Structure and Activity of Drug Substance, Active Pharmaceutical Ingredients

Buzder-Lantos Péter
Chinoin - Sanofi
Drug discovery-Medicinal chemistry
World of abbreviations

COA
Caco2
ADME
ADME
CYP inhibition
API
TPP
PK
HTS
IC50
EC50
PAMPA
PAMPA
CYP inhibition
LD50
DMPK
Clinical POC
CMC
DC
PAMPA
CYP inhibition
MTS
Lead
EC50
Cherrypicking
PerOs
POC
EC50
Half life
IV
COA
DMF
Hit
PerOs
Half life
Falsh positive
Clinical POC
In Vivo
In vitro
CYP inhibition
CEREP
Hit
CYP inhibition
CEREP
In vitro
Falsh positive
CYP inhibition
CEREP
In vitro
Content

- Types, characteristics and classification of Drugs and APIs
  - Drug product forms and application methods
  - Molecular mechanism of APIs
- Structure-activity relationships
  - Path and fate of the drug in the body, toxicity
- Drug discovery
  - Strategies (HTS, design, fragment screen etc.)
  - Hit, Lead, DC
    - definitions, selection criterion
  - H2L, L2C optimization. Types, characteristics and classification of Drugs and APIs
Types, characteristics and classification of Drugs and APIs
Classification of drugs

Definitions

- **Finished Dosage Form (FDF) or Drug Product (DP)**
  A Finished Pharmaceutical Product (FPP), prepared for consumer applications, containing excipients and the Active Pharmaceutical Ingredient (API).

- **Active Pharmaceutical Ingredient (API)**
  A substance used in a Finished Pharmaceutical Product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings. (WHO Technical Report Series, No. 961, Annex 10)

- **Excipient**
  Any substances, other than the Active Pharmaceutical Ingredient (API), that have been appropriately evaluated for safety and are included in a drug delivery system to either aid the processing of the drug during its manufacture or protect, support or enhance stability, bioavailability, or patient acceptability, assist in product identification, or enhance any other attribute of the overall safety and effectiveness of the drug delivery system during storage and use.
In Europe, the term is "medicinal product", and it is defined by EU law as:

- "(a) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or
- (b) Any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis."

In the US, a "drug" is:

- A substance recognized by an official pharmacopoeia or formulary.
- A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.
- A substance (other than food) intended to affect the structure or any function of the body.
- A substance intended for use as a component of a medicine but not a device or a component, part or accessory of a device.
- Biological products are included within this definition and are generally covered by the same laws and regulations, but differences exist regarding their manufacturing processes (chemical process versus biological process.)
Classifications of Drugs
Types of classification

• Level of control
• Basis of their origin
• Mechanism of action
• Route of administration
• Biological system affected
• Anatomical Therapeutic Chemical Classification System (ATC system)
• Dosage form
• By patent status
Classifications of Drugs

Level of control

- **Prescription drug**
  
  Prescription drug is a pharmaceutical drug that legally requires a medical prescription to be dispensed. Prescription drugs are often dispensed together with a monograph (EP, USP, IP...) that gives detailed information about the drug.

- **Over-the-counter (OTC) drug**
  
  OTC drugs are medicines sold directly to a consumer without a prescription, from a healthcare professional, as compared to prescription drugs, which may be sold only to consumers possessing a valid prescription. In many countries, OTC drugs are selected by a regulatory agency to ensure that they are ingredients that are safe and effective when used without a physician's care. OTC drugs are usually regulated by active pharmaceutical ingredients (APIs), not final products. By regulating APIs instead of specific drug formulations, governments allow manufacturers freedom to formulate ingredients, or combinations of ingredients, into proprietary mixtures.
“Formerly, ‘Regular’ and ‘Extra-Strength’."

“It may surprise you to hear that, actually, morphine is the best medicine.”
Classifications of Drugs
Basis of their origin

- **Drug from natural origin**: Herbal or plant or mineral origin, some drug substances are of marine origin.
- **Drug from chemical as well as natural origin**: Derived from partial herbal and partial chemical synthesis Chemical, example steroidal drugs
- **Drug derived from chemical synthesis**
- **Drug derived from animal origin**: For example, hormones, and enzymes.
- **Drug derived from microbial origin**: Antibiotics
- **Drug derived by biotechnology genetic-engineering**, hybridoma technique for example
- **Drug derived from radioactive substances.**
Classifications of Drugs
Mechanism of action

Mechanism of Action (MoA) refers to the specific biochemical interaction through which a drug substance produces its pharmacological effect.

- specific molecular targets to which the drug binds (enzyme or receptor)
- Receptor sites
- specific action

(A: ACE inhibitors with calcium channel blocking agents, ACE inhibitors with thiazides, adamantane antivirals, adrenal cortical steroids, adrenal corticosteroid inhibitors, adrenergic bronchodilators, agents for hypertensive emergencies, agents for pulmonary hypertension, aldosterone receptor antagonists, alkylation agents, allergens, alpha-glucosidase inhibitors, alternative medicines, amebicides, aminoglycosides, aminopenicillins, aminosalicylates, AMPA receptor antagonists, amylin analogs, analgesic combinations, analgesics, androgens and anabolic steroids, angiotensin converting enzyme inhibitors, angiotensin II inhibitors with calcium channel blockers, angiotensin II inhibitors with thiazides, angiotensin receptor blockers, angiotensin receptor blockers and neprilysin inhibitors, anorectal preparations, anorexants, antacids, anthelmintics, anti-angiogenic ophthalmic agents, anti-CTLA-4 monoclonal antibodies, anti-infectives, antiadrenergic agents (central) with thiazides, antiadrenergic agents (peripheral) with thiazides, antiadrenergic agents, centrally acting, antiadrenergic agents, peripherally acting, antiandrogens, antianginal agents, antiarrhythmic agents, antiasthmatic combinations, antibiotics/antineoplastics, anticholinergic antiemetics, anticholinergic antiparkinson agents, anticholinergic bronchodilators, anticholinergic chronotropic agents, anticholinergics/antispasmodics, anticoagulant reversal agents, anticoagulants, anticonvulsants, antidepressants, anti diabetic agents...)

Example:

Aspirin

The mechanism of action of aspirin involves irreversible inhibition of the enzyme cyclooxygenase; therefore suppressing the production of prostaglandins and thromboxanes, thus reducing pain and inflammation. This mechanism of action is specific to aspirin, and is not constant for all nonsteroidal anti-inflammatory drugs (NSAIDs). Rather, aspirin is the only NSAID that irreversibly inhibits COX-1.
Classifications of Drugs

Route of administration

- **Oral**
  The most convenient and carries the lowest cost.

- **Topical**
  By delivering drugs almost directly to the site of action, the risk of systemic side effects is reduced. Skin irritation may result, the dosage is difficult to control.

- **Sublingual**
  This method refers to the pharmacological route of administration by which drugs diffuse into the blood through tissues under the tongue. For ex.: cardiovascular drugs, steroids, barbiturates, enzymes and increasingly, vitamins and minerals.

- **Inhalation**
  Inhaled medications can be absorbed quickly, and act both locally and systemically. Proper technique is necessary to achieve the correct dose. Inhalation is the most rapid way to deliver drugs to the brain, as the substance travels directly to the brain without being diluted in the systemic circulation.

- **Injection**
  The term injection encompasses **intravenous (IV)**, **intramuscular (IM)**, and **subcutaneous (SC)** administration.

  Injections act rapidly, with onset of action in 15–30 seconds for IV, 10–20 minutes for IM, and 15–30 minutes for SC, with 100% of bioavailability, and can be used for drugs that are poorly absorbed or ineffective when given orally.
Classifications of Drugs

Biological system affected

- **Gastrointestinal tract** - digestive system
  - **Upper digestive tract**: reflux suppressants, proton pump inhibitors (PPIs), H2-receptor antagonists
  - **Lower digestive tract**: antispasmodics, antidiarrhoeals, bile acid sequestrants, opioid

- **Cardiovascular system**
  - **General**: β-receptor blockers ("beta blockers"), calcium channel blockers, diuretics, vasodilators.
  - **Affecting blood pressure/(antihypertensive drugs)**: ACE inhibitors, angiotensin receptor blockers, beta-blockers, calcium channel blockers,
  - **Coagulation**: anticoagulants, heparin, antiplatelet drugs
  - **HMG-CoA reductase inhibitors**: hypolipidaemic agents.

- **Central nervous system**
  - Hypnotics, anaesthetics, antipsychotics, antidepressants

- **For pain and consciousness (analgesic drugs)**
  - The main classes of painkillers are NSAIDs, opioids and Local anesthetics.

- **Musculo-skeletal disorders**
  - The main categories of drugs for musculoskeletal disorders are: NSAIDs (including COX-2 selective inhibitors), muscle relaxants, neuromuscular drugs
Classifications of Drugs

Biological system affected cont’

- For the eye
  - Antibacterial, antiviral, anti-fungal, anti-inflammatory, anti-allergy, anti-glaucoma drugs

- For the ear, nose
  - Antibiotics, antihistamines, NSAIDs, corticosteroids, antiseptics, local anesthetics

- Respiratory system
  - bronchodilators, antitussives, mucolytics, decongestants, inhaled and systemic corticosteroids,

- Endocrine system
  - androgens, antiandrogens, estrogens, corticosteroids, human growth hormone, insulin, antidiabetics, thyroid hormones, antithyroid drugs

- Reproductive and urinary system
  - antifungal, quinolones, antibiotics, cholinergics, anticholinergics, fertility medications

- For the skin
  - antifungals, disinfectants, systemic antibiotics, hormones, sunscreens, antiperspirants, corticosteroids, immune modulators

- For the immune system
  - Vaccines, immunoglobulins, immunosuppressants, interferons, monoclonal antibodies
  - For infections: antibiotics, antifungals, antituberculous drugs, antimalarial, antivirals, probiotics
  - For allergic disorders: anti-allergics, antihistamines, NSAIDs, Corticosteroids
Classifications of Drugs
Dosage form

- **Oral**
  - Pill, i.e. tablet or capsule syrups
  - Specialty tablet like buccal or sub-lingual
  - Thin film
  - Liquid solution or suspension (e.g., drink or syrup)
  - Powder or liquid or solid crystals
  - Pastes (e.g., Toothpaste)

- **Parenteral**
  - Intradermal (ID)
  - Subcutaneous (SC)
  - Intramuscular (IM)
  - Intraosseous (IO)
  - Intraperitoneal (IP)
  - Intravenous(IV)

- **Ophthalmic**
  - Drops
  - Cream
  - Liquid solution

- **Topical**
  - Cream, gel, liniment or balm, lotion
  - Ear drops (otic)
  - Eye drops (ophthalmic)
  - Skin patch (transdermal)
  - Vaginal rings
  - Dermal patch

- **Inhalational**
  - Aerosol
  - Inhaler
  - Nebulizer
  - Smoking
  - Vaporizer

- **Suppository**
  - Vaginal (e.g., douche, pessary, etc.)
  - rectal
  - Urethral suppositories
  - Nasal suppositories
  - Ear cones
Classifications of Drugs
By patent status

● **Original drug**
  - Discovered and Developed by a pharmaceutical company
  - Patented
    - Novelty
    - Inventive
    - Priority
  - Exclusivity on the market for 20 years (can be prolonged by 5 years)

● **Generic drug**
  - Identical in dose, strength, route of administration, safety, efficacy, and intended use with original drug product
  - Bioequivalent
    - Pharmacokinetic properties
    - Pharmacodynamic properties
  - Biosimilar
    - Generic biological drugs
Drug product forms and application methods
Target Product Profile (TPP)

- A Target Product Profile (TPP) is a planning tool for therapeutic candidates based on FDA Guidance for Industry and Review Staff Target Product Profile — A Strategic Development Process Tool

- Describes Ideal results and minimum acceptable results
  - Primary indication
  - Patient population
  - Treatment duration
    - Chronic for example
  - Delivery mode /Dosage form/Regimen
    - Oral/capsule/1-2X daily
  - Efficacy
  - Risk
Therapeutic index (TI) is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity.

\[ TI = \frac{TD_{50}}{ED_{50}} \]

- TD$_{50}$ - toxic dose in 50% of subjects
- ED$_{50}$ - efficacious dose in 50% of subjects

In contrast, in a drug development setting
TI is calculated based on plasma exposure levels.

A higher therapeutic index is preferable to a lower one

A drug with a narrow therapeutic range (i.e. having little difference between toxic and therapeutic doses) may have its dosage adjusted according to measurements of the actual blood levels achieved in the person taking it. This may be achieved through therapeutic drug monitoring (TDM) protocols. TDM is recommended for use in the treatment of psychiatric disorders with lithium due to its narrow therapeutic range.
Drug formulation

- API and excipients mixed in a controlled way to ensure proper bioavailability of the proper dosage and to enable the proper administration.
  - Shelf life depends on the stability of the API and the stability of the drug substance
  - Stability of the API within the given formula is depending on the compatibility of excipient and the API
  - Formulation often depends on physicochemical properties of the API
    • Particle size distribution, pH, Polimorphism, solubility, bulk density

- Tablets, capsules
  - uniform appearance, with an acceptable taste, tablet hardness, or capsule disintegration
  - Wet granulation,
    • Water, ethanol, isopropanol
  - direct pressing, dry granulation

- Injectables
  - API is dissolved in a liquid
  - Often needs refrigaeration to keep stability
Tablets - composition and release

- **Tablet composition**
  - 5-10% of the drug: API
  - 80% of fillers, disintegrants, lubricants, glidants, and binders; and
  - 10% of compounds which ensure easy disintegration, disaggregation, and dissolution of the tablet in the stomach or the intestine.

- **Sustained release**
  - **Special coatings** can make the tablet resistant to the stomach acids resulting disintegration in the latter trackts of the gastointestinal system
  - Embeddign the active ingredient in an **insoluble porous matrix**, such that the dissolving drug must make its way out of the matrix before it can be absorbed.
  - Matrix swelling
    - To form a **gel** through which the drug exits.
  - Osmotic controlled-release oral delivery system where the active compound is encased in a water-permeable membrane with a laser drilled hole at one end. As water passes through the membrane the drug is pushed out through the hole and into the digestive tract where it can be absorbed.

- **Coating**
  - Sugar, varnish or wax to disguise the taste
BCS Classification
Solubility-Permeability

- Class I - High Permeability, High Solubility
- Class II - High Permeability, Low Solubility
- Class III - Low Permeability, High Solubility
- Class IV - Low Permeability, Low Solubility

**CLASS BOUNDARIES**

- A drug substance is considered **HIGHLY SOLUBLE** when the highest dose strength is soluble in < 250 ml water over a pH range of 1 to 7.5.
- A drug substance is considered **HIGHLY PERMEABLE** when the extent of absorption in humans is determined to be > 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose.
- A drug product is considered to be **RAPIDLY DISSOLVING** when > 85% of the labeled amount of drug substance dissolves within 30 minutes using USP apparatus I or II in a volume of < 900 ml buffer solutions.
Molecular mechanism of API
Types of APIs

- **Biologicals**
  - Enzymes
  - Monoclonal antibodies
  - siRNAs
  - Large peptides

- **Vaccines**

- **Small molecules**
  - Enzyme inhibitors
  - Receptor antagonists
  - Receptor agonists
  - Hormones
  - Kinase blockers
  - Effectors on RNA transcription
Enzyme therapy

- **Pompe disease**
  - Pompe disease is a rare inherited neuromuscular disorder that causes progressive muscle weakness in people of all ages
  - Pompe disease is caused by a defective gene that results in a deficiency of an enzyme, acid alpha-glucosidase (GAA). The absence of this enzyme results in excessive buildup of glycogen, stored in a specialized compartment of muscle cells.

- **Hurler syndrome**
  - Genetic disorder that results in the buildup of glycosaminoglycans due to a deficiency of alpha-L iduronidase.

- **Hunter syndrome**
  - Very similar to Hurler syndrome. Deficiency of iduronate-2-sulfatase (I2S). The accumulated substrates in Hunter syndrome are heparan sulfate and dermatan sulfate.

- **Gaucher disease**
  - The disorder is characterized by bruising, fatigue, anemia, low blood platelet count and enlargement of the liver and spleen, and is caused by a hereditary deficiency of the enzyme glucocerebrosidase.
Antibodies (immunoglobulins IGs)

- **Types of antibodies**
  - Chimeric
  - Recombinant
  - Fully human
  - Humanized

- **Production of antibodies**
  - Fermentation
Purification of antibodies

Antibody purification involves selective enrichment or specific isolation of antibodies from serum (polyclonal antibodies), ascites fluid or cell culture supernatant of a hybridoma cell line (monoclonal antibodies).

Purification methods range from very crude to highly specific and can be classified as follows:

- **Physicochemical fractionation** – differential precipitation, size-exclusion or solid-phase binding of immunoglobulins based on size, charge or other shared chemical characteristics of antibodies in typical samples. This isolates a subset of sample proteins that includes the immunoglobulins.

- **Class-specific affinity** – solid-phase binding of particular antibody classes (e.g., IgG) by immobilized biological ligands (proteins, lectins, etc.) that have specific affinity to immunoglobulins. This purifies all antibodies of the target class without regard to antigen specificity.

- **Antigen-specific affinity** – affinity purification of only those antibodies in a sample that bind to a particular antigen molecule through their specific antigen-binding domains. This purifies all antibodies that bind the antigen without regard to antibody class or isotype.
Monoclonal antibodies

- **Effective in protein protein interactions**
  - Large surfaces with many weak interactions separated in space but resulting in very specific and strong interaction
  - Difficult and in most cases impossible to influence by small molecules

- **Main indications**
  - Cancer treatment
  - Autoimmune diseases
  - Anti-inflammatory
  - Diagnostics

- **Potential side effects of antibodies**
  - Antibodies are much more selective than small molecules, hence less adverse effect are associated with them
  - Risk of a side effect associated with an antibody is related to the long half-life in the body
  - Potential side effect might be higher rate of infections compared to control group taking placebo
# Examples
## Anti-inflammatory Mabs

<table>
<thead>
<tr>
<th>Mab</th>
<th>Indications</th>
<th>Mechanism</th>
<th>Type</th>
</tr>
</thead>
</table>
| infliximab  | • Rheumatoid arthritis  
• Crohn's disease  
• Ulcerative Colitis  
• Ankylosing spondylitis | Inhibits TNF-α                                | Chimeric   |
| adalimumab  | • Rheumatoid arthritis  
• Crohn's disease  
• Ulcerative Colitis  
• Ankylosing spondylitis | Inhibits TNF-α                                | Human      |
| basiliximab | • Acute rejection of kidney transplants | Inhibits IL-2 on activated T cells             | Chimeric   |
| daclizumab  | • Acute rejection of kidney transplants | Inhibits IL-2 on activated T cells             | Humanized  |
| ignasimab   | • Bistue's Syndrome                                                      | Inhibits Manent receptor on activated T cells  | Humanized  |
| omalizumab  | • Moderate-to-severe allergic asthma                                      | Inhibits human immunoglobulin E (IgE)         | Humanized  |
# Examples
## Anticancer drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indications</th>
<th>Targets</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>gemtuzumab</td>
<td>• relapsed acute myeloid leukemia</td>
<td>targets myeloid cell surface antigen CD33 on leukemia cells</td>
<td>humanized</td>
</tr>
<tr>
<td>alemtuzumab</td>
<td>• B cell leukemia</td>
<td>targets an antigen CD52 on T- and B-lymphocytes</td>
<td>humanized</td>
</tr>
<tr>
<td>rituximab</td>
<td>• non-Hodgkin's lymphoma, rheumatoid arthritis</td>
<td>targets phosphoprotein CD20 on B lymphocytes</td>
<td>chimeric</td>
</tr>
<tr>
<td>trastuzumab</td>
<td>• breast cancer with HER2/neu overexpression</td>
<td>targets the HER2/neu (erbB2) receptor</td>
<td>humanized</td>
</tr>
<tr>
<td>nimotuzumab</td>
<td>• Approved in squamous cell carcinomas, Glioma, Clinical trials for other indications underway</td>
<td>EGFR inhibitor</td>
<td>humanized</td>
</tr>
<tr>
<td>cetuximab</td>
<td>• Approved in squamous cell carcinomas, colorectal carcinoma</td>
<td>EGFR inhibitor</td>
<td>chimeric</td>
</tr>
<tr>
<td>bevacizumab</td>
<td>• Anti-angiogenic cancer therapy</td>
<td>inhibits VEGF</td>
<td>humanized</td>
</tr>
</tbody>
</table>
Many diseases develop from the undesirable production of specific proteins. Protein production in the cell begins with transcription. This process generates a messenger RNA (mRNA), which is then translated into protein in the cytoplasm.

Typical mRNA produces approximately 5,000 copies of a protein. Consequently, targeting mRNA rather than the protein itself is potentially a much more efficient approach to block protein function.

- RNAs of 21-23 nucleotides in length, called small interfering RNAs (siRNAs), are snipped from longer dsRNA chains by an enzyme called Dicer. The antisense strand of the siRNA is used by an RNA-induced silencing complex (RISC) to guide messenger RNA (mRNA) cleavage, so promoting mRNA degradation.
- siRNA associates with RISC and directs it to the target mRNA. The siRNA-associated RISC binds to the target mRNA through a base-pairing interaction and degrades it. RISC complex is catalytic and can cleave multiple target mRNAs.

**Administration**

- Synthetic RNA (siRNA) can be injected into the cell
- A viral vector encoding a short hairpin RNA (shRNA) can be used to deliver siRNA into the cell
- siRNA -coding DNA constructs can be incorporated into the genome
Peptides

More than 7000 naturally occurring peptides have been identified, and often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives. In general, **peptides are selective and efficacious signaling molecules** that bind to specific cell surface receptors, such as G protein-coupled receptors (GPCRs) or ion channels, where they trigger intracellular effects. Given their attractive pharmacological profile, peptides represent an excellent starting point for the design of novel therapeutics and their specificity has been seen to translate into excellent safety, tolerability, and efficacy profiles in humans.

Naturally occurring peptides are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short circulating plasma half-life. Some of these weaknesses have been successfully resolved through what we term the ‘traditional design’ of therapeutic peptides (see table: **SWOT analysis**). Besides traditional design, a range of peptide technologies has been emerging that represent the opportunities and future directions within the peptide field. These include multifunctional and cell penetrating peptides, as well as peptide drug conjugates.
Examples for peptide therapeutics

**Insulin**

*Oxytocin*

*Ecallantide*

is a drug used for the treatment of hereditary angioedema (HAE) and in the prevention of blood loss in cardiothoracic surgery. It is an inhibitor of the protein kallikrein and a 60-amino acid polypeptide.
Vaccines

A vaccine is a biological preparation that provides active acquired immunity to a particular disease.

Types

- **Inactivated**
  
  These vaccines contain inactivated, but previously virulent, micro-organisms that have been destroyed with chemicals, heat, radiation, or antibiotics. E.g. influenza, cholera, bubonic plague, polio, hepatitis A, and rabies.

- **Attenuated**
  
  Some vaccines contain live, attenuated microorganisms. Many of these are active viruses that have been cultivated under conditions that disable their virulent properties, or that use closely related but less dangerous organisms to produce a broad immune response. E.g. yellow fever, measles, rubella, and mumps, and the bacterial disease typhoid.

  Attenuated vaccines have some advantages and disadvantages. They typically provoke more durable immunological responses and are the preferred type for healthy adults. But they may not be safe for use in immunocompromised individuals, and may rarely mutate to a virulent form and cause disease.

- **Toxoid**
  
  Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism. E.g. tetanus and diphtheria. Toxoid vaccines are known for their efficacy.

- **Subunit**
  
  Protein subunit – rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine), a fragment of it can create an immune response. Examples include the subunit vaccine against Hepatitis B virus that is composed of only the surface proteins of the virus (previously extracted from the blood serum of chronically infected patients, but now produced by recombination of the viral genes into yeast), the virus-like particle (VLP) vaccine against human papillomavirus (HPV) that is composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus. Subunit vaccine is being used for plague immunization.
Vaccines cont'

- **Conjugate**
  Conjugate – certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g., toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the Haemophilus influenzae type B vaccine.

- **Experimental (vaccines under development)**
  - **Dendritic cell vaccines** combine dendritic cells with antigens in order to present the antigens to the body’s white blood cells, thus stimulating an immune reaction. (brain tumors and malignant melanoma)
  - **DNA vaccination** – an alternative, experimental approach, created from an infectious agent’s DNA. The proposed mechanism is the insertion of viral or bacterial DNA into human cells. Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them. Because these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system.
  - **T-cell receptor peptide vaccines** are under development for several diseases such as atopic dermatitis. These peptides have been shown to modulate cytokine production and improve cell mediated immunity.

- **Valence**
  Vaccines may be monovalent or multivalent. A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism, or against two or more microorganisms.

- **Heterotypic**
  These are vaccines that are pathogens of other animals that either do not cause disease or cause mild disease in the organism being treated. The classic example is Jenner's use of cowpox to protect against smallpox. A current example is the use of BCG vaccine made from Mycobacterium bovis to protect against human tuberculosis.
Small molecules enzyme inhibitors

- **An enzyme inhibitor** is a molecule that binds to an enzyme and decreases its activity
  - Competitive inhibition
    - Affinity towards the active site of the enzyme
  - Uncompetitive inhibition
    - inhibitor binds only to the substrate-enzyme complex
  - Non-competitive inhibition
    - binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate
  - Mixed inhibition
    - Allosteric binding

- **Examples**
  - Ritonavir
    - Peptidomimetic HIV-1 protease inhibitor
  - Tipranavir
    - Non-peptide based HIV-1 protease inhibitor
  - Captopril
    - ACE (Angiotensin Converting Enzyme) inhibitor
  - Axitinib
    - Protein kinase inhibitor
Small molecules
Receptor agonists, antagonists

Receptors are protein molecules with the ability of receiving chemical signal (ligand binding) and upon the chemical signal they induce a response or a cascade of responses (signal transduction)

Types of receptors
- Based on location
  - External - Cell surface receptors
  - Plasma receptors
  - Nuclear receptors
- Based on molecular actions
  - GPCR (G-protein coupled receptor)
  - Ionotropic receptors
  - Tyrosine kinase linked receptors, Enzyme linked receptors
  - Nuclear receptors
Small molecules
Receptor ligands

- **Agonist**
  - Activates the receptor resulting in a maximal biological response

- **Partial agonist**
  - Activates the receptor resulting in a partial or none maximal response

- **Inverse-agonist**
  - Blocks the constitutive activity of the receptor
  - A receptor which is capable of producing a biological-response in the absence of a bound-ligand is said to display "constitutive-activity".

- **Antagonist**
  - Binds to the receptor but do not activates it. Inhibits the binding of agonist or inverse-agonist
  - Reversible (competitive)
  - Irreversible (covalent binding)

- **Allosteric modulators**
  - They do not bind to the agonist-binding site of the receptor but instead on specific allosteric-binding sites, through which they modify the effect of the agonist, e.g. benzodiazepines (BZDs) bind to the BZD-site on the GABA-A receptor and potentiate the effect of endogenous-GABA
A GPCR is a protein with 7 transmembrane domains
Upon the ligand binding G-protein is released and dissociates
Alfa domain of G-protein induces adenylate cyclase activity (ATP is transformed to c-AMP)
C-AMP induces ion-flux by activating ion channels Na+ or Ca+
G-protein can also induce other mechanism resulting in kinase activity and arrestin activity
Examples of ionotropic receptors

- **Extracellular**
  - Nicotinic acetylcholine receptor
    - Acetylcholine, Nicotine
      - Na+, K+, Ca2+
  - Glycine receptor (GlyR)
    - Glycine, Strychnine
      - Cl−, HCO3−
  - GABA receptors:
    - GABA-A, GABA-C GABA
      - Cl−, HCO3−
  - Glutamate receptors: NMDA receptor, AMPA receptor, and Kainate receptor
    - Glutamate
      - Na+, K+, Ca2+
  - 5-HT3 receptor
    - Serotonin
      - Na+, K+
  - P2X receptors
    - ATP
      - Ca2+, Na+, Mg2+
Examples of ionotropic receptors cont.

- **Intracellular**
  - cyclic nucleotide-gated ion channels
    - cGMP, cAMP and cGTP
    - Na+, K+
  - IP3 receptor
    - IP3
      - Ca2+
  - Intracellular ATP receptors
    - ATP (closes channel)
      - K+
  - Ryanodine receptor
    - Ca2+
    - Ca2+
Small molecules
Ion channel activators / blockers

- **Ion channels** are pore-forming membrane proteins whose functions include establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane.

- **Ligand gated**
  - Ionotropic receptors

- **Voltage gated**
  - Sodium
  - Calcium
  - Potassium
  - Proton

- **Examples**
  - Calcium channel blockers for indications such as cardiovascular diseases
    - Dihydropyridines, phenylalkylamines, benzothiazepines
  - Sodium channel blockers
    - Antiepileptics
      - Carbamazepine
Small molecules Effectors on RNA transcription

- **Drugs that modulates gene transcription**
  - Hence translation to and expression of proteins
  - Selectivity is major issue

- **Immunosuppressants**
  - Immunosuppressants inhibit T-cell activation and proliferation, which play a central role in both immune responses and autoimmune diseases.

- **Estrogen agonists-antagonists**

- **Antiinflammatory drugs**
  - Aspirin and salicilates
Thank you