ONLINE SCIENTIFIC WORKSHOP

SPECIFICS OF DEVELOPMENT AND TESTING OF BIOLOGICAL ORIGIN PRODUCTS

SPEAKER: DR. TETIANA LUTSENKO

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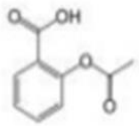
INTRODUCTION

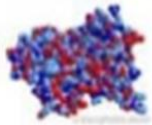
- For many years, the pharmaceutical industry traditionally developed chemical drugs (also referred to as small molecules), including well-known medicines such as acetylsalicylic acid, to treat a wide range of illnesses.
- Since the 1970's a revolution in Biotechnology resulted in New class of medicine: ***The Biologics***
- What are Biologic Medicines? A large molecule typically derived from living cells and used in the treatment or prevention of disease. The term biological product means virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein, or analogous product or arsphenamine or derivative of arsphenamine, applicable to the prevention, treatment or cure of a disease or condition of human beings.
- Examples: Biological products are used for a wide range of disease and conditions, including serious and life threatening conditions such as cancers and rheumatoid arthritis.

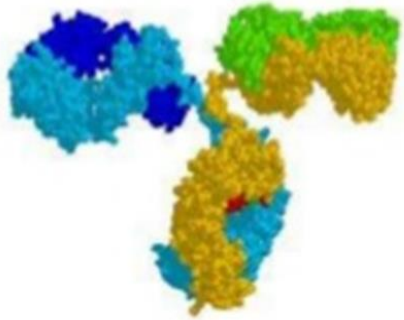
Biological origin products often 200 to 1,000 times the size of a small molecule chemical drug and far more complex structurally.

WHAT IS THE DIFFERENCE BETWEEN CHEMICAL ORIGIN PRODUCTS AND BIOLOGICAL ORIGIN PRODUCTS?

Biologicals : Differences to Small molecules





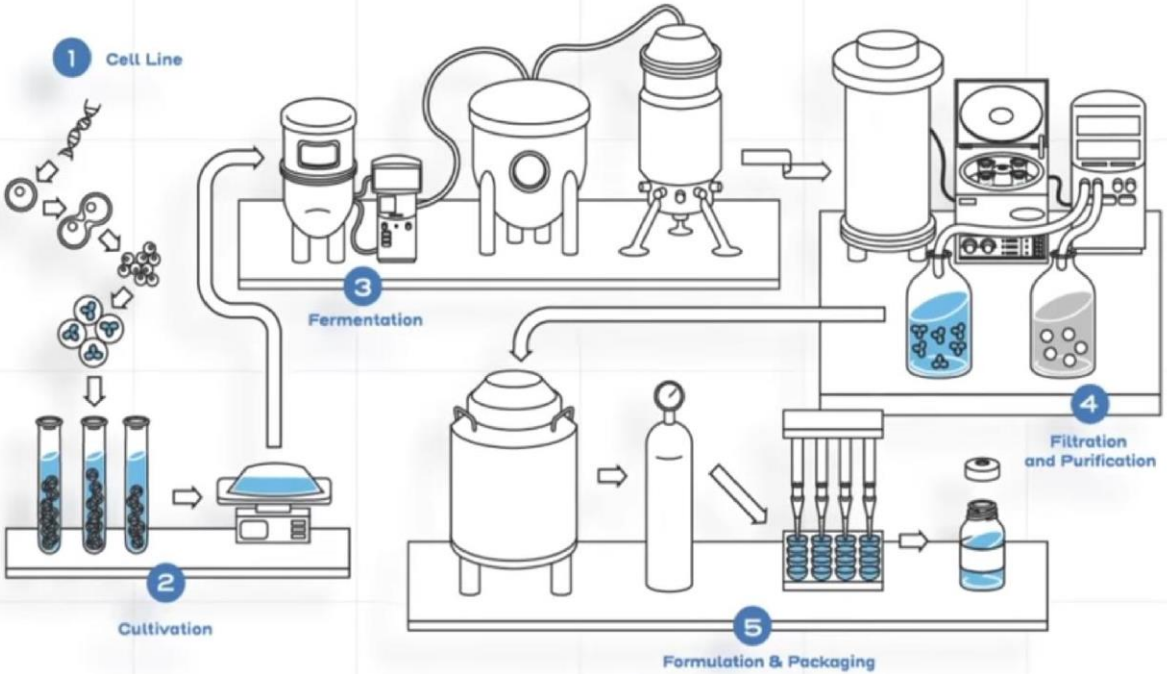


	Aspirin	Growth hormone	Monoclonal Antibody
Size	21 atoms	3,000 atoms	25,000 atoms
Structure	1º	1º, 2º, 3º, 4º	
Bonding	Strong (covalent)	Strong & Weak (covalent & non-covalent)	

COMPARISION OF TRADITIONAL SMALL MOLECULE DRUGS AND BIOLOGIC DRUGS

Feature	Small Molecule Drug	Biologic Agent
Example	Acetylsalicylic acid (180 Da)	Monoclonal antibody (~150,000 Da)
Entity	Chemical	Protein
Structure	Small, simple, well characterized	Large, complex, heterogeneous
Stability	Stable	Unstable
Mode of administration	Usually amenable to ingestion	Usually requires injection or infusion
Manufacturing process	Predictable and precise method; identical copies in batches	Living cell-based complex technology; batch-to-batch variation, sensitive to storage and handling
Immunogenicity	Mostly nonimmunogenic	Immunogenic

BIOLOGICAL DRUG PROCESS



SMALL MOLECULE DRUG PROCESS


SIMPLE BIOLOGICS
COMPLEX BIOLOGICS
EPO
EPOGEN (EPOETIN ALFA)
PROCRIT EPOETIN ALFA
Aranesp (darbepoetin alfa)
G – CSF
Neupogen
Neulasta (pegfilgrastym)
HGH
Genotropin
norditropin
IFN – A
INTRON A
PEGASYS
Peginterferon alfa-2a
Pegintron
IFN – B
AVONEX (interferon beta-1a)
Rebif
ETANERCEPT
Enbrel etanercept
ADALIMUMAB
HUMIRA adalimumab
BEVACIZUMAB
AVASTIN (bevacizumab)
CETUXIMAB
ERBITUX CETUXIMAB
INFLIXIMAB
Remicade
RITUXIMAB
Rituxan Rituximab
TRASTUZUMAB
Herceptin trastuzumab

SOURCES OF BIOLOGICALS PRODUCTS


Mammalian cell cultue


Humans


Avian cell culture


Mice


Transgenics


Insect cell culture

PRODUCTION OF VARIOUS BIOLOGICALS

- Recombinant DNA and Hybridoma technologies …..
  - Production of commercially viable biologicals
  - Definite need for skill in formulation
- Biomolecule formulation development - challenging area

  Critical processing events:

  - Purification
  - Production and
  - Physical and chemical stability

  Objective:

  Safe, stable and maintains efficacy
- Loss of activity during …..
  - Processing
  - Packaging
  - Shipping and
  - Long-term storage

THE PROBLEMS WITH BIOLOGICAL ORIGIN PRODUCTS

- Stability - 'The tendency to maintain a native (biologically active) conformation'.
- Are only marginally stable
- X- ray structure analysis of biologicals -

  - Structure is held together by weak non-covalent forces.

  - When these forces becomes weak, get broken apart leading to unfolding and/or inactivation of biologicals.

- Chemical instability (Covalent):

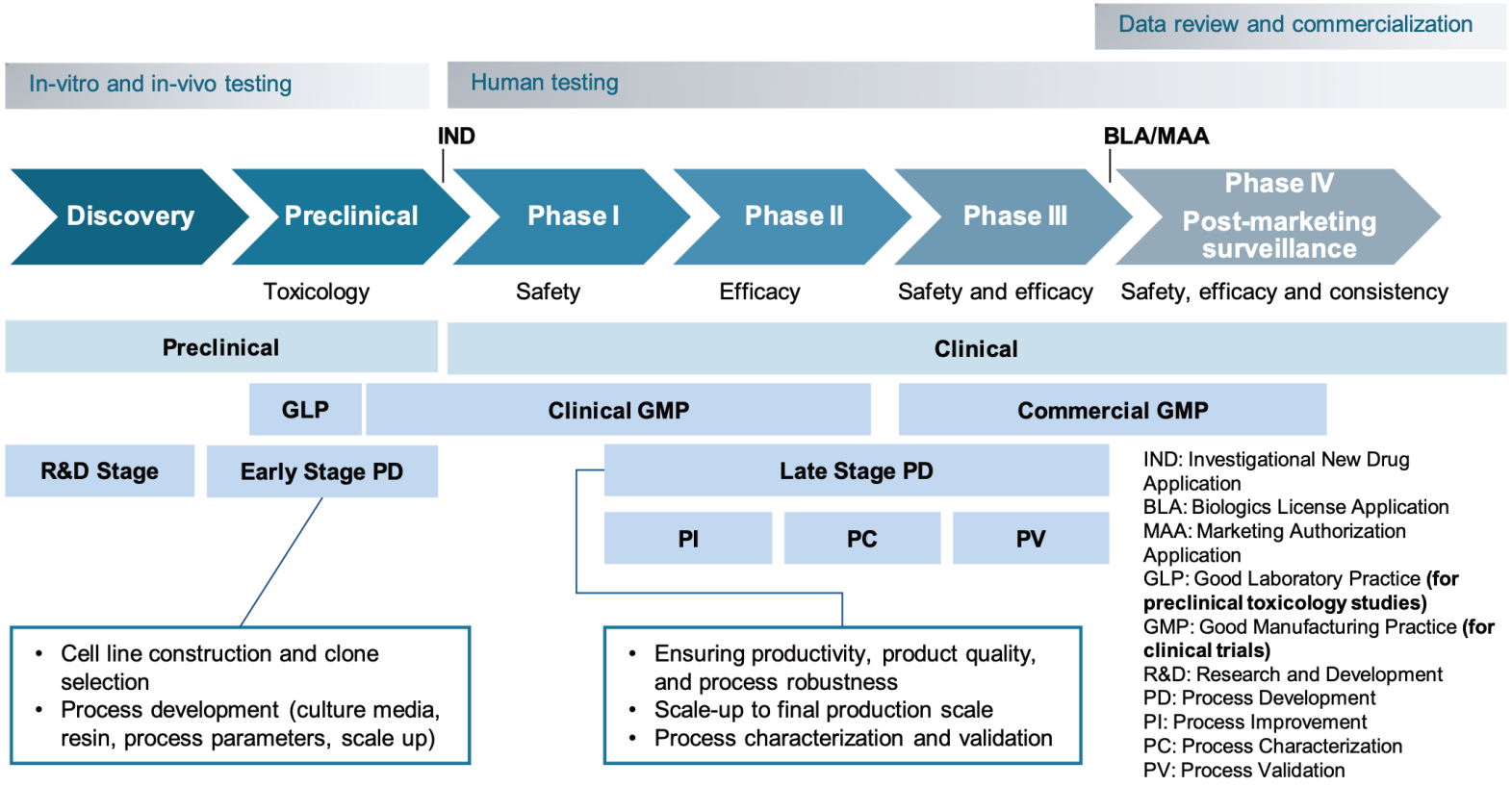
  Deamidation, Oxidation., Disulfide exchange and Proteolysis.

- Physical instability (Non-covalent):

  Aggregation and precipitation, Adsorption to surface, and unfolding.

- High sensitivity to both chemical and physical degradation - observed both in the production process and in the final packaging.

THE DEVELOPMENT CYCLE OF BIOLOGICAL ORIGIN PRODUCTS AND THE CONTENT OF EARLY/LATE-STAGE PROCESS DEVELOPMENT

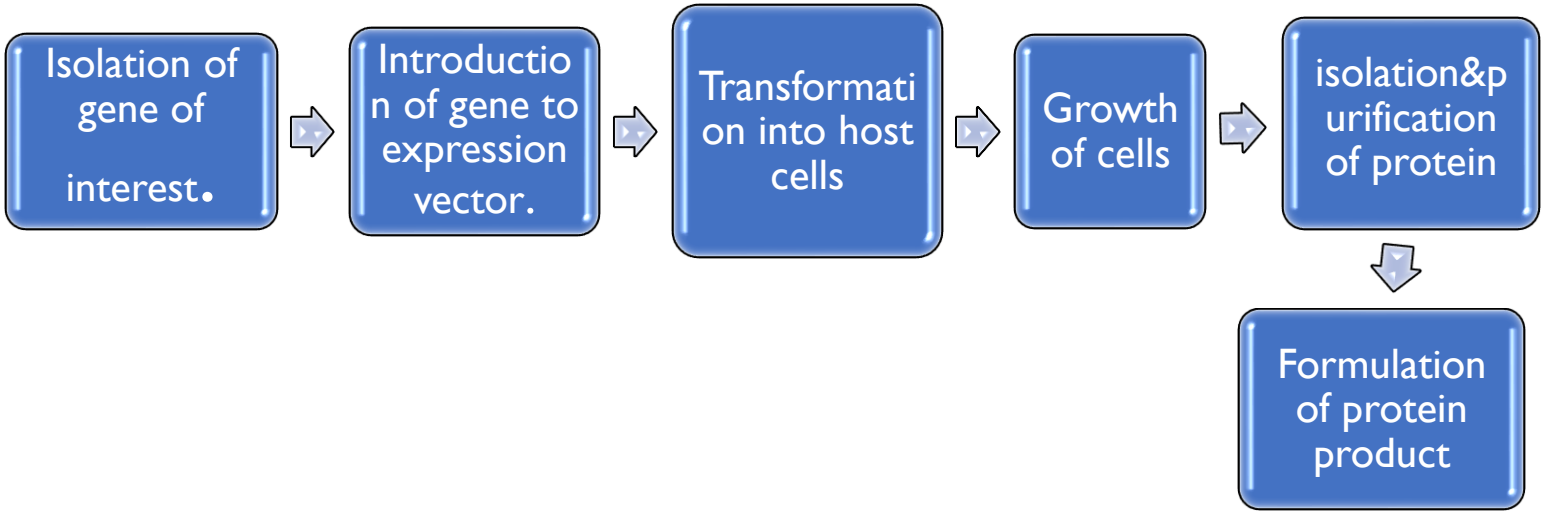


IND: Investigational New Drug Application
BLA: Biologics License Application
MAA: Marketing Authorization Application
GLP: Good Laboratory Practice **(for preclinical toxicology studies)**
GMP: Good Manufacturing Practice **(for clinical trials)**
R&D: Research and Development
PD: Process Development
PI: Process Improvement
PC: Process Characterization
PV: Process Validation

STEPS INVOLVED IN PRODUCTION OF BIOLOGICAL ORIGIN PRODUCTS

Develop Host	• A host cell is developed by isolating the DNA sequence that codes for the desired protein,
Establish a Cell Bank	• A cell bank is then established using elaborate cell screening and selection process
Protein Production System	• The “engineered” cells are then cultured on a large scale under growth conditions to optimize cellular production
Purification	• Fractions containing the desired protein are harvested and isolated, and the undesired proteins and impurities are separate
Analysis	• Protein molecules are analyzed for uniformity in terms of structure, character, and potency;
Formulation	• Therapeutic protein is then formulated

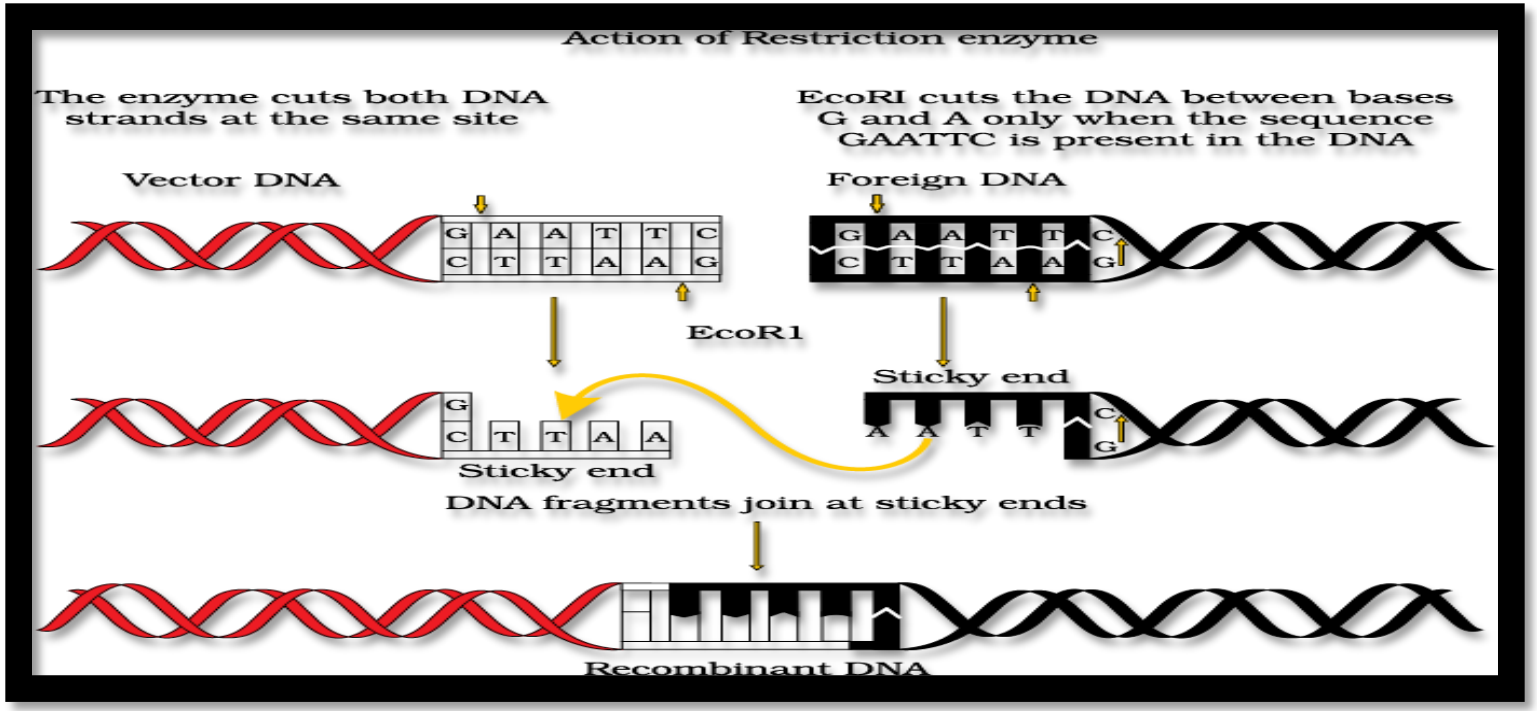
Process of Development of Recombinant Protein Biotech Product:



Isolation of gene of interest:

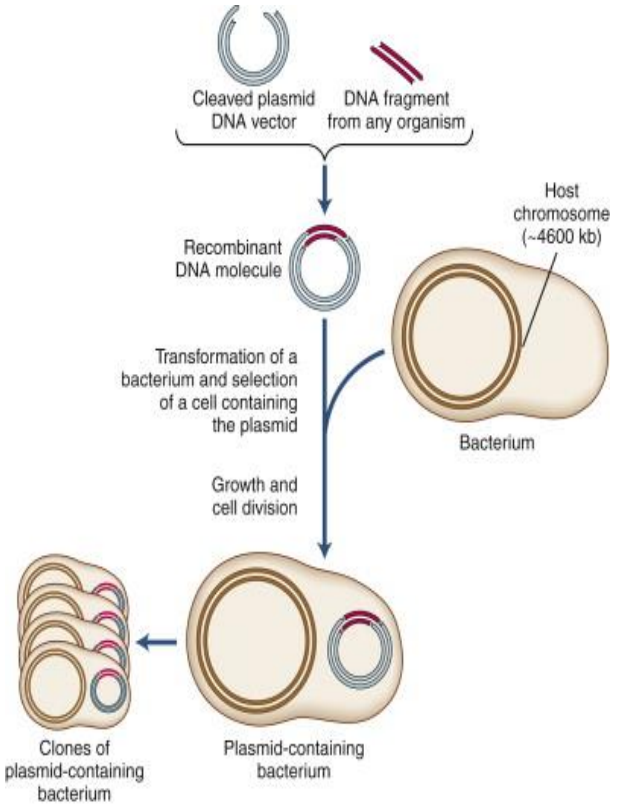
- **Genes** are pieces of DNA which store information for specific proteins that control specific traits. Genes are present in nucleus chromosomes which code for one polypeptide.
- The double strand of DNA are cut at specific site by special enzymes which are known as **restriction endonucleases.**
- **Ligases** enzymes used to joined pieces of DNA through covalent bond.
- Through these enzymes DNA containing genes of interest is cut and joined with vector.
- A vector, as related to molecular biology, is a DNA molecule (often plasmid or virus) that is used as a vehicle to carry a particular DNA segment into a host cell as part of a cloning or recombinant DNA technique

RESRTICTION ENZYME



DNA RECOMBINATION

- DNA recombination is a process in which the pieces of DNA from different organisms are artificially mixed to create Recombinant DNA.
- Steps involved in Recombinant DNA technology are:
- DNA extraction
- Purification
- Fragmentation



Transfer of Gene to Expression Vector:

- Different organism may be used to express a target protein.
- The most commonly used organism is Escherichia coli
- However not all proteins can be successfully expressed in E coli and other systems may therefore be used such as
- Yeasts e.g commonly used for protein expression
- in Baculovirus
- Mammalian cell culture
- Plants
- Animals

Growth of Cells:

- After the transfer of rDNA into host of choice , i.e bacteria, yeast, Ecoli they are cultivated in culture medium.
- For research (small scale):
- For growth on small scale incubators are used. The most commonly used is shake flask incubator
- For productions (large scale):
- Fermentors and Bioreactors are used.

Culture media

- It is important to provide nutritional conditions that exist in bacterial natural habitat
- Common components:
- Water
- Source of carbon and energy
- Source of nitrogen
- Trace elements
- Growth factors
- Buffer
- For growth on small scale incubators are used, the most commonly used is shake flask incubator

Fermentors and Bioreactors

Bioreactor or fermentor is a container in which substrate is turned into product where gene of interest is mass produced.

- Types of production process are:
- Batch
- Continuous
- Fed batch

Batch process

- In this process the bioreactor is only fed once
- The bioreactor will be allowed to run till completion
- Very difficult to achieve in real life because there should be no input to or withdrawal from the bioreactor even for sampling

Advantages and disadvantages of batch operations

- **Advantages**:
- Ease in operating
- Genetic stability of organism could b controlled if it is genetically engineered biocatalyst
- Lower contamination risk
- **Disadvantages**:
- Non productive down time
- Batch to batch variability is problem
- Accumulation of inhibitory product

Continuous process

- The bioreactor is fed continuously
- The amount of feed introduced into the bioreactor equals the removed volume.
- The process is sensitive and subjected to influence from various factors.

Advantages and disadvantages of continuous operations

- **Advantages:**
- Efficient, higher productivity
- Uniform quality of product
- No accumulation of inhibitory products
- **Disadvantages:**
- Destruction of biocatalyst
- Higher contamination risk

Fed batch process

- In fed batch process, one or more nutrients are fed to the bioreactor during cultivation and in which the product remain in the bioreactor until the end of run
- Possible to control the rate of growth of the microorganisms or the concentration of the biomass by controlling the fee parameters
- Most commonly used process in industry

Isolation & Purification of Protein:

- Protein is isolated from microorganism and purified.
- For isolation of extracellular products destruction of cell is not necessary.
- For intracellular cell disruption is needed which is done through following methods,
- Detergents lysis
- Enzymatic lysis
- Osmotic lysis
- Freeze-thaw cycles
- Ultrasonication
- Homogenization

Formulation of protein products:

- Freeze drying or lyophillization is the common technique used for protein product formulation.
- Finally protein biotech drug are prepared.

PRODUCTION OF MONOCLONAL ANTIBODIES

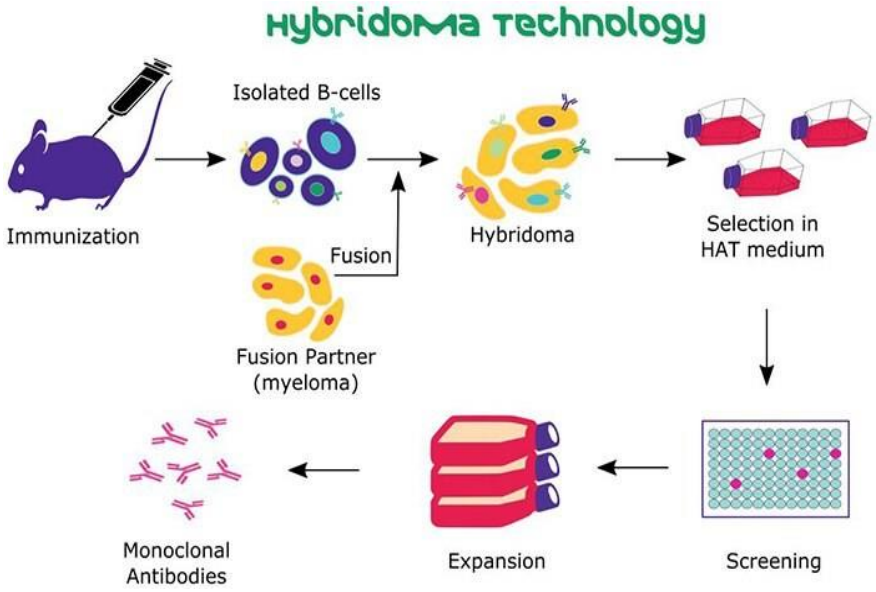
- Monoclonal antibodies are produced by Hybridoma technology.

HYBRIDOMA TECHNOLOGY:

- Hybridoma technology involves fusion of plasma B cells and myeloma cells resulting in production of hybrid cells.

STEPS INVOLVED IN MONOCLONAL ANTIBODY PRODUCTION

- Immunization Of Mice
- Screening Of Mice For Antibody Production
- Fusion of Myeloma Cells with Immune Spleen Cells
- Separation Of fused Hybridoma Cell and Screening
- Cloning of Hybridoma cells



Step 1: - Immunization of Mice & Selection Of Mouse Donor For Generation Of Hybridoma cells

- The first step in making a hybridoma is to generate antibody producing B cells. This is done by immunizing a mouse against the antigen of interest. IP (intra peritoneal injections) are more commonly used route of administration for immunization.

Step 2: - Screening Of Mice for Antibody Production

- After several weeks of immunization, tests are performed and examined for the presence of antibodies
- If the host is producing the desired antibody, the spleen is removed and dissociated in culture medium to release the resident B cells.

Step 3:- Fusion of Myeloma Cells with Immune Spleen Cells

- Plasma cells producing antibodies are fused with myeloma cells to produce hybridoma cells. Hybridoma cells take the advantage of both cells to mass produce antibodies of interest.
- Fusion can be enhanced using various methods;
- Chemical agents e.g. PEG (Polyethylene glycol)
- Physical agents e.g. Electro fusion

Step 4:- Isolation Of Fused Hybridoma Cell And Screening

- After fusion three types of cells are present in mixture;
- Un fused myeloma cells
- Un fused spleen cells
- Fused (hybridoma) cells
- It is necessary to separate hybridoma cells from un fused myeloma and plasma cells. Selection of hybridoma cells is performed by using HAT (hypoxanthine, aminopterin, thymidine) medium.

Step 5: - Cloning of Hybridoma Cell Lines

- Clone each positive culture
- Test each supernatant for antibodies
- Expand positive cultures either

✓In vitro low concentrations are produced (1-10 microgram/ml)

✓In vivo high concentrations are produced (1-10 mg/ml.

Gene therapy

- **Gene therapy:**
- It is an experimental technique for correcting defective genes that are responsible for disease development.

- **Types of gene therapy:**
- Somatic gene therapy
- Germ line gene therapy

Somatic gene therapy

- Somatic gene therapy involves transfer of a section of DNA to somatic cells of the body.
- Not passed to future generation.
- Short lived
- Appropriate and acceptable for many disorders such as:
  - ✓Cystic fibrosis
  - ✓Muscular dystrophy cancer
  - ✓Infectious diseases

Types of somatic gene therapy

***Ex vivo* gene therapy:**

- Cells are modified outside the body and then transplanted back into the body.

***In vivo* gene therapy:**

- Genes are changed in cells when the cells are still in the body.

Germ line gene therapy

- Germ line gene therapy is transfer of a section of DNA to germ line cells.
- Effects of gene therapy Result in permanent changes.
- Effects passed to future progeny.
- Possibility of eliminating some diseases from a particular family.

Issues in biotech products

- Issues related to the use of protein based drugs:

❑ **Drug delivery**

- Denaturation or chemical alteration
- Rapid liver clearance

❑ **antigenicity**

- Foreign protein may induce allergic reactions

❑**Stability**

- Denaturation leads to loss of 3D conformation of protein
- Covalent bond breaking at high pressure and low pH

Sterility consideration of biotech drugs

- It is impossible to sterilize the end product therefore it is important that

a) All the raw material should be sterilized

b) All the equipments should be sterilized

c) processing should be carried out in aseptic environment

Quality control

- **Viral decontamination**

There is no well determined mean to detect viruses in cell culture

- **Bacterial decontamination**

Filtration sterilization of the final product by bacterial filter “0.22 micrometer membrane filter

- **Pyrogen removal**

Applications of biotechnological products

Vaccines

used to stimulate the immune system against a particular disease

- Recombinant DNA vaccine eg
- Hepatitis A and hepatitis B vaccine and lyme disease vaccines are commonly available.

Monoclonal antibodies

Trastuzumab used to treat metastatic breast cancer

Gene therapy

- The potential scope of gene therapy is enormous. Following are examples of potential gene therapies
- Immune deficiencies
- Hereditary blindness
- Hemophilia
- Parkinson’s disease
- Cancer
- Diabetes

Forensic applications:

DNA fingerprinting is classic example of forensic application. It is most widely used for law enforcement and crime scene investigation

Nonclinical Studies

- The Committee for Medicinal Products for Human Use (CHMP) has adopted ICH S6 as a guideline governing preclinical testing of biologics.
- The CHMP adopted the addendum to this guideline, and the addendum came into effect in Europe in 2011.
- The addendum complements, clarifies, and updates ICH S6 and is intended to further harmonize the standards for nonclinical studies.

General Principles

The addendum covers the following five topics：

- Species selection
- Study design
- Immunogenicity
- Reproductive and developmental toxicity
- Carcinogenicity

Species Selection

- According to the addendum the factors that sponsors should consider are as follows:
  - ✓ subsequent *in vitro* assays making qualitative and quantitative cross species comparisons of relative target binding affinities.
  - ✓ receptor– ligand occupancy.
  - ✓ kinetics.
  - ✓ Sponsors also should assess functional activity.
- Specific instructions are provided for mAbs, for which a short term safety study in one species and no additional toxicity studies are recommended.
- If there are two relevant species (one rodent and one non rodent), the sponsor should conduct short-term studies in both.
- If the toxicologic findings from these studies are similar for both species, long-term studies may involve one of those species.

Study Design

- When selecting the high dose for toxicity testing , the high dose should be the higher of:

  ✓ The dose providing the maximum intended pharmacologic effect in the preclinical species.

  ✓ The dose providing an approximately 10-fold exposure over the maximum exposure to be achieved in the clinic.

  ✓ When no PD endpoint is available, the sponsor should select the high dose based on PK data.

- Generally, repeat-dose toxicity tests should have a duration of 6 months; studies of longer duration are not considered valuable.
- Finally, the sponsor may need to assess subject recovery from the medicine's pharmacologic and toxicologic effects.

Immunogenicity

- As noted in ICH S6, non clinical studies are not useful in predicting potential immunogenicity of human or humanized proteins in humans.
- The sponsor should measure antidrug antibodies (ADAs), namely when

  (1) There is evidence of altered PD activity.

  (2) There are unexpected changes in exposure in the absence of a PD marker.

  (3) there is evidence of immune-mediated reactions.
- Collection of appropriate samples during the study is recommended.

Reproductive and Developmental Toxicity

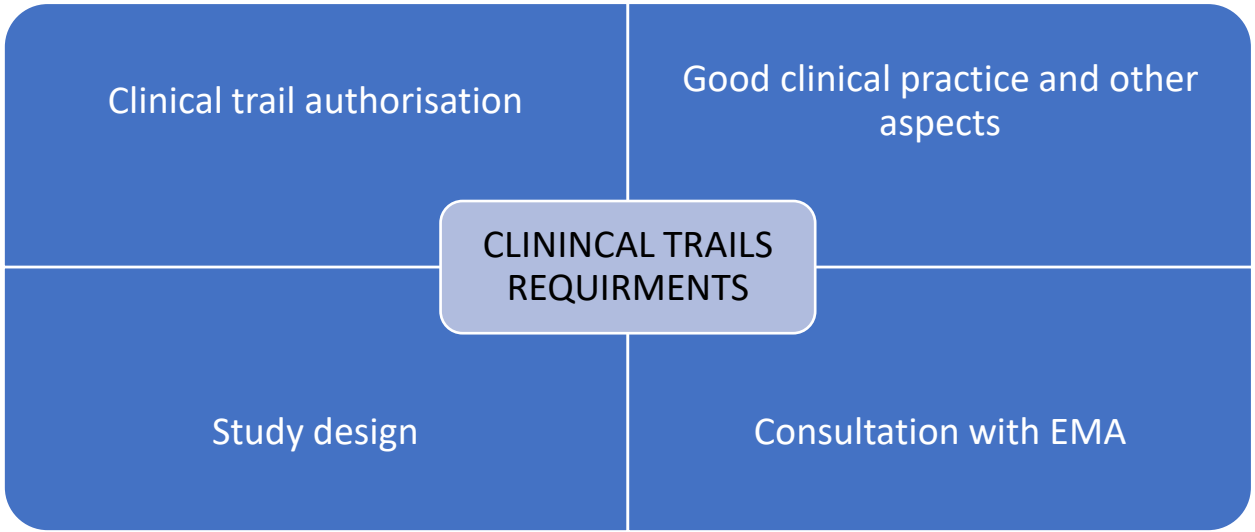
- The addendum first provides general advice on reproductive and developmental testing and then discusses more specific recommendations for
  - ✓ Fertility studies.
  - ✓ Embryo–fetal development (EFD) studies.
  - ✓ pre-and post natal development (PPND)studies.
  - ✓ Studies in nonhuman primates (NHPs).
- Reproductive studies should occur in a relevant species, but no such studies are required for products directed at foreign targets, such as bacteria and viruses.
- Sponsors should not use NHPs in developmental testing unless they are the only relevant species, and even then, the sponsor can provide scientific justification to use an alternative model.
- If no relevant species exists, you may consider using transgenic mice.
- If the weight of the evidence suggests an adverse effect on fertility or pregnancy, and additional studies might not be warranted.
- When the product's mechanism of action raises serious developmental toxicity concerns and NHPs are the only relevant species.
- No study is necessary. instead, the labeling should disclose the concern, and the sponsor should avoid administering the candidate to women of child bearing potentia

Carcinogenicity

- As noted, carcinogenicity assessments of biologics are not always warranted, but the addendum provides advice for use in situations when they are appropriate.
- According to the addendum, the sponsor may design a strategy addressing potential carcinogenicity based on a weight of evidence, including a review of relevant information such as :
- Literature.
- Target biology and mechanisms of action.
- *In vitro* data .
- Clinical data.
- Data from chronic toxicity studies.
- In situations the weight of the evidence supports them, the hazard should be addressed through product labeling and risk management practices.
- If the weight of the evidence is unclear, the sponsor can propose additional studies to address it.
- If this assessment instead suggests no carcinogenicity concern, additional nonclinical testing is not recommended.

Clinical Studies in Compliance with the Clinical Trials Directive

- This section summarizes the requirements of the Clinical Trials Directive.



Clinical Trial Authorization

A clinical trial may commence only if :

- ✓ The anticipated therapeutic and public health benefits outweigh risks and inconveniences to the subjects.
- ✓ The trial subjects understand the objectives and risks of the trial and give informed, written consent to participate.
- ✓ The trial safeguards the physical and mental integrity of the subjects.
- ✓ Insurance covers the liability of the sponsor and investigator.

- In general, the sponsor must take responsibility for the following:
  - ✓ Trial conduct
  - ✓ Appointment of an appropriate investigator
  - ✓ selection of the institution that will conduct the trial
  - ✓ data collection standards etc.,
- The trial may begin only if

(1) The ethics committee has issued a favorable opinion.

(2) No competent authority has informed the applicant of grounds for non acceptance.

Good Clinical Practices and Other aspects for Clinical Trials

- Clinical trials of biologics must comply with Good Clinical Practice and the ICH E6.
- Investigators must obtain freely given informed consent.
- Clinical trial information must be handled, recorded, and stored with respect for relevant confidentiality and privacy rules.
- Trials must comply with the ethical principles.
- Confirm that the benefits of the trial outweigh the risks, and ensure safety to the subjects.
- CHMP has issued a guideline on quality :
  - ✓ An adequate description of the process and process controls.
  - ✓ A description and justification of “any reprocessing during manufacture of the drug substance”.
- For first-in-human (FIH) clinical trials, sponsors should use product representative of the material used during the nonclinical testing phase.

Study Design Considerations

- General guidance on study design applies to biologics as well as small molecule medicines.
- Phase I usually involves the initial safety and pk studies.
- Phase II study is a therapeutic exploratory study that explores efficacy.
- Phase III typically involves therapeutic confirmatory studies.

Consultation with the EMA

- A sponsor may obtain, from the EMA, scientific advice regarding clinical trial protocols.
- Generally focus on matters such as the selection of endpoints and comparator, the duration of treatment or follow-up, and the design of pivotal studies.
- If the applicant decides not to follow the EMA's advice, it should justify this decision in its MAA.

The Marketing Authorization Application- Contents and Approval Standard

The requirements of the EU centralized procedure are :

- ✓ The approval standards for Biotechnology products are the same as for chemically synthesized medicines.
- ✓ Both types of products must be safe and effective and have appropriate quality.

MAAs for biologics also must meet special requirements:

- ✓ The applicant must thoroughly describe the manufacturing process , facilities and equipment.
- ✓ provide information on the origin and history of the starting materials.
- ✓ demonstrate that the active substance complies with specific measures for preventing the transmission of animal spongi form encephalopathies.
- ✓ if cell banks are used, demonstrate that cell characteristics remain unchanged at the passage level for production (and beyond).
- ✓ describe the origin, criteria, and procedures for the collection, transportation, and storage of the starting material if they derived from human blood and plasma

REGULATORY STRATEGIES FOR WORLDWIDE MARKETING OF BIOLOGICAL PRODUCTS

- **Acceptance of Foreign Clinical Studies in the Europe**

- Europe Directive 2001/83/EC allows for clinical trials conducted outside the European Union.
- If such trials have been designed, implemented, and reported based on principles equivalent to those of the Clinical Trials Directive with regard to good clinical practice and ethical principles.

PRACTICAL CASES:

Group No. 1.

You are developing a monoclonal antibody. Make a list of the main stages of the development of a biological drug.

Outline what research is needed at each stage (eg, preclinical and clinical research).

Group No. 2.

You are developing a skin product based on a recombinant protein. Design an experiment to evaluate the safety and efficacy of a biologic in laboratory animals.

Design a virtual experiment using an animal model to evaluate the toxicity and efficacy of a selected biologic.


Thank
You

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