effect on antibody level but does not increase the percentage of sensitized cells in one or more T-cell subsets

Immunological Parameters

- *Cytokines*
  - *They are hormone of the immune system*
  - *The family of cytokines covers a large number of smallish proteins usually less than 20kDa*
  - *They serve a hormone like function in enabling cells to communicate with each other*
  - *Hormones that are produced in one organ and act on a distant tissue are said to be acting in an endocrine manner*

- *kDa = Dalton is an alternative name for atomic mass unit*
- *kDa is kilodalton, i.e., 1000 Dalton*
- *Thus a peptide with a mass of 64kDa has a molecular weight of 64000 grams per mole*

Immunological Parameters

- *Cytokines do not act in an endocrine manner*
- *They act locally*
- *They are produced by cells in a particular tissue and acts on cells in that tissue (this is paracrine or autocrine manner of functioning)*
- *Paracrine action means that the cytokine binds to receptors on cells close to those producing the cytokine*
- *'Close' means few microns to 1 mm*
- *Autocrine means that cytokine actually binds to receptors of the cell that produced the cytokine*
- *Thus the role of cytokines is to enable cells within each other in a local environment*

Cytokine families[16]

Family	Members	Comments
Interleukin (IL)	IL-1 to IL-35	Different IL have different functions and are secreted by different cells.
Interferon (IFN)	IFNα IFNβ IFNγ	Leukocyte IFN. Inhibits viral replication. Fibroblast IFN. Inhibits viral replication. Secreted by lymphocytes. Many immunoregulatory functions.
Tumour necrosis factor (TNF)	TNFα	Secreted by monocytes and other cells. Factor activates macrophages and endothelium.
	TNFβ	Secreted by T cells. Similar activity to TNFα.
Colony-stimulating factor (CSF)	G-CSF, M-CSF, GM-CSF and others	Originally identified by ability to make bone marrow cells differentiate into particular cell type, e.g. neutrophil. Also have effects on mature cells of same lineage, e.g. monocytes, macrophages, neutrophils.
Chemokine	MCP, Eotaxin and many others	Very important in controlling the migration of cells between and within tissues. Also influence function of many cells.
Growth factor	TGF, IGF and many others	Originally identified because of non-immune-related function but may have effects on immune cells.

G-CSF, granulocyte-CSF; M-CSF, macrophage-CSF; GM-CSF, granulocyte/monocyte-CSF; MCP, macrophage chemotactic protein; TGF, transforming growth factor; IGF, insulin-like growth factor.

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T Cell Subsets

- These lymphoid cells mature in thymus
- Basically there are two subsets named as CD4 and CD8 cells
- Specific Cytokines are involved in shaping the two subsets of the T-cell system. CD4+ T helper (Th) and CD8+ Cytotoxic T Lymphocytes (CTL)

Assays

- Assays
  - Assays are done for total or functional antibody for demonstrating immune responses
  - The method should be acceptable to the NRA
  - The assays may be:
    - Commercially available
    - Non- commercial validated method
    - Assays comparable to a reference assays established in a WHO reference laboratory
  - Clinical trial protocol should document the assay that will be used

References

9(a). WHO. Vaccine Safety Basics, pp. 51

10. National Institute on Ageing. What are Clinical Trials and Studies. Available at https://www.nia.nih.gov/health/what-are-clinical-trials-and-studies Accessed on 19 Dec 2020

11. (F.No. 12-01/09-DC-(Pt-32), Directorate General of Health Services, Office of Drugs Controller General (India), (New Drug **Division)**

12. MedlinePlus. https://medlineplus.gov/ency/imagepages/9071.htm

13. WHO. Guidelines on Clinical Evaluation of Vaccines – Regulatory Expectations, Annex 9, replacement of Annex 1 of WHO Technical Report Series, No. 924, pp. 11

14. European Medicines Agency. Guideline on Clinical Evaluation of New vaccines, 18 Oct 2006, pp. 14

15. Wood P. Understanding Immunology, 3rd Ed, pp. 43. Prentice Hall, England

16. Ibid, pp. 31

End of Part 2