Program	B. Pharmacy
Semester	VI
Subject /Course	Pharmacology-III
Subject/Course ID	BP602T
Module No.	04
Module Title	Chemotherapy and immunology
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Learning Outcome of Module-4

LO	Learning Outcome (LO)	Course Outcome Code
LO1	Students understand the fundamental knowledge of various aspects of infectious diseases and immunopharmacology.	BP602.4
LO2	Learn about the drug use as immunosuppressants and immunostimulants, their mechanism of action, clinical uses, side effects and contraindications.	BP602.4
LO3	Students understand the concept of Monoclonal antibodies, Protein drugs, biosimilars and drugs targeting antigens	BP602.4
LO4	To Understand the Pharmacokinetics and pharmacological action of different class of drugs.	BP602.4

Content Table

Topic
• Urinary tract infections and sexually transmitted diseases. • Chemotherapy of malignancy • immunopharmacology • Immunostimulants, Immunosuppressant • Protein drugs • monoclonal antibodies • target drugs to antigen • biosimilars

DRUGS USED IN URINARY TRACT INFECTION

The general principles of use of AMAs for urinary tract infections (UTIs) remain the same as for any other infection. Some specific considerations are highlighted below.

Most UTIs are caused by gram-negative bacteria, especially coliforms. Majority of acute infections involve a single organism (commonest is E. coli); chronic and recurrent infections may be mixed infections. Acute infections are largely self limiting; high urine flow rates with frequent bladder voiding may suffice. Many single dose antimicrobial treatments have been successfully tried, but a three day regimen is considered optimal for lower UTIs. Upper UTIs require more aggressive and longer treatment. In any case, treatment for more than 2 weeks is seldom warranted.

Bacteriological investigations are very important to direct the choice of drug. Though, treatment may not wait till report comes, urine sample must be collected for bacteriology before commencing therapy. Most AMAs attain high concentrations in urine, smaller than usual doses may be effective in lower UTIs—antibacterial action in urine is sufficient, mucosa takes care of itself. In upper UTI (pyelonephritis) antimicrobial activity in kidney tissue is needed—doses are similar to any systemic infection.

The least toxic and cheaper AMA should be used just long enough to eradicate the pathogen. It is advisable to select a drug which does not disrupt normal gut and perineal flora. If recurrences are frequent, chronic suppressive treatment with cotrimoxazole, nitrofurantoin, methenamine, cephalexin or norfloxacin may be given.

The commonly used antimicrobial regimens for empirical therapy of uncomplicated acute UTI are given in the box.

The status of AMAs (other than urinary antiseptics) in urinary tract infections is summarized below:

Sulfonamides

Dependability in acute UTIs has decreased: not used now as single drug. May occasionally be employed for suppressive and prophylactic therapy.

Antimicrobial regimens for acute UTI (all given orally for 3–5 days)*

1. Norfloxacin 400 mg 12 hourly
2. Ciprofloxacin 250 mg 12 hourly
3. Cotrimoxazole 960 mg 12 hourly
4. Cephalexin 250 mg 6 hourly
5. Cefpodoxime proxetil 200 mg 12 hourly
6. Amoxicillin + clavulanic acid (500 + 125 mg) 8 hourly
7. Nitrofurantoin 50 mg 8 hourly or 100 mg 12 hourly × 5–7 days

* For upper UTI, the same drugs may be given for 10–14 days. Nitrofurantoin is not suitable for pyelonephritis.

Cotrimoxazole

Though response rate and use have declined, it may be employed empirically in acute UTI without bacteriological data, because majority of urinary pathogens, including C. trachomatis, are covered by cotrimoxazole. It should not be used to treat UTI during pregnancy.

Quinolones

The first generation FQs, especially norfloxacin and ciprofloxacin are highly effective and currently the most popular drugs, because of potent action against gram-negative bacilli and low cost. Nalidixic acid is also employed. However, to preserve their efficacy, use should be restricted. FQs are particularly valuable in complicated cases, those with prostatitis or indwelling catheters and for bacteria resistant to cotrimoxazole/ampicillin. The FQs should not be given to pregnant women.

Ampicillin/Amoxicillin

Frequently used in the past as first choice drug for initial treatment of acute infections without bacteriological data, but higher failure and relapse rates have made them unreliable for empirical therapy. Many E. coli strains are now ampicillin-resistant. Amoxicillin + clavulanic acid is more frequently employed.

Cloxacillin

Use is restricted to penicillinase producing staphylococcal infection, which is uncommon in urinary tract.

Piperacillin/Carbenicillin

Only in serious Pseudomonas infection in patients with indwelling catheters or chronic obstruction, and in hospitalized patients.

Cephalosporins

Use is increasing, especially in women with nosocomial Klebsiella and Proteus infections; should normally be used only on the basis of sensitivity report, but empirical use for community acquired infection is also common. Some guidelines recommend them as alternative drugs.

Gentamicin

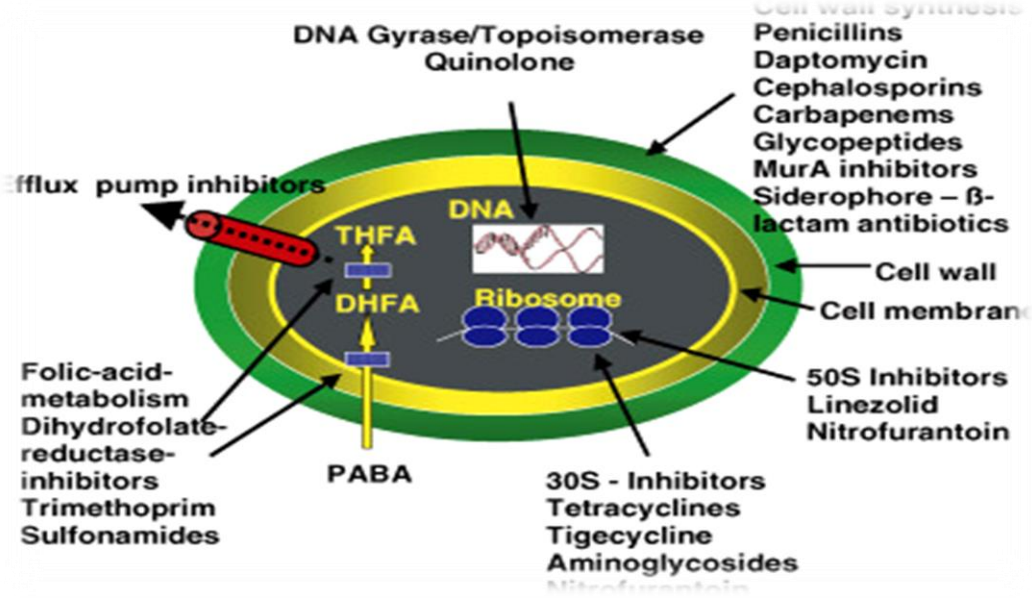
Very effective against most urinary pathogens including Pseudomonas. However, because of narrow margin of safety and need for parenteral administration, it is generally used only on the basis of in vitro bacteriological sensitivity testing. The newer aminoglycosides may be needed for hospital-acquired infections.

Chloramphenicol

Though effective in many cases, use should be restricted, for fear of toxicity, to pyelonephritis in cases where the causative bacteria is sensitive only to this antibiotic.

Tetracyclines

They are seldom effective now, because most urinary pathogens have become resistant. Though broad spectrum, they are used only on the basis of sensitivity report and in trachomatis cystitis.

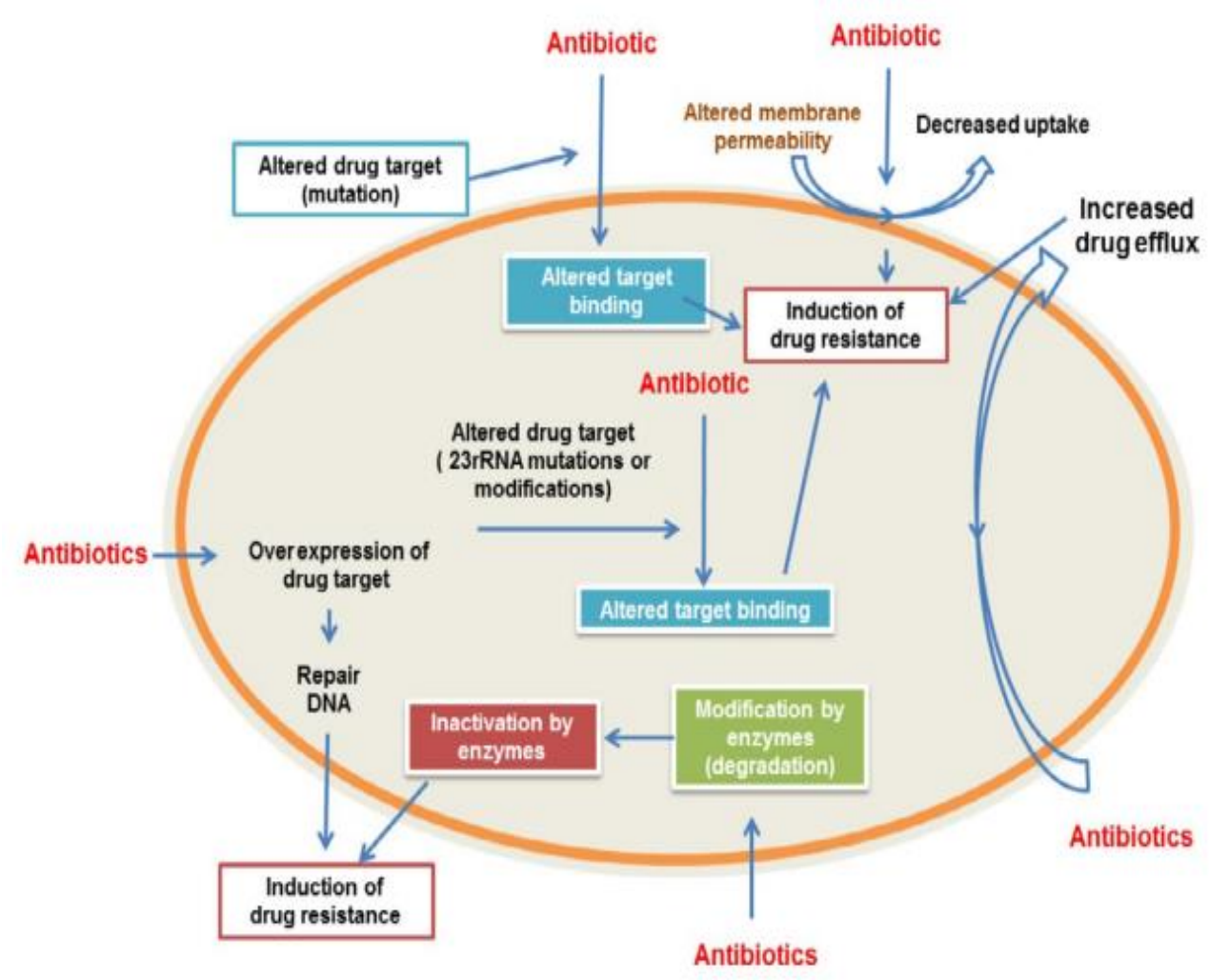


Mechanism of drugs used in UTIs

URINARY PH IN RELATION TO USE OF AMAS

Certain AMAs act better in acidic urine, while others in alkaline urine. However, specific intervention to produce urine of desired reaction (by administering acidifying or alkalinizing agents) is seldom required (except for methenamine), because most drugs used in UTI attain high concentration in urine and minor changes in urinary pH do not affect clinical outcome. In case of inadequate response or in complicated cases, measurement of urinary pH and appropriate corrective measure may help.

In certain urease positive Proteus (they split urea present in urine into NH3) infections it is impossible to acidify urine. In such cases, acidification should not be attempted and drugs which act better at higher pH should be used.



Mechanism of antibacterial resistance

URINARY INFECTION IN PATIENTS WITH RENAL IMPAIRMENT

This is relatively difficult to treat because most AMAs attain lower urinary concentration. Methenamine mandelate, tetracyclines (except doxycycline) and certain cephalosporins are contraindicated.

Nitrofurantoin, nalidixic acid and aminoglycosides are better avoided. Still, every effort must be made to cure the infection, because if it persists, kidneys may be further damaged. Bacteriological testing and followup cultures are a must to select the appropriate drug and to ensure eradication of the pathogen. Potassium salts and acidifying agents are contraindicated.

PROPHYLAXIS FOR URINARY TRACT INFECTION

This may be given when:

- Catheterization or instrumentation inflicting trauma to the lining of the urinary tract is performed; bacteremia frequently occurs and injured lining is especially susceptible.
- Indwelling catheters are placed.
- Uncorrectable abnormalities of the urinary tract are present.
- Inoperable prostate enlargement or other chronic obstruction causes urinary stasis.

SEXUALLY TRANSMITTED DISEASES

Need of the topic- Sexually Transmitted Diseases is one of the rarely discussed topic among young population although it must be one of those topic which should be discuss on priority basis, because this disease may be very lethal and depressive to the person who have infected with these, so there is no sense to feel shy or guilty to discuss on Sexually Transmitted Diseases. "Prevention is always better than cure and discussion is best medicine for preventing such disease". There are other reasons which makes this topic relevant to be posted

1. Every person wants to know more about it but feel shy to ask about it.
2. There are no such people who come forward to discuss on it (Relatively)
3. Privacy issue is major concern
4. Shy nature of young people and fear of social rejection or doubt.
5. Lack of reliable source which may provide detail information about Sexually Transmitted Diseases and many more reasons.

Sexually transmitted disease- As name suggest sexually transmitted disease is collection of all those disease which may cause by specific types of microorganisms and get transmitted from one person to another through unsafe sexual relationship or by transfusing body fluids via several sources. The causes of STDs are bacteria, parasites, yeast, and viruses. Generally Sexually Transmitted Diseases doesn't differentiate between male or female, it may affect both, but it is generally seen that the causing rate in female is higher than male. And if there is strong possibility to transfer this Sexually Transmitted Diseases to the fetus if any woman is pregnant.

There is some medicine which may treat STD which are caused by- Bacteria, Fungus, yeast but there is not yet treatment for virus generated STD but medicines can often help with the symptoms and keep the disease under control. Correct usage of latex condoms greatly reduces, but does not completely eliminate, the risk of catching or spreading STDs. The most reliable way to avoid infection is to not have unsafe and un-natural relationship.

Many Sexually Transmitted Diseases have no signs or symptoms (asymptomatic). Even with no symptoms, however, you can pass the infection to your partners. So it's important to use protection, such as a condom, during relationship. And visit your doctor regularly for STD screening, so you can identify and treat an infection before you can pass it on.

Simple classification based in their causative agents

Causative Agents	Examples
STDs caused by Bacteria	Cancroid *(Haemophilus ducreyi),* Chlamydia (*Chlamydia trachomatis*) Gonorrhea (*Neisseria gonorrhea*), Granuloma inguinal (*Calymmatobacterium granulomatous*), Lymph granuloma venereum (*Chlamydia trachomatis*), Syphilis (*Treponema palladium*)
STDs caused by Viruses	Genital herpes (herpes simplex virus), Genital warts (human papillomavirus virus) Hepatitis B and D, (hepatitis viruses, types A-E) HIV/AIDS , Molluscum contagiosum (poxvirus)
STD caused by Protozoan	Trichomoniasis (*Trichomoniasis Vaginalis*)
STD's caused by Parasites	Pubic lice or crabs (*Pediculosis pubis*), Scabies *Sarcoptes scabies*

Common sign and symptoms of sexually transmitted disease (Doesn't take it confirmatory signs)

1. Pain while urination
2. Lower abdominal pain especially in woman
3. Genital discharge in women

4. Discharge from the Genital parts in men
5. Pain during sexual intercourse in women
6. Bleeding between periods in women
7. Testicular pain in men

Types wise symptoms of sexually transmitted disease

1. Chlamydia
2. Cancroids
3. Crabs (pubic lice)
4. Genital herpes
5. Genital warts
6. Hepatitis B
7. Human immunodeficiency virus and acquired immunodeficiency syndrome
8. Human papillomavirus (HPV)
9. Trichomoniasis (parasitic infection)
10. Molluscum contagiosum
11. Pelvic inflammatory disease (PID)
12. Syphilis, gonorrhea
13. Trichomoniasis (trich)

Chlamydia

Also known as chlamydial infection, chlamydia is an STD caused by *Chlamydia trachomatis* (*C. trachomatis*), this is one type of bacterial infection which humans exclusively. Chlamydia is the one of most common infectious reason for genital and eye worldwide, generally symptoms is not easily seen but if there are any, they are usually non-specific and may include:

1. Cystitis
2. A change in vaginal discharge (color and smell)
3. Mild lower abdominal pain
4. Painful sexual intercourse, either intermittently or all the time
5. Bleeding between menstrual periods

Genital Herpes

This STD is caused by the herpes simplex virus (HSV). The virus affects the skin, cervix, genitals, and some other parts of the body.

The signs and symptoms associated with genital include:

1. Blisters and ulceration on the cervix
2. Vaginal discharge
3. Pain on urinating
4. Fever
5. Generally feeling unwell (malaise)
6. Cold sores around the mouth - for type 1 HSV

Trichomoniasis is a common sexually transmitted disease that can affect both males and females. However, women are more likely to experience symptoms because of their physiological and anatomical structure. This infection is caused by a single-celled protozoan parasite, **Trichomoniasis Vaginalis. For women,**

The most common site of infection is the vagina, while for men it is the urethra (urine canal). Transmission may occur by sexual intercourse

Signs and symptoms of trichomoniasis include:

1. Vaginal odor
2. Vaginal discharge
3. Pain or discomfort during sexual intercourse
4. Pain when urinating

A woman with trichomoniasis has more possibilities to suffer from HIV infection if she is exposed to the virus. A woman with trichomoniasis and HIV is also more likely to pass the HIV virus onto other sexual partners.

Scabiesis

Scabiesis a contagious skin condition caused by Sarcoptes scabies, a tiny mite. They burrow into the skin and lay their eggs. The patient how get infected with this disease may experience skin rashes, pain, swelling and other issue related o skin. People with scabies are often unaware of their condition for several weeks after initial infection, which means scabies infestations spread rapidly. Scabies is most commonly transmitted through close body contact, such as holding hands for a long time or sexual intercourse. Signs and symptoms may not become apparent for several weeks after initial infection, and may include:

1. A skin rash - small red spots, known as burrow marks; they look like tiny insect bites. Some people may think it is eczema.
2. Intense itching, which gets worse at night or after taking a hot shower.
3. The burrow marks, which typically appear as a small line of at least four tiny spots, appear on the elbows, wrists,, near the genitals (in men), and in between the toes and fingers.
4. After scratching the rash, the area can become inflamed, and crusty sores may develop.
5. Less commonly, the rash may appear on the buttocks, ankles, axillae (armpits), genitalia (in women), groin, the inside of the elbow, scalp, neck, face, head, shoulders, waist, soles of the feet, lower leg, and knees.

Gonorrhea- Also known as the clap or the drip, this sexually transmitted bacterial infection usually attacks the mucous membranes. Gonorrhea is the second most common STD in the U.S., after Chlamydia. The bacterium, which is highly contagious, resides in the warm and moist cavities of the body. Signs and symptoms of gonorrhea may appear from 2-10 days after initial infection, in some cases, it may take 30 days.

Males may have the following signs and symptoms:

1. Burning during urination
2. Testicular pain and/or swelling
3. A green, white, or yellow discharge from the penis

Women are less likely to show symptoms, but if they do, they may include:

1. Spotting after sexual intercourse
2. Swelling of the vulva
3. Irregular bleeding (between periods)
4. Pink eye (conjunctivitis)
5. Pain in the pelvic area
6. Burning or pain during urination

Diagnosis of sexually transmitted disease

Type of STD	Diagnosis Procedure
Chlamydia and gonorrhea	Chlamydia and gonorrhea screening is done either through a urine test or through a swab inside the penis in men or from the cervix in women. The sample is then analyzed in a laboratory. Screening is important, because if you don't have signs or symptoms, you can be unaware that you have either infection.
HIV, syphilis and hepatitis	by taking either a blood sample or a swab from any genital sores you might have. The sample is examined in a laboratory. A blood sample is taken to test for HIV and hepatitis.
Human papillomavirus (HPV)	There's no routinely used HPV screening test for men, in whom the infection is diagnosed by visual inspection or biopsy of genital warts. In women, HPV testing involves: **Pap test.** Pap tests, which check the cervix for abnormal cells, are recommended every three years for women between ages 21 and 65. **HPV test.** Women over 30 may be offered the option to have the HPV test along with a Pap test every five years if previous tests were normal. Women between 21 and 30 will be given an HPV test if they've had abnormal results on their Pap test.

Treatment/ Prevention of sexually transmitted disease

Type of STD	Possible Treatment *
HIV/AIDS	Abacavir, Didanosine, Lamivudine , Stavudine, Zalcitabine , Zidovudine , Indinavir, Nelfinavir, Ritonavir, Saquinavir, Lopinavir Plus Ritonavir, Delavirdine, Efavirenz, Nevirapine
Chlamydia	Azithromycin, Erythromycin, Doxycycline
Gonorrhea	Ceftriaxone, Cefixime, Ciprofloxacin, Ofloxacin
Pelvic Inflammatory Disease	Cefotetan or Cefoxitin Plus Doxycycline, Clindamycin Plus Gentamicin, Ofloxacin Plus Metronidazole
Human Papillomavirus	Imiquimod, Podophyllin, Podofilox, Fluorouracil (5-FU), Trichloroacetic Acid (TCA), Interferon
Genital Herpes	Acyclovir, Famciclovir, Valacyclovir
Syphilis	Penicillin. Doxycycline or Tetracycline only if allergic to penicillin

Treatment of STDs

ANTICANCER DRUGS

The anticancer drugs either kill cancer cells or modify their growth. However, selectivity of majority of drugs is limited and they are one of the most toxic drugs used in therapy.

Treatment of malignant diseases with drugs is a rather recent development—started after 1940 when nitrogen mustard was used, but progress has been rapid, both in revealing patho-biology of the diseases and in discovery of new drugs. In addition, attempts have been made to define optimal combinations, treatment strategies and patient support measures. Cancer chemotherapy is now of established value and a highly specialized field; only the general principles and an outline will be presented here.

In addition to their prominent role in leukaemias and lymphomas, drugs are used in conjunction with surgery, radiotherapy and immunotherapy in the combined modality approach for many solid tumours, especially metastatic. In malignant diseases, drugs are used with the aim of:

1. **Cure or Prolonged Remission Chemotherapy is the primary treatment modality that can achieve cure or prolonged remission in:**

In Children

- Acute leukemias

- Wilm's tumour
- Ewing's sarcoma
- Retinoblastoma
- Rhabdomyosarcoma

Other Ages

- Choriocarcinoma
- Hodgkin's disease
- Lymphosarcoma
- Burkitt's lymphoma
- Testicular teratomas
- Seminoma
- Mycosis fungoides

2. Palliation Gratifying results are obtained (shrinkage of evident tumour, alleviation of symptoms) and life is prolonged by chemotherapy in:

- Breast cancer
- Chronic lymphatic leukemia
- Ovarian carcinoma
- Chronic myeloid leukemia
- Endometrial carcinoma
- Non-Hodgkin lymphomas
- Myeloma
- Head and neck cancers
- Prostatic carcinoma
- Lung (small cell) cancer

Many other malignant tumours are less sensitive to drugs—life may or may not be prolonged by chemotherapy. Tumours that are largely refractory to presently available drugs are:

- Colorectal carcinoma
- Malignant melanomas
- Carcinoma pancreas
- Bronchogenic carcinoma

- Carcinoma stomach
- Carcinoma esophagus
- Hepatoma
- Renal carcinoma
- Sarcoma

3. Adjuvant Chemotherapy Drugs are used to mop up any residual malignant cells (micro metastases) after surgery or radiotherapy. This is routinely employed now.

Classification

A.Drugs acting directly on cells (Cytotoxic drugs)

1. Alkylating agents

Nitrogen mustards:

Mechlorethamine

(Mustine HCl)

Cyclophosphamide,

Ifosfamide,

Chlorambucil,

Melphalan

Ethylenimine: Thio-TEPA

Alkyl sulfonate: Busulfan

Nitrosoureas: Carmustine (BCNU), Lomustine (CCNU), Triazine Dacarbazine (DTIC)

2. Antimetabolites

Folate antagonist: Methotrexate (Mtx)

Purine antagonist: 6-Mercaptopurine (6-MP), 6-Thioguanine (6-TG), Azathioprine, Fludarabine

Pyrimidine antagonist: 5-Fluorouracil (5-FU), Cytarabine (cytosine arabinoside)

3. Vinca alkaloids: Vincristine (Oncovin), Vinblastine

4. Taxanes: Paclitaxel, Docetaxel

5. Epipodophyllotoxin: Etoposide

6. Camptothecin analogues: Topotecan, Irinotecan

7. Antibiotics: Actinomycin D (Dactinomycin), Doxorubicin, Daunorubicin (Rubidomycin), Mitoxantrone, Bleomycins, Mitomycin C

8. Miscellaneous

Anticancer Drugs

Different way of Classification = Cytotoxic Drugs

Alkylating Agents	Nitrogen Mustered	Mechlorethamine, Cyclophosphamide, Ifosphamide, Chlorambucil, Bendamustine, Melphalan
	Ethylenimine	Thiotepa, Altretamine
	Alkylsulonate	Busulfan
	Nitrosourease	Carmustine, Lomustine
	Triazine	Dacarbazine, Temozolomide
	Methyl hydrazine	Procarbazine
Platinum coordination complexes = Cisplatin, Carboplatin, Oxaliplatin,		
Antimetabolites	Folate antagonist	Methotrexate, Pemetrexed
	Purine antagonist	6- Mercaptopurine, 6- Thioguanine, Azathioprine, Fludarabine
	Pyrimidine antagonist	5-Flurouracil, Capecitabine, Doxyfluridine, Cytarabine, Gemcitabine
Microtubule damaging agent	Vinca Alkaloid	Vincristine, Vinblastine, Vinorelbine
	Taxanes	Paclitaxel, Docetaxel
	Estramustine	

: Hydroxyurea, Procarbazine, L-Asparaginase,Cisplatin, Carboplatin, Imatinib

Different way of Classification = Cytotoxic Drugs

Topoisomerase- 2 Inhibitors	Etoposide
Topoisomerase- 1 Inhibitors	Topotecan, Irinotecan
Antibiotics	Actinomycin D, Doxorubicin, Daunorubicin, Idarubicin, Epirubicin, Aclarubicin, Mitoxantrone , Blemomycins, Mitomycin C
Miscellaneous	Hydroxyurea, Tretinoin, Arsenic trioxide

Different way of Classification = Targeted Drugs

BCR-ABL Tyrosine kinase inhibitors	Imatinib, Dasatinib, Nilotinib
EGF (HER) receptor inhibitors	Gefitinib, Erlotinib, Cetuximab, Trastuzumab, Lapatinib
Angiogenesis Inhibitors	Bevacizumab, Sunitinib, Sorafenib
Protease Inhibitors	Bortezomib,
CD20 Inhibitors	Rituximab

B. Drugs altering hormonal milieu

1. Glucocorticoids: Prednisolone and others
2. Estrogens: Fosfestrol, Ethinylestradiol
3. Selective estrogen receptor modulators: Tamoxifen, Toremifene
4. Selective estrogen receptor down regulators: Fulvestrant
5. Aromatase inhibitors: Letrozole, Anastrozole, Exemestane
6. Antiandrogen: Flutamide, Bicalutamide
7. 5-α reductase: Finasteride, inhibitor Dutasteride
8. GnRH analogues: Nafarelin, Triptorelin
9. Progestins: Hydroxyprogesterone acetate, etc.

Glucocorticoids	Prednisolone and others
SERM (Selective Estrogen Receptor Modulators)	Tamoxifen, Toremifene
Estrogen	Ethinyl estradiol, Fosfestrol
SER- Down Regulator	Fulvestrant
Aromatase inhibitors	Letrozole, Anastrozole, Exemestane
Antiandrogens	Flutamide, Bicalutamide
5Alfa Reductase inhibitors	Finasteride, Dutasteride
GnRH Analogues	Nafarelin, Leuprorelin, Triptorelin
Progestin	Hydroxy-progesterone acetate and Others

General Toxicity of Cytotoxic Drugs

Majority of the cytotoxic drugs have more profound effect on rapidly multiplying cells, because the most important target of action are the nucleic acids and their precursors; rapid nucleic acid synthesis occurs during cell division. Many cancers (especially large solid tumours) have a lower growth fraction (lower percentage of cells are in division) than normal bone marrow, epithelial linings, reticuloendothelial (RE) system and gonads. These tissues are particularly affected in a dose-dependent manner by majority of drugs; though, there are differences in susceptibility to individual members.

Bone Marrow: Depression of bone marrow results in granulo-cytopenia, agranulocytosis, thrombocytopenia, aplastic anaemia. This is the most serious toxicity; often limits the dose that can be employed. Infections and bleeding are the usual complications.

Lymphoreticular Tissue: Lymphocytopenia and inhibition of lymphocyte function results in suppression of cell mediated as well as humoral immunity.

Because of action (1) and (2) + damage to epithelial surfaces, the host defence mechanisms (specific as well as nonspecific) are broken down → susceptibility to all infections is increased. Of particular importance are the opportunistic infections due to low pathogenicity organisms. Infections by fungi (Candida and others causing deep mycosis), viruses (Herpes zoster, cytomegalo virus), Pneumocystis jiroveci (a fungus) and Toxoplasma are seen primarily in patients treated with anticancer drugs.

Oral Cavity: The oral mucosa is particularly susceptible to cytotoxic drugs because of high epithelial cell turnover. Many chemotherapeutic drugs produce stomatitis as an early manifestation of toxicity. The gums and oral mucosa are regularly subjected to minor trauma, and breaches are common during chewing. Oral microflora is large and can be the source of infection. Neutropenia and depression of immunity caused by the drug indirectly increase the chances of oral infections. Thrombocytopenia may cause bleeding gums. Xerostomia due to the drug may cause rapid progression of dental caries.

GIT: Diarrhoea, shedding of mucosa, haemorrhages occurs due to decrease in the rate of renewal of the mucous lining. Drugs that frequently cause mucositis are—bleomycin, actinomycin D, daunorubicin, doxorubicin, fluorouracil and methotrexate.

Nausea and vomiting are prominent with many cytotoxic drugs. This is due to direct stimulation of CTZ by the drug as well as generation of emetic impulses/mediators from the upper g.i.t. and other areas.

Skin: Alopecia occurs due to damage to the cells in hair follicles. Dermatitis is another complication.

Gonads: Inhibition of gonadal cells causes oligozoospermia and impotence in males; inhibition of ovulation and amenorrhoea are common in females.

Damage to the germinal cells may result in mutagenesis.

Foetus: Practically all cytotoxic drugs given to pregnant women profoundly damage the developing foetus → abortion, foetal death, teratogenesis.

Carcinogenicity: Secondary cancers, especially leukaemias, lymphomas and histocytic tumours appear with greater frequency many years after the use of cytotoxic drugs. This may be due to depression of cell mediated and humoral blocking factors against neoplasia.

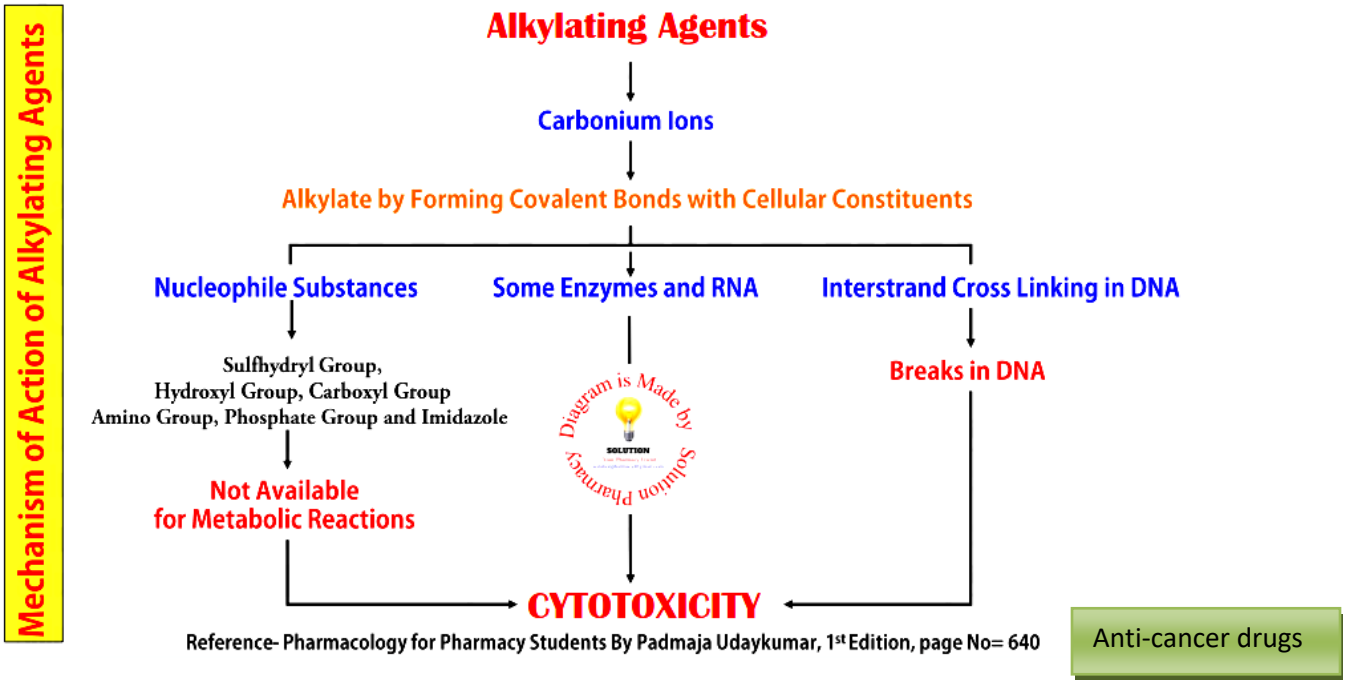
Hyperuricaemia: This is secondary to massive cell destruction (uric acid is a product of purine metabolism). Gout and urate stones in the urinary tract may develop. Allopurinol is protective by decreasing uric acid synthesis.

In addition to these general toxicities, individual drugs may produce specific adverse effects, e.g. neuropathy by vincristine, cardiomyopathy by doxorubicin, cystitis and alopecia by cyclophosphamide.

ALKYLATING AGENTS

These compounds produce highly reactive carbonium ion intermediates which transfer alkyl groups to cellular macromolecules by forming covalent bonds. The position 7 of guanine residues in DNA is especially susceptible, but other molecular sites are also involved. Alkylation results in cross linking/abnormal base pairing/ scission of DNA strand. Cross linking of nucleic acids with proteins can also take place.

Alkylating agents have cytotoxic and radiomimetic (like ionizing radiation) actions. Many are cell cycle nonspecific, i.e. act on dividing as well as resting cells. Some have CNS stimulant and cholinergic properties.



Anti-cancer drugs

Mechlorethamine (Mustine HCl)

It is the first nitrogen mustard; highly reactive and local vesicant—can be given only by i.v. route. It produces many acute effects like nausea, vomiting and haemodynamic changes. Extravasation during i.v. injection may cause sloughing.

Cyclophosphamide

It is inactive as such: produces few acute effects and is not locally damaging. Transformation into active metabolites (aldophosphamide, phosphoramide mustard) occurs in the liver, and a wide range of antitumour actions is exerted. It has prominent immunosuppressant property. Thus, it is one of the most popular anticancer drugs. It is less damaging to platelets, but alopecia and cystitis (due to another metabolite acrolein) are prominent. Chloramphenicol retards the metabolism of cyclophosphamide.

Ifosfamide

This congener of cyclophosphamide has a longer and dose-dependent t½. It has found utility in bronchogenic, breast, testicular, bladder, head and neck carcinomas, osteogenic sarcoma and some lymphomas. The dose limiting toxicity of ifosphamide is haemorrhagic cystitis. To prevent the same mesna is routinely given with it. Mesna is a –SH compound that is excreted in urine—binds and inactivates the vasicotoxic metabolites of ifosfamide and cyclophosphamide. Ifosfamide causes less alopecia and is less emetogenic than cyclophosphamide.

Chlorambucil

It is a very slow acting alkylating agent, especially active on lymphoid tissue: Myeloid tissue is largely spared. It is the drug of choice for long-term maintenance therapy for chronic lymphatic leukaemia; Hodgkin's disease and some solid tumours also resolve. It has some immunosuppressant property.

Melphalan

It is very effective in multiple myeloma and has been used in advanced ovarian cancer. Bone marrow depression is the most important toxicity. Infections, diarrhoea and pancreatitis are the complications.

Thio-TEPA

It is an ethylenimine: does not require to form an active intermediate. It has high toxicity: seldom used now.

Busulfan

It is highly specific for myeloid elements; granulocyte precursors being the most sensitive, followed by those of platelets and RBC. It produces little effect on lymphoid tissue and g.i.t. Hyperuricaemia is common and pulmonary fibrosis is a specific adverse effect. Sterility also occurs. It is the drug of choice for chronic myeloid leukaemia.

Nitrosoureas

These are highly lipid soluble alkylating agents with a wide range of antitumour activity. They cross bloodbrain barrier—are effective in meningeal leukaemias and brain tumours. Nausea, vomiting are common and CNS effects also occur. Bone marrow depression is peculiarly delayed, taking nearly 6 weeks to develop. Visceral fibrosis and renal damage can occur:

Dacarbazine (DTIC)

It is different from other alkylating agents in having primary inhibitory action on RNA and protein synthesis (others mainly affect DNA). It is activated in the liver. The most important indication is malignant melanoma; also used in Hodgkin's disease. Nausea and vomiting are prominent side effects.

ANTIMETABOLITES

These are analogues related to normal components of DNA or of coenzymes involved in nucleic acid synthesis. They competitively inhibit utilization of the normal substrate or get themselves incorporated forming dysfunctional macromolecules.

FOLATE ANTAGONIST

Methotrexate (Mtx)

It is one of the oldest and highly efficacious antineoplastic drugs; inhibits dihydrofolate reductase (DHFRase)—blocking the conversion of dihydrofolic acid (DHFA) to tetrahydrofolic acid (THFA) which is an essential coenzyme required for one carbon transfer reactions in de novo purine synthesis and amino acid interconversions. The inhibition is pseudoirreversible because Mtx has 50,000 times higher affinity for the enzyme than the normal substrate.

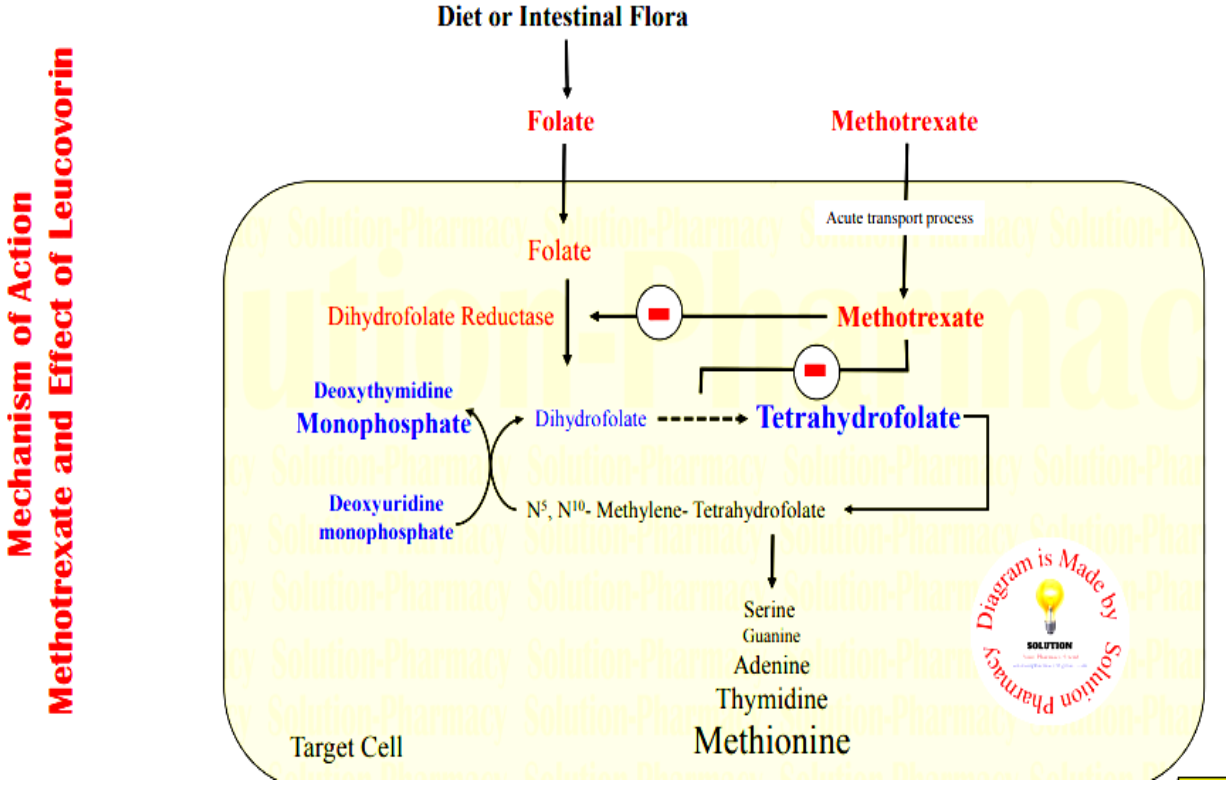
Methotrexate has cell cycle specific action— kills cells in S phase; primarily inhibits DNA synthesis, but also affects RNA and protein synthesis. It exerts major toxicity on bone marrow—low doses given repeatedly cause megaloblastic anaemia, but high doses produce pancytopenia. Desquamation and bleeding may occur in g.i.t.

Methotrexate is absorbed orally, 50% plasma protein bound, little metabolized and largely excreted unchanged in urine. Salicylates, sulfonamides, dicumerol displace it from protein binding sites. Aspirin and sulfonamides enhance toxicity of Mtx by decreasing its renal tubular secretion.

The toxicity of Mtx cannot be overcome by folic acid, because it will not be converted to the active coenzyme form. However, Folinic acid (N5 formyl THFA, cirtrovorum factor) rapidly reverses the effects. Thymidine also counteracts Mtx toxicity.

Methotrexate is apparently curative in choriocarcinoma: 15–30 mg/day for 5 days orally or 20–40 mg/m2 BSA i.m. or i.v. twice weekly. It is highly effective in maintaining remission in children with acute leukaemias, but not good for inducing remission: 2.5–15 mg/day. It is also useful in other malignancies, rheumatoid arthritis, psoriasis and as immunosuppressant.

The use of folinic acid rescue has permitted much higher doses of Mtx and has enlarged its scope to many difficult to treat neoplasms. A nearly 100 times higher dose (250–1000 mg/m2 BSA) of Mtx is infused i.v. over 6 hours, followed by 3–15 mg i.v. calcium leucovorin within 3 hours, repeated as required. This procedure can be repeated weekly.



PURINE ANTAGONISTS

Mercaptopurine (6-MP) and Thioguanine (6-TG)

These are highly effective anti-neoplastic drugs. They are converted in the body to the corresponding mono-ribonucleotides which inhibit the conversion of inosine monophosphate to adenine and guanine nucleotides. There is also feedback inhibition of de novo purine synthesis.

They are especially useful in childhood acute leukaemia, choriocarcinoma and have been employed in some solid tumours as well. In acute leukaemia, both have been used in combination regimens to induce remission and 6MP to maintain it as well.

Azathioprine

It has marked effect on T-lymphocytes; suppresses cell mediated immunity (CMI) and is used primarily as immuno-suppressant in organ transplantation, rheumatoid arthritis, etc.

All antipurines are absorbed orally (though incompletely). Azathioprine and 6MP are metabolized by xanthine oxidase; their metabolism is inhibited by allopurinol; dose has to be reduced to ¼–½ if allopurinol is given concurrently. Thioguanine is not a substrate for xanthine oxidase; follows a different (S-methylation) metabolic path and its dose need not be reduced if allopurinol is given.

Methylation by thiopurine methyl transferase (TPMT) is an additional pathway of 6MP metabolism. Genetic deficiency of TPMT makes the individual more susceptible to 6MP toxicity, while over expression of TPMT is an important mechanism of 6MP resistance by acute leukaemia cells. Toxicity of azathioprine is also enhanced in TPMT deficiency.

The main toxic effect of antipurines is bone marrow depression, which develops slowly. Mercaptopurine causes more nausea and vomiting than 6TG. It also produces a high incidence of reversible jaundice. Hyperuricaemia occurs with both; can be reduced by allopurinol.

Fludarabine

This newer purine anti-metabolite is phosphorylated intracellularly to the active triphosphate form which then inhibits DNA polymerase as well as gets incorporated to form dysfunctional DNA. Tumour cell apoptosis is promoted by multiple mechanisms confering activity even in slow growing neoplasm. It is indicated in chronic lymphatic leukaemia and non-Hodgkin's lymphoma that have recurred after treatment. Prominent adverse effects are chills, fever and vomiting after injection, myelosuppression and opportunistic infections.

PYRIMIDINE ANTAGONISTS

Pyrimidine analogues have varied applications as antineo-plastic, antifungal and antipsoriatic agents.

Fluorouracil (5-FU) is converted in the body to the corresponding nucleotide 5fluoro2deoxyuridine monophosphate, which inhibits thymidylate synthase and blocks the conversion of deoxyuridilic acid to deoxythymidylic acid. Selective failure of DNA synthesis occurs due to nonavailability of thymidylate: thymidine can partially reverse its toxicity. Fluorouracil itself gets incorporated into nucleic acids and this may contribute to its toxicity. Even resting cells are affected, though rapidly multiplying ones are more susceptible.

It has been particularly used for many solid tumours—breast, colon, urinary bladder, liver, etc. Topical application in cutaneous basal cell carcinoma has yielded gratifying results.

Cytarabine

It is phosphorylated in the body to the corresponding nucleotide which inhibits DNA synthesis. The triphosphate of cytarabine is an inhibitor of DNA polymerase and blocks generation of cytidilic acid. However, it is now believed that its incorporation into DNA is more important for the expression of cellular toxicity. It also interferes with DNA repair. Cytarabine is cell cycle specific and acts primarily during S phase. Its main use is to induce remission in acute leukaemia in children, also in adults. Other uses are—Hodgkin's disease and non-Hodgkin lymphoma.

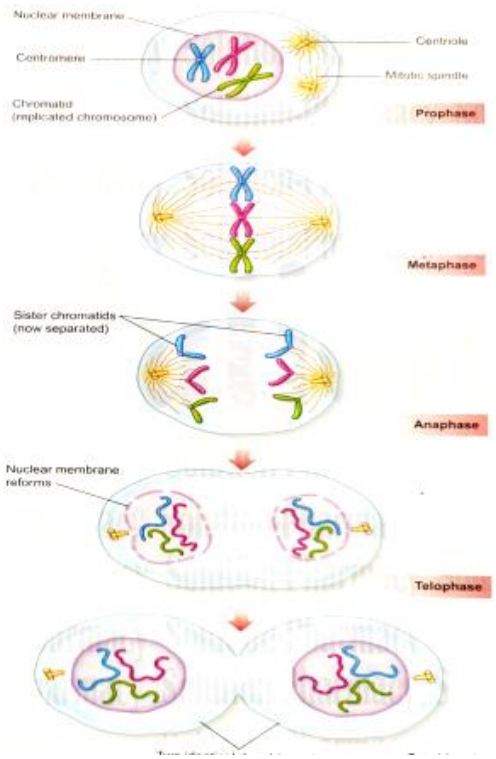
Both 5FU and cytarabine exert primary toxicity on bone marrow and g.i.t. Genetic deficiency of dihydropyrimidine dehydrogenase (DPD) predisposes to severe 5FU toxicity.

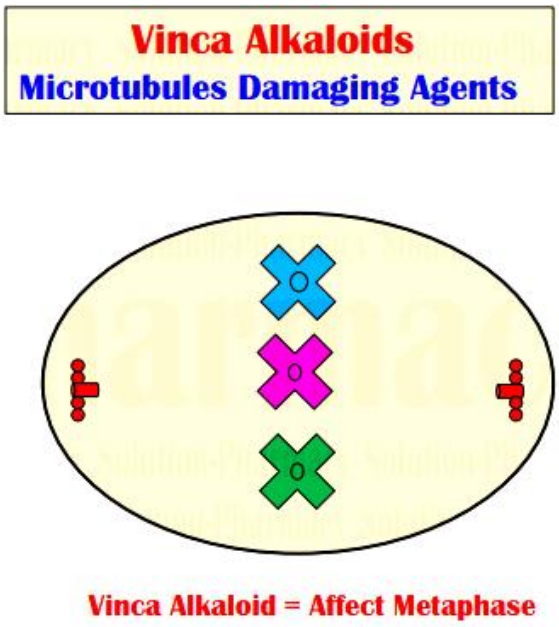
VINCA ALKALOIDS

These are mitotic inhibitors, bind to microtubular protein—'tubulin', prevent its polymerization and assembly of microtubules, cause disruption of mitotic spindle and interfere with cytoskeletal function. The chromosomes fail to move apart during mitosis: metaphase arrest occurs. They are cell cycle specific and act in the mitotic phase. Vincristine and vinblastine, though closely related chemically, have somewhat different spectrum of anti-tumour activity and toxicity.

Vincristine (oncovin)

It is a rapidly acting drug, very useful for inducing remission in childhood acute leukaemia, but is not good for maintenance therapy. Other indications are lymphosarcoma, Hodgkin's disease, Wilms' tumour, Ewing's sarcoma and carcinoma lung. Prominent adverse effects are peripheral neuropathy and alopecia. Bone marrow depression is minimal.





Vinblastine

It is primarily employed with other drugs in Hodgkin's disease and testicular carcinoma. Bone marrow depression is more prominent while neurotoxicity and alopecia are less marked than with vincristine.

TAXANES

Paclitaxel

It is a complex diterpin taxane obtained from bark of the Western yew tree, which exerts cytotoxic action by a novel mechanism. It enhances polymerization of tubulin: a mechanism opposite to that of vinca alkaloids. The microtubules are stabilized and their depolymerization is prevented. This stability results in inhibition of normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic functions. Abnormal arrays or 'bundles' of microtubules are produced throughout the cell cycle. Cytotoxic action of paclitaxel emphasizes the importance of tubulin-microtubule dynamic equilibrium.

The approved indications of paclitaxel are metastatic ovarian and breast carcinoma after failure of first line chemotherapy and relapse cases. It has also shown efficacy in advanced cases of head and neck cancer, small cell lung cancer, esophageal adenocarcinoma and hormone refractory prostate cancer.

The major toxicity is reversible myelosuppression and 'stocking and glove' neuropathy. Chest pain, arthralgia, myalgia, mucositis and edema can be troublesome.

Docetaxel

More potent congener of paclitaxel with the same mechanism of action. It has been found effective in breast and ovarian cancer refractory to first line drugs. Small cell cancer lung, pancreatic, gastric and head/neck carcinomas are the other indications. Major toxicity is neutropenia, but neuropathy is less frequent. Arrhythmias, fall in BP and heart failure are also reported.

EPIPODOPHYLLOTOXINS

Etoposide

It is a semisynthetic derivative of podophyllotoxin, a plant glycoside. It is not a mitotic inhibitor, but arrests cells in the G2 phase and causes DNA breaks by affecting DNA topoisomerase II function. While the cleaving of double stranded DNA is not interfered, the subsequent resealing of the strand is prevented. It has been primarily used in testicular tumours, lung cancer, Hodgkin's and other lymphomas, carcinoma bladder. Alopecia, leucopenia and g.i.t. disturbances are the main toxicity.

CAMPTOTHECIN ANALOGUES

Topotecan and Irinotecan are two recently introduced semisynthetic analogues of camptothecin, an antitumour principle obtained from a Chinese tree. They act in a manner similar to etoposide, but interact with a different enzyme (DNA topoisomerase I). Their binding to this nuclear enzyme allows single strand breaks in DNA, but not its resealing after the strand has untwisted. They damage DNA during replication; act in the S phase and arrest cell cycle at G2 phase.

Topotecan is used in metastatic carcinoma of ovary and small cell lung cancer after primary chemotherapy has failed. The major toxicity is bone marrow depression, especially neutropenia. Other adverse effects are pain abdomen, vomiting anorexia and diarrhoea.

Irinotecan

It is a prodrug; is decarboxylated in liver to the active metabolite. Cholinergic effects are produced in some patients because it inhibits AChE. These effects can be suppressed by prior atropinization. Irinotecan is indicated in metastatic/advanced colorectal carcinoma, cancer lung/cervix/ovary, etc.

Dose limiting toxicity is diarrhoea; neutropenia, thrombocytopenia, haemorrhage, bodyache and weakness are the other adverse effects.Individuals expressing the UGT1A1*28 allele of glucuronyl transferase enzyme are more susceptible to irinotecan induced diarrhoea and neutropenia.

ANTIBIOTICS

These are products obtained from microorganisms and have prominent antitumour activity. Practically all of them intercalate between DNA strands and interfere with its template function.

Actinomycin D (Dactinomycin)

It is a very potent antineoplastic drug, highly efficacious in Wilms' tumour and rhabdomyosarcoma. It has also produced good results in Mtx resistant choriocarcinoma and few other malignancies. Prominent adverse effects are vomiting, stomatitis, diarrhoea, erythema and desquamation of skin, alopecia and bone marrow depression.

Daunorubicin (Rubidomycin), Doxorubicin

These are antitumour antibiotics with quite similar chemical structures. However, utility of daunorubicin is limited to acute leukaemia (in which it is highly active) while doxorubicin, in addition, is effective in many solid tumours. They are capable of causing breaks in DNA strands by activating topoisomerase II and generating quinone type free radicals. They have mutagenic and carcinogenic potential. Maximum action is exerted at S phase, but toxicity is usually exhibited in G2 phase.

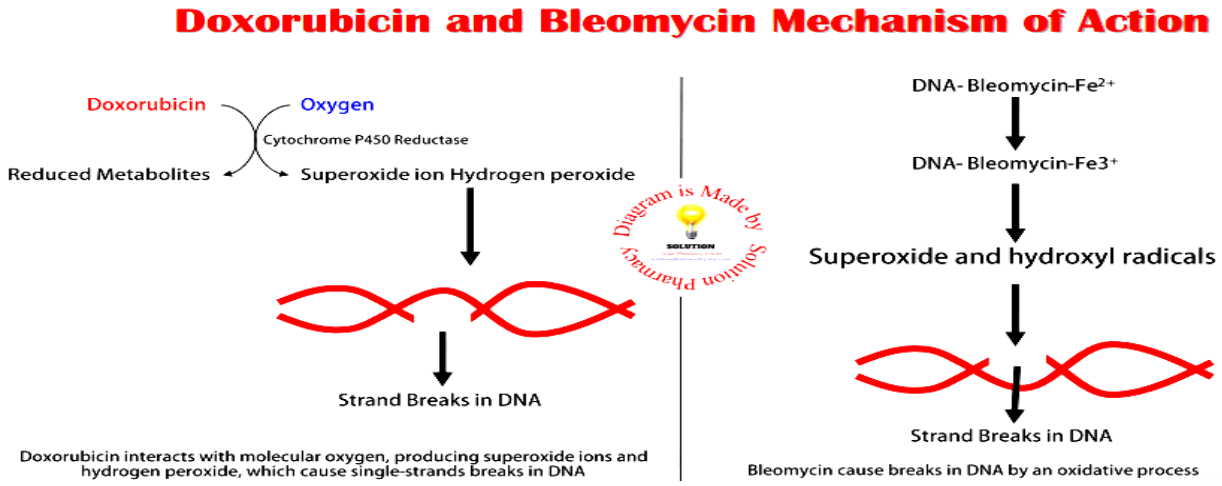
Both these antibiotics produce cardiotoxicity as a unique adverse effect. This can manifest either acutely with ECG changes, arrhythmias and hypotension which are reversible, or be delayed—congestive heart failure (related to the total dose administered). CHF is due to cardiomyopathy and may be fatal. Marrow depression, alopecia, stomatitis, vomiting and local tissue damage (on extravasation) are other adverse effects.

Mitoxantrone

A recently introduced analogue of doxorubicin with lower cardiotoxicity, probably because it does not produce quinone type free radicals. It has a narrow range of utility: in acute nonhaemolytic leukaemia, chronic myelogenous leukaemia, nonHodgkin lymphoma and carcinoma breast. Though cardiomyopathy can occur, major toxicity is marrow depression and mucosal inflammation.

Bleomycin

This is a mixture of closely related glycopeptide antibiotics having potent antitumour activity. It chelates copper or iron, produces superoxide ions and intercalates between DNA strands—causes chain scission and inhibits repair. It is highly effective in testicular tumour and squamous cell



Mechanism of action of doxorubicin and bleomycin

carcinoma of skin, oral cavity, head and neck, genitourinary tract and esophagus; also useful in Hodgkin's lymphoma.

Mucocutaneous toxicity and pulmonary fibrosis, but little myelosuppression are the special features.

Mitomycin C

This highly toxic drug is used only in resistant cancers of stomach, cervix, colon, rectum, bladder, etc. It is transformed intracellularly to a form which acts as an alkylating agent and kills cells in G1M phases. Bone marrow and g.i.t. are the primary targets of toxicity.

MISCELLANEOUS CYTOTOXIC DRUGS

These drugs (except L-asparaginase) have been developed by random synthesis and testing for antitumour activity.

Hydroxyurea

It blocks the conversion of ribonucleotides to deoxyribonucleotides by inhibiting the enzyme ribonucleoside diphosphate reductase—thus interferes with DNA synthesis; exerts Sphase specific action. Its primary therapeutic value is in chronic myeloid leukaemia, psoriasis, polycythaemia vera and in some solid tumours. Myelosuppression is the major toxicity.

Procarbazine

After metabolic activation (it is inactive as such), procarbazine depolymerizes DNA and causes chromosomal damage. Inhibition of nucleic acid synthesis also occurs. Because of damage to DNA, mutagenic and carcinogenic action is noted experimentally. Procarbazine is a component of the popular MOPP regimen for Hodgkin's disease. It is also useful in non-Hodgkin lymphomas and oat cell carcinoma of lung.

Procarbazine is a weak MAO inhibitor, produces some CNS effects and interacts with foods and drugs. Alcohol causes hot flushing and a disulfiram like reaction in patients receiving procarbazine. Vomiting, leucopenia, thrombocytopenia and dermatitis are the prominent toxicities.

L-Asparaginase

It was introduced on the basis of a qualitative difference observed between normal cells and those from childhood lymphoblastic leukemia—the leukaemia cells are deficient in L-asparagine synthase and depend on the supply of L-asparagine from the medium. The enzyme L-asparaginase (from E. coli) degrades L-asparagine to L-aspartic acid, depriving leukaemic cells of an essential metabolite—may cause cell death. However, the clinical response to L-asparaginase has been disappointing. Though, remission is induced in acute leukaemia, it is short lasting. Thus, it is now used when other drugs have failed to induce remission. It is ineffective in solid tumours.

Many of the typical adverse effects of anticancer drugs are not seen with L-asparaginase (no leucopenia, alopecia or mucosal damage); but it produces liver damage, pancreatitis and CNS symptoms (due to defective protein synthesis). Being a foreign protein, it produces allergic reactions in a significant percentage of patients— even anaphylaxis can occur.

Cisplatin

It is a platinum coordination complex that is hydrolysed intracellularly to produce a highly reactive moiety which causes cross linking of DNA. The favoured site is N7 of guanine residue. It can also react with –SH groups in proteins and has radiomimetic property. It is bound to plasma proteins, enters tissues and is slowly excreted unchanged in urine with a t½ about 72 hrs. Negligible amounts enter brain.

Cisplatin is very effective in metastatic testicular and ovarian carcinoma. It has found use in many other solid tumours as well.

Cisplatin is a highly emetic drug. Antiemetics are routinely administered before infusing it. The most important toxicity is renal impairment which is dependent on total dose administered. Renal toxicity can be reduced by maintaining good hydration. Tinnitus, deafness, neuropathy and hyperuricaemia are other problems. Shock like state sometimes occurs during i.v. infusion.

Carboplatin

It is a less reactive second generation platinum compound that is better tolerated and has a toxicity profile different from cisplatin. Nephrotoxicity, ototoxicity and neurotoxicity are low. Nausea and vomiting is milder and is delayed: only infrequently limits the dose. The doselimiting toxicity is thrombocytopenia and less often leucopenia. Because of less plasma protein binding, it is rapidly eliminated by the kidney (t½ 4–6 hr). It is primarily indicated in ovarian carcinoma of epithelial origin, and has shown promise in squamous carcinoma of head and neck, small cell lung cancer and seminoma.

Imatinib

This novel antineoplastic drug inhibits the tyrosine protein kinases in chronic myeloid leukaemia (CML) cells and the ones that are activated by platelet derived growth factor (PDGF) receptor, stem cell receptor and ckit receptor found in gastrointestinal stromal tumour (GIST), a rare tumour. Stricking success has been obtained in chronic phase of CML as well as in accelerated phase, and in metastatic kitpositive GIST. Adverse effects are fluid retention, edema, vomiting, abdominal pain, myalgia and liver damage.

HORMONES

They are not cytotoxic, but modify the growth of hormone-dependent tumours. All hormones are only palliative.

Glucocorticoids

They have marked lympholytic action—are primarily used in acute child hood leukaemia and lymphomas. They induce remission rapidly but relapses inevitably occur after variable intervals and gradually the responsiveness is lost. Considerable palliative effects are obtained in Hodgkin’s disease. Glucocorticoids have a secondary role in some hormone responsive breast cancers.

Corticosteroids are also valuable for the control of complications like hypercalcaemia, haemolysis, bleeding due to thrombocytopenia, increased intracranial tension and mediastinal edema due to radiotherapy. Moreover, they afford symptomatic relief by antipyretic and mood

elevating action and potentiate the antiemetic action of ondansetron/metoclopramide. Prednisolone/ dexamethasone are most commonly used; doses are high—hypercorticism may occur.

Estrogens

They produce symptomatic relief in carcinoma prostate, which is an androgen-dependent tumour. However, relapses occur, but life is prolonged. Estrogens have been superseded in carcinoma prostate by GnRH agonists used with an antiandrogen.

Fosfestrol

It is the phosphate derivative of stilbestrol; has been specifically used in carcinoma prostate.

- Selective Estrogen Receptor Modulators (Tamoxifen)
- Selective Estrogen Receptor Down Regulators (Fulvestrant)
- Aromatase Inhibitors (Letrozole, Etc).

The above three classes of drugs are the sheet anchor of adjuvant and palliative therapy of carcinoma breast, as well as for primary and secondary prevention of breast cancer

Antiandrogen

Flutamide and bicalutamide antagonise androgen action on prostate carcinoma and have palliative effect in advanced/metastatic cases. Because they increase androgen levels, combination with orchiectomy or GnRH analogues is required to produce full therapeutic effect.

5-α Reductase Inhibitor

Finasteride and dutasteride inhibit conversion of testosterone to dihydrotestosterone in prostate (and other tissues), have palliative effect in advanced carcinoma prostate; occasionally used.

GnRH Agonists

They indirectly inhibit estrogen/androgen secretion by suppressing FSH and LH release from pituitary and have palliative effect in advanced estrogen/androgen dependent carcinoma breast/prostate. They are generally used in combination with antiandrogens or SERMS.

Progestins

They bring about temporary remission in some cases of advanced, recurrent (after surgery/radiotherapy) and metastatic endometrial carcinoma. High doses are needed. They have also been used in palliative treatment of metastatic carcinoma breast that has become unresponsive to tamoxifen.

GENERAL PRINCIPLES IN CHEMOTHERAPY OF CANCER

1. In cancer chemotherapy, analogy is drawn with bacterial chemotherapy; the malignant cell being viewed as an invader. However, there are two main differences—

a) Bacterial metabolism differs markedly from that of the host, while malignant cells are in fact host cells with deranged regulation of growth and differentiation and only minor other differences. Therefore, selectivity of drugs is limited. A number of measures which enhance selectivity of drugs for the tumour need to be exploited. However, lately some unique tumour antigens and oncogenes (like the CML-tyrosine protein kinase gene) have been identified, which provide specific targets for drug therapy. b) Infecting microorganisms are amenable to immunological and other host defence mechanisms. This is absent or minimal against cancer cells.

b) Human interferon α-2 and other cytokines (interleukin2, tumour necrosis factor, etc.) that can modify the biological responses to tumour cells are being used as adjuvants in treating neoplasms. They appear to have some direct inhibitory effect on malignant cells, in addition to reinforcing immunological defence against these.

2. A single clonogenic malignant cell is capable of producing progeny that can kill the host. To affect cure, all malignant cells must be killed or removed. Survival time is related to the number of cells that escape chemotherapeutic attack.

3. In any cancer, sub-populations of cells differ in their rate of proliferation and susceptibility to cytotoxic drugs. These drugs kill cancer cells by first order kinetics, i.e. a certain fraction of cells present are killed by one treatment.

4. Drug regimens or number of cycles of combined chemotherapy which can effectively palliate large tumour burdens may be curative when applied to minute residual tumour cell population after surgery and/or irradiation. This is the basis of the combined modality approach.

5. Whenever possible, complete remission should be the goal of cancer chemotherapy: drugs are often used in maximum tolerated doses. Intensive regimens used earlier yield better results.

6. Formerly cancers were treated with one drug at a time. Now a combination of 2–5 drugs is given in intermittent pulses to achieve total tumour cell kill, giving time in between for normal cells to recover.

Synergistic combinations and rational sequences are devised by utilizing:

- Drugs which are effective when used alone.
- Drugs with different mechanisms of action.
- Drugs with differing toxicities.
- Empirically by trial and error; optimal schedules are mostly developed by this procedure.
- Drugs with different mechanisms of resistance.
- Drugs with known synergistic biochemical interactions.
- Kinetic scheduling: On the basis of cell cycle specificity/nonspecificity of the drugs and the phase of cell cycle (see box) at which the drug exerts its toxicity.

Cytotoxic drugs are either cell cycle specific (CCS) or cell cycle nonspecific (CCNS).

Cell Cycle Nonspecific: Kill resting as well as dividing cells, e.g. nitrogen mustard, cyclophosphamide, chlorambucil, carmustine, dacarbazine, busulfan, L-asparaginase, cisplatin, procarbazine, actinomycin D.

Cell Cycle Specific: Kill only actively dividing cells. Their toxicity is generally expressed in S phase. However, these drugs may show considerable phase selectivity, e.g.—

G1: Vinblastine.

S : Mtx, cytarabine, 6TG, 6MP, 5FU, hydroxyurea, mitomycin C, doxorubicin, daunorubicin.

G2: Daunorubicin, bleomycin, etoposide, topotecan.

M: Vincristine, vinblastine, paclitaxel, docetaxel.

Phases of cell cycle

It is logical to use cell cycle specific drugs in short courses (pulses) of treatment. This allows noncycling cells (which are generally less susceptible to drugs) to reenter the cycle between drug courses. The CCS drugs are generally scheduled after a course of CCNS drug(s) to improve the cell kill. The CCS drugs are more effective in haematological malignancies and in solid tumours with a large growth fraction, while the CCNS drugs are effective in these as well as in solid cancers with a small growth fraction.

- Many regimens have been devised by taking into consideration the above factors and by observing patient response.
- One popular combination has been the MOPP regimen, which has yielded over 80% response rate in Hodgkin's disease. For optimum remission 6–11 cycles may be needed. Maintenance therapy thereafter does not produce additional benefit.

- Similarly many other regimens have been devised for different tumours.
- **VAMP:** Vincristine + Amethopterine (Mtx) + 6MP + Prednisolone: used in acute leukaemia.
- **COAP:** Cyclophosphamide + Oncovin (Vincristine) + AraC (Cytarabine) + Prednisolone.
- **POMP:** Prednisolone + Oncovin + Mtx + Purinethol (6MP).
- **CART:** Cytarabine + Asparaginase + Rubidomycin (Daunorubicin) + 6TG.
- **BACOP:** Bleomycin + Adriamycin (Doxorubicin) + Cyclophosphamide + Vincristine + Prednisolone.

7. Tumours often become resistant to any drug that is used repeatedly due to selection of less responsive cells. Such selection is favoured if low dose of a single drug is used.

Several mechanisms of tumour resistance have been recognized. Mutations altering the target biomolecule confer specific (to single drug) resistance. An important mechanism of multidrug resistance is overexpression of MDR 1 gene which increases the concentration of Pglycoprotein (an efflux transporter) on the surface of cancer cells, resulting in pumping out of the chemotherapeutic agents, especially natural products like vinca alkaloids, anthracycline antibiotics, taxanes, etc.

Toxicity Amelioration

High doses and intensive regimens are being employed aiming at cure of the malignancy. The associated toxicity may be ameliorated to some extent by—

1. Toxicity Blocking Drugs: Folinic acid rescue has permitted administration of > 100 fold dose of Mtx. It is professed that normal cells are rescued more than the cancer cells— therapeutic index is increased.
2. Cystitis caused by cyclophosphamide and ifosphamide can be blocked by systemically administered mesna and by irrigating the bladder with acetylcysteine. Both these are –SH containing compounds that combine with and detoxify the toxic metabolites in the bladder. Generous fluid intake and frequent bladder voiding also helps.
3. For controlling cytotoxic drug induced vomiting, ondansetron, a 5HT3 antagonist, has surpassed the efficacy of metoclopramide, which nevertheless is still used (see Ch.

No. 47). Addition of dexamethasone and/or lorazepam further enhances the protection against vomiting.

4. Hyperuricaemia occurring as a consequence of rapid destruction of bulky tumour masses and degradation of large amount of purines can be reduced by allopurinol, alkalinization of urine and plenty of fluids. Corticosteroids also reduce hyperuricemia.
5. Hypercalcaemia occurring as a complication of certain malignancies like myeloma, cancer breast/prostate, etc. may be aggravated by chemotherapy. It is treated by vigorous hydration and i.v. bisphosphonates.
6. Drugs given in pulses with 2–3 week intervals for normal cells to recover improve the efficacy of therapy: malignant cells recovering more slowly.
7. Selective exposure of tumour to the drug by intraarterial infusion into a limb or head and neck; intrapleural/intraperitoneal injection— especially for rapidly accumulating pleural effusion or ascitis; topical application on the lesion—on skin, buccal mucosa, vagina, etc. may reduce systemic toxicity.
8. Platelet and/or granulocyte transfusion after treatment—to prevent bleeding or infection.
9. Use of biological response modifiers like recombinant GMCSF/GCSF hastens recovery from cytotoxic drug induced myelosuppression.
10. Interleukin2 (Il2) is a cytokine biological response modifier that itself has antitumour property by amplifying killer Tcell response.
11. Bone marrow transplantation after treatment with high doses of myelosuppressant drugs.
12. Thalidomide (banned in 1960 for its teratogenic effect) has anxiolytic, antiemetic, adjuvant analgesic/antipyretic properties and has been found to counteract cancer associated cachexia. It probably acts by suppressing TNFα and by modulating IL-2

IMMUNOSUPPRESSANT DRUGS

These are drugs which inhibit cellular/humoral or both immune response and have their major use in organ transplantation and autoimmune diseases. The drugs are:

1. Calcineurin Inhibitors (Specific Tcell inhibitors): Cyclosporine (Ciclosporin), Tacrolimus

2. Antiproliferative Drugs (Cytotoxic drugs): Azathioprine, Cyclophosphamide, Methotrexate, Chlorambucil, Mycophenolate mofetil (MMF)

3. Glucocorticoids: Prednisolone and others

4. Antibodies: Muromonab CD3, Antithymocyte globulin (ATG), Rho (D) immuneglobulin

The development of immune response and the sites of action of different immunosuppressants is summarized in Fig.

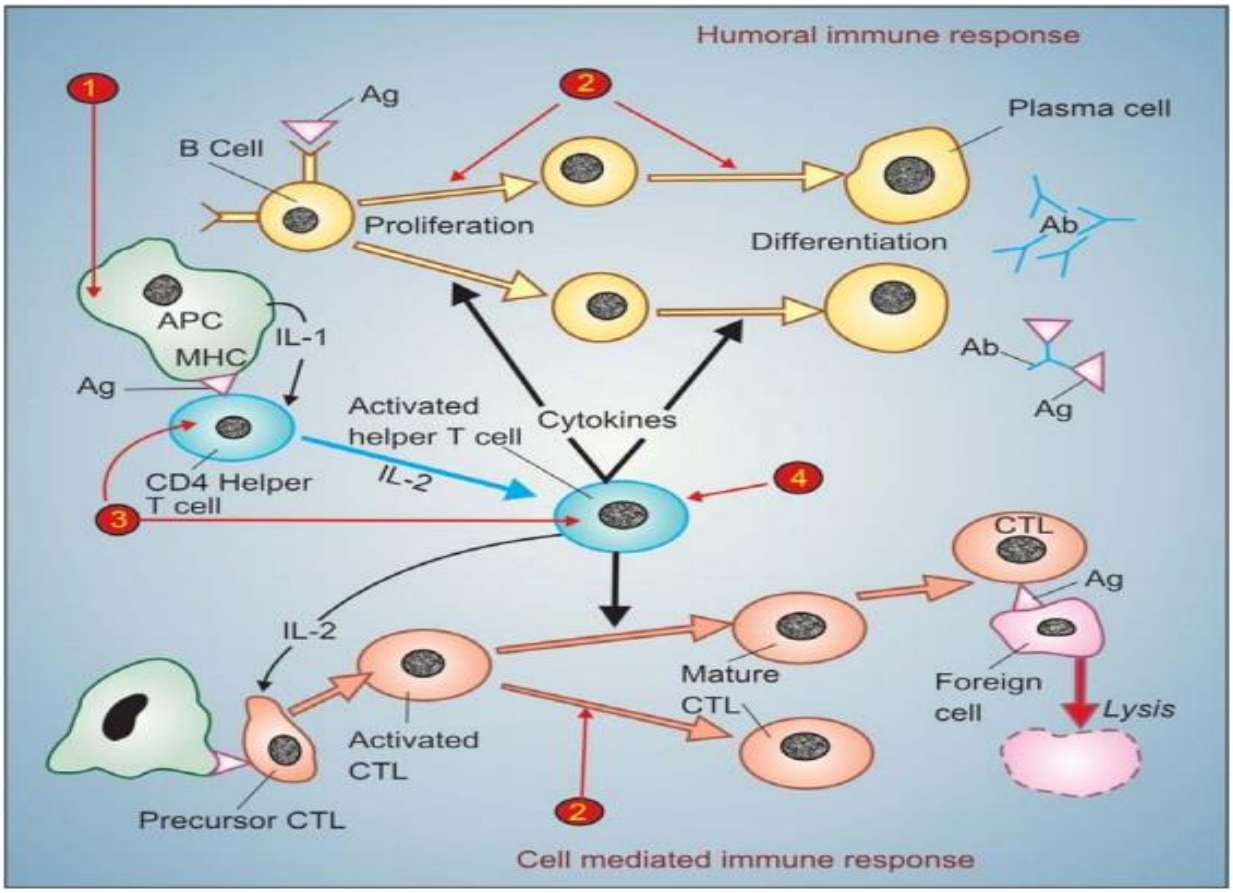


Fig. 63.1: Generation of humoral and cell-mediated immune response and sites of action of immunosuppressant drugs

The antigen (Ag) is processed by macrophages or other antigen presenting cells (APC), coupled with class II major histocompatibility complex (MHC) and presented to the CD4 helper cell which are activated by interleukin-I (IL-1), proliferate and secrete cytokines—these in turn promote proliferation and differentiation of antigen activated B cells into antibody (Ab) secreting plasma cells. Antibodies finally bind and inactivate the antigen.

In cell-mediated immunity—foreign antigen is processed and presented to CD4 helper T cell which elaborate IL-2 and other cytokines that in turn stimulate proliferation and maturation of precursor cytotoxic lymphocytes (CTL) that have been activated by antigen presented with class I MHC. The mature CTL (Killer cells) recognize cells carrying the antigen and lyse them.

1. Glucocorticoids inhibit MHC expression and IL-1, IL-2, IL-6 production so that helper T-cells are not activated.
2. Cytotoxic drugs block proliferation and differentiation of T and B cells.
3. Cyclosporine and tacrolimus inhibit antigen stimulated activation and proliferation of helper T cells as well as expression of IL-2 and other cytokines by them.
4. Antibodies like muromonab CD3, antithymocyte globulin specifically bind to helper T cells, prevent their response and deplete them.

Calcineurin inhibitors (Specific Tcell inhibitors)

Cyclosporine

It is a cyclic polypeptide with 11 amino acids, obtained from a fungus and introduced in 1977 as a highly selective immuno-suppressant which has markedly increased the success of organ transplantations. It profoundly and selectively inhibits T lymphocyte proliferation, IL2 and other cytokine production and response of inducer T cells to IL1, without any effect on suppressor Tcells. Lymphocytes are arrested in G0 or G1 phase.

The CD4 molecule associated with T cell receptor on helper T cells anchors the major histocompatibility complex class II (MHC II) carrying the antigen peptide so that it is able to activate the T cell receptor. Stimulation of T cell receptor produces a cascade of Ca2+ dependent events and protein kinase C (PKC) activation. The Ca2+ ions after binding to calmodulin activate a membrane associated serine/ threonine phosphatase called calcineurin which dephosphorylates regulatory protein 'nuclear factor of activated Tcell' (NFAT), permitting its intranuclear migration and transcription of cytokine genes leading to production of IL2 along with other interleukins, GMCSF, TNFα, interferon, etc. Cyclosporine enters target cells and binds to cyclophilin, an immunophilin class of protein. The complex then binds to and inactivates calcineurin → response of the helper T cell to antigenic stimulation fails. Cyclosporine also enhances expression of an inhibitor of IL2 which attenuates IL2 stimulated Tcell proliferation and production of killer lymphocytes. Cyclosporine is most active when administered before antigen exposure, but can, in addition, suppress the responses of primed helper T cells; hence useful in autoimmune diseases as well.

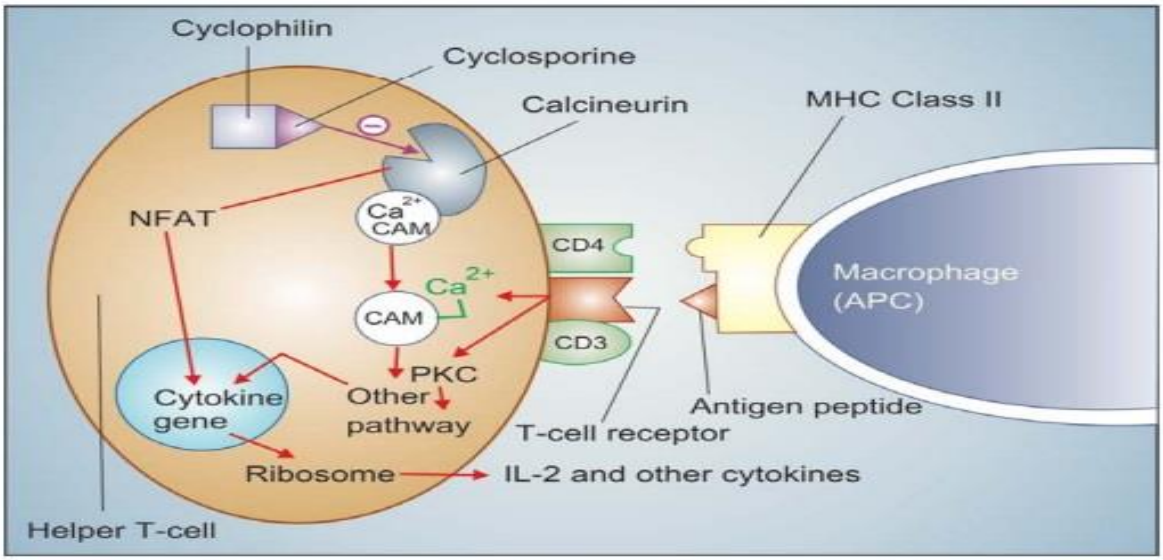


Fig. 63.2: Interaction between macrophage antigen presenting cell (APC) and helper T-cell in the immune response and mechanism of action of cyclosporine.

Cyclosporine binds to an intracellular protein 'Cyclophilin' and this complex inhibits Ca^{2+}-Calmodulin (Ca^{2+}-CAM) activated enzyme 'Calcineurin'. Normally, after activation through T-Cell receptor calcineurin activates cytokine genes through a 'Nuclear factor of activated T-cells' (NFAT) resulting in transcription of cytokine genes and production of IL-2 and other cytokines. These pathways become unoperational in the presence of cyclosporine

Cyclosporine selectively suppresses cell-mediated immunity, prevents graft rejection and yet leaves the recipient with enough immune activity to combat bacterial infection. Unlike cytotoxic immunosuppressants, it is free of toxic effects on bone marrow and RE system. Humoral immunity remains intact. However, it is nephrotoxic—the major limitation, and impairs liver function. Other adverse effects are sustained rise in BP, precipitation of diabetes, anorexia, lethargy, hyperkalaemia, opportunistic infections, hirsutism, gum hyperplasia, tremor and seizures.

Cyclosporine is the most effective drug for prevention and treatment of graft rejection reaction. It is routinely used in renal, hepatic, cardiac, bone marrow and other transplantations. For induction it is started orally 12 hours before the transplant and continued for as long as needed. When graft rejection has started, it can be given i.v., because oral bioavailability is low, dependent on presence of bile and is highly variable. It is concentrated in WBCs and RBCs, metabolized in liver by CYP3A4 and excreted in bile. The plasma t½ is biphasic 4–6 hr and 12–18 hr.

Dose: 10–15 mg/kg/day with milk or fruit juice till 1–2 weeks after transplantation, gradually reduced to maintenance dose of 2–6 mg/kg/day. Therapy may be started with 3–5 mg/kg i.v. infusion.

IMUSPORIN 25, 50, 100 mg soft gelatin cap. Absorption from this preparation is slower and more variable. A newer microemulsion formulation SANDIMMUN NEORAL, PANIMUN

BIORAL 25, 50, 100 mg caps, has more consistent bioavailability. For i.v. use cyclosporine is dispersed in cremaphor emulsion: SANDIMMUN, PANIMUN 100 mg/ml inj in 1 ml, 5 ml, 50 ml vial, which is diluted and infused over 4–6 hours. An acute reaction consisting of chills, fever, bodyache and dyspnoea often occurs because of the solvent; i.v. cyclosporine is used only in emergency, and is substituted by oral medication as soon as possible.

Cyclosporine is a second line drug in autoimmune diseases, like severe rheumatoid arthritis, uveitis, bronchial asthma, inflammatory bowel disease, dermatomyositis, etc. and in psoriasis, especially to suppress acute exacerbations. It is often used along with corticosteroids or Mtx. Good results have been obtained in some cases of aplastic anaemia. For these conditions, lower doses (2–5 mg/kg/day) are needed and adverse effects are mild. However, it is not curative and relapses occur when the drug is withdrawn.

Drug interactions with a large number of drugs occur. All nephrotoxic drugs like aminoglycosides, vancomycin, amphotericin B and NSAIDs enhance its toxicity. By depressing renal function, it can reduce excretion of many drugs. Phenytoin, phenobarbitone, rifampin and other enzyme inducers lower its blood levels so that transplant rejection may result. On the other hand, CYP3A4 inhibitors erythromycin, ketoconazole and related drugs inhibit its metabolism to increase bioavailability and cause toxicity. Pot. supplements and K+ sparing diuretics can produce marked hyperkalaemia in patients on cyclosporine.

Tacrolimus (FK506)

It is a newer immunosuppressant chemically different from cyclosporine, but having the same mechanism of action, and is ~100 times more potent. It binds to a different cytoplasmic immunophilin protein labelled ‘FKBP’, but the subsequent steps are the same, i.e. inhibition of helper T cells via calcineurin.

Tacrolimus is administered orally as well as by i.v. infusion. Oral absorption is variable and decreased by food. It is metabolized by CYP3A4 and excreted in bile with a longer t½ of 12 hour. Therapeutic application, clinical efficacy as well as toxicity profile are similar to cyclosporine. It is particularly valuable in liver transplantation because its absorption is not dependent on bile. Because of more potent action, it is also suitable for suppressing acute rejection that has set in. Hypertension, hirsutism and gum hyperplasia are less marked than cyclosporine, but tacrolimus is more likely to precipitate diabetes, cause neurotoxicity, alopecia and diarrhoea. Dose limiting toxicity is renal.

ANTIPROLIFERATIVE DRUGS (Cytotoxic Immunosuppressants)

Certain cytotoxic drugs used in cancer chemotherapy exhibit prominent immunosuppressant action, mainly by preventing clonal expansion of T and B lymphocytes (see Fig. 63.1).

Azathioprine

It is a purine antimetabolite which has more marked immunosuppressant than anti-tumour action. The basis for this difference is not clear, but may be due to its selective uptake into immune cells and intracellular conversion to the active metabolite 6mercaptopurine, which then undergoes further transformations to inhibit de novo purine synthesis and damage to DNA. It selectively affects differentiation and function of T cells and inhibits cytolylic lymphocytes; cell-mediated immunity is primarily depressed.

The most important application of azathioprine is prevention of renal and other graft rejection, but it is less effective than cyclosporine; generally combined with it or used in patients developing cyclosporine toxicity. It has also been used in progressive rheumatoid arthritis and some other autoimmune diseases.

Cyclophosphamide

This cytotoxic drug has more marked effect on B cells and humoral immunity compared to that on T cells and cell-mediated immunity. It has been particularly utilized in bone marrow transplantation in which a short course with high dose is generally given. In other organ transplants it is employed only as a reserve drug. In rheumatoid arthritis, it is rarely used, only when systemic manifestations are marked. Low doses are occasionally employed for maintenance therapy in pemphigus, systemic lupus erythematosus and idiopathic thrombocytopenic purpura.

Methotrexate (Mtx.)

This folate antagonist is a potent immunosuppressant which markedly depresses cytokine production and cellular immunity, and has anti-inflammatory property. It has been used as a first line drug in many autoimmune diseases like rapidly progressing rheumatoid arthritis, severe psoriasis, pemphigus, myasthenia gravis, uveitis, chronic active hepatitis. Low dose Mtx maintenance therapy is relatively well tolerated.

Chlorambucil

It has relatively weak immunosuppressant action which is sometimes utilized in autoimmune diseases and transplant maintenance regimens.

Mycophenolate mofetil (MMF)

It is a new immunosuppressant; prodrug of mycophenolic acid which selectively inhibits inosine monophosphate dehydrogenase an enzyme essential for de novo synthesis of guanosine nucleotides in the T and B cells (these cells, unlike others, do not have the purine salvage pathway). Lymphocyte proliferation, antibody production and cellmediated immunity are inhibited. As 'add on' drug to cyclosporine + glucocorticoid in renal transplantation, it has been found as good or even superior to azathioprine, but should not be combined with azathioprine. It can help to reduce the dose of cyclosporine and thus its toxicity. Vomiting, diarrhoea, leucopenia and predisposition to CMV infection, g.i. bleeds are the prominent adverse effects.

GLUCOCORTICOIDS

Glucocorticoids have potent immunosuppressant and anti-inflammatory action; inhibit several components of the immune response. They particularly inhibit MHC expression and proliferation of T lymphocytes. Expression of several IL and other cytokine genes is regulated by corticosteroids and production of adhesion molecules is depressed. The short-lived rapid lymphopenic effect of steroids is due to sequestration of lymphocytes in tissues. Accordingly, they have a more marked effect on CMI.

The corticosteroids are widely employed as companion drug to cyclosporine in various organ transplants. In case graft rejection sets in—large doses of corticoids i.v. are employed for short periods. They are used in practically all cases of severe autoimmune diseases, especially during exacerbation. Long-term complications are the greatest limitations of steroid use.

IMMUNOSUPPRESSANT ANTIBODIES

Muromonab CD3

It is a murine monoclonal antibody against the CD3 glycoprotein located near to the T cell receptor on helper T cells (see Fig. 63.2). Binding of muromonab CD3 to the CD3 antigen obstructs the binding of MHC IIantigen complex to the T cell receptor: antigen recognition is interfered, so that participation of T cells in the immune response is prevented and T cells rapidly disappear from circulation leading to an immune blocked state. The response to this monoclonal antibody is less variable than to the polyclonal anti-thymocyte globulin. It is also less likely to produce allergic reactions.

Muromonab CD3 has been used as induction therapy together with corticosteroids and azathioprine with delayed use of cyclosporine in 'sequential regimen' for organ transplantation. This serves to postpone potential nephro and hepatotoxicity of cyclosporine. This sequential regimen has been found to be more effective than the standard triple therapy in renal and hepatic, but not in cardiac transplant recipients. It is also valuable for steroid-resistant rejection reactions and has been used to deplete T cells from the donor bone marrow before transplantation.

The initial doses of muromonab CD3 are associated with 'cytokine release' syndrome with flu like symptoms: chills, rigor and wheezing. Occasionally aseptic meningitis, intragraft thrombosis, pulmonary edema, seizures and a shock like state are produced. High dose corticosteroid pretreatment reduces the reaction.

Antithymocyte Globulin (ATG)

It is a polyclonal antibody purified from horse or rabbit immunized with human thymic lymphocytes which binds to T lymphocytes and depletes them. It is a potent immunosuppressant and has been used primarily to suppress acute allograft rejection episodes, especially in steroid-resistant cases or is combined with them. It can also be used in induction regimens, but responses are less consistent than with muromonab CD3, and it has the potential to produce serum sickness or anaphylaxis, but is less expensive than muromonab CD3.

Anti-D Immuneglobulin

It is human IgG having a high titer of antibodies against Rh (D) antigen. It binds the Rho antigens and does not allow them to induce antibody formation in Rh negative individuals. It is used for prevention of postpartum/postabortion formation of antibodies in RhoD negative, DU negative women who have delivered or aborted an RhoD positive, DU positive baby/foetus. Administered within 72 hours of delivery/ abortion, such treatment prevents Rh haemolytic disease in future offspring. It has also been given at 28th week of pregnancy.

Higher doses (1000–2000 μg) are needed for Rh negative recipients of inadvertantly administered Rh positive blood. It should never be given to the infant or to RhoD positive, DU positive individuals.

IMMUNOSUPPRESSION IN ORGAN TRANSPLANTATION

Use of immunosuppressants is essential for successful organ transplantation. In general 3 types of regimens are used depending upon the stage of transplantation.

Induction Regimen: This is given in the perioperative period: starting just before the transplant to about 2–12 weeks after it. Accelerated rejection develops in the first week, while acute rejections are most likely from 2–12 weeks. The most common regimens include triple therapy cyclosporine + prednisolone + azathioprine (with or without muromonab CD3/ATG), but 2 drug and single drug regimens are also used. Many experts do not give cyclosporine preoperatively, and try to dealy its induction as far as possible to avoid nephrotoxicity, particularly in renal transplantation. If no rejection develops, the doses are gradually reduced after 2 weeks and this phase merges imperceptably with maintenance phase.

Maintenance Regimen: This is given for prolonged periods, may be lifelong. Triple drug regimen is favoured because each component is needed in lower doses—reduces toxicity and cost. Cyclosporine is the most costly and its nephrotoxicity is often the limiting factor. Long-term steroid therapy has its own problems. The component which produces toxicity in a given patient is curtailed or dropped. Two drug and one drug regimens are also used, but are associated with more episodes of acute rejection. After 1 year, cyclosporine is generally dropped, but its continuation is associated with fewer acute rejections. In case of intolerance to the first line drugs cyclosporine, azathioprine and prednisolone, the second line drugs like cyclophosphamide, MMF, chlorambucil are substituted.

Anti-Rejection Regimen: This is given to suppress an episode of acute rejection. Steroid pulse therapy (methylprednisolone 0.5–1 g i.v. daily for 3–5 days) is effective in majority of cases. In case of no response, muromonab CD3/ATG is given as rescue therapy or the antibodies are combined with steroids. Tacrolimus, MMF have also been used in rescue therapy of steroid resistant rejection. If the maintenance regimen had not included cyclosporine, its addition can treat acute rejection, but can be damaging to the transplanted kidney.

Adverse Effects

The two general untoward effects of immunosuppressant therapy are:

- ✓ Increased risk of bacterial, fungal, viral (especially CMV) as well as opportunistic infections.
- ✓ Development of lymphomas and related malignancies after a long latency

MONOCLONAL ANTIBODIES

The body naturally produces antibodies, which are elements of the immune system produced by B-lymphocytes, that bind to foreign proteins in the body known as antigens, which the aim of eliminating them.

They naturally circulate in the body searching for foreign bodies (antigens) and ones they attach to the antigen, they destroy the antigen using various immune mechanisms.

On the other hand, monoclonal antibodies are proteins prepared in the laboratory to target specific antigens on body cells such as receptors and other foreign proteins on the surface of normal and cancer cells in the body.

So what are monoclonal antibodies? Monoclonal antibodies are artificial antibodies that are produced from a single clone of cells by fusing B-lymphocytes to myeloma cells. The fusion of B-lymphocytes with myeloma cells by somatic cell hybridization secretes desired antibody-producing elements which are immortalized cell-lines known as a hybridoma. These hybridomas produce homogenous monoclonal antibodies.

Monoclonal antibodies (mAbs) have the ability to recognize unique binding sites (epitopes) found on the specific antigens. This differentiates monoclonal antibodies from polyclonal antibodies i.e monoclonal antibodies are derived from a single B-cell clone to target single epitopes, unlike polyclonal antibodies that target multiple epitopes.

Monoclonal antibodies (mAbs) have been produced to target receptors or other foreign proteins that are present on the surface of normal cells and cancer cells.

Monoclonal antibodies are used in the treatment of many diseases including some cancers.

The specificity of monoclonal antibodies (mAbs) allows them to bind to cancerous cells coupled with a cytotoxic agent such as a strong radioactive agent. The radioactive agent seeks to destroy the cancer cells without harming the healthy ones.

Types of monoclonal antibodies

Being synthetically manufactured to act like human antibodies in the immune system, monoclonal antibodies are prepared in 4 different ways and they are named after what they are made of.

A. Murine monoclonal antibodies

- They were the first monoclonal antibodies to be produced on a lab-scale by the hybridoma technology in 1975.

- They were named murine because of their origin from rodent hosts (mice and rats) belonging to the Muridae family.
- Preclinical trials for its use in the treatment of Cryptococcus neoformans.
- The murine mAbs have played a crucial role in the development of modern antibody production techniques and the potential applications of these artificial immunoglobulins in therapeutics and analytical applications.
- In therapeutic applications, they are used as a framework for the development of antibody and engineering techniques that include chimerization, humanization, and development of bispecific antibodies from antibody fragments known as single-chain antibody fragments (scFvs).
- Preclinical trials to use murine mAbs on the capsular polysaccharide and enhance the therapeutic activity of amphotericin B administration.
- The name of their treatments ends with -omab.

B. Chimeric monoclonal antibodies

- These are structural chimeras that are made of a combination of mouse parts and human parts, by fusion. This involves fusing variable regions of one species such as mice and the constant regions of the other species such as the human species.
- Chimeric monoclonal antibodies are produced to reduce immunogenicity and to increase the serum half-life when preparing them for therapeutic reasons.
- Chimeric antibodies retain the original antibody's antigen specificity and affinity.
- The name of their treatments ends with -ximab.

C. Humanized monoclonal antibodies

- They are an extension of the chimeric monoclonal antibodies whereby, all regions of the mouse antibody in chimeric mAbs are replaced with human ones except for the complementarity-determining regions (CDRs) which are the amino acids that make direct contact with the antigen. This means that humanized mAbs have small parts of the mouse protein that are attached to the human protein.
- The names of treatments end in -zumab.

D. Human monoclonal antibodies

- These are fully human proteins that have been manipulated by molecular biological techniques so as to modify the amino acid sequences.
- This alters the specificity, affinity, or biological functions, acquiring sequences that are not part of the human repertoire.
- The name of the treatments of human mAbs end with -umab.
- Monoclonal antibodies can also be classified based on the functions they play, such as monoclonal antibodies used in cancer treatment include:

A. Naked monoclonal antibodies

- These are mAbs the do not have a drug or radioactive agent attached to them. they are the most common mAbs used in the treatment of cancer.
- Most naked mAbs attach to the antigens on the cancer cells and others bind to antigens or other non-cancerous cells or free-floating proteins.
- These naked mAbs function differently for example:
- Boost immune response against the cancer cells by attaching to the cancer cells and act as markers for the body's immune systems to destroy them e.g Alemtuzumab (Campath®) used for treating Chronic lymphocytic leukemia (CLL). This drug Alemtuzumab binds to the CD52 antigen found on the lymphocytes including leukemic cells, thereby attracting immune cells to them and thus destroying them.
- Boosting the immune response by targeting the immune system checkpoints
- Attaching to and blocking the antigens on cancer cells that facilitate growth and spread of cancer cells and other neighboring cells. eg trastuzumab (Herceptin) is a mAb designed against HER2 protein of breast and stomach cancer and blocks their activation. This HER2 protein facilitates the growth of the cancer cells.

B. Conjugated (labeled or tagged or loaded) monoclonal antibodies

- Conjugated mAbs are conjugated or combined with chemotherapy drugs or a radioactive agent.
- They are mainly used as homing devices in driving the chemotherapy drug directly to the cancer cells.

- Conjugated mAbs circulate freely throughout the body until it finds and attaches (hooks) onto the target antigen and delivers the antigen to immune elimination processes.
- The advantage of conjugated monoclonal antibodies is that they reduce the risks of damaging normal cells in other body parts.
    - Radiolabeled monoclonal antibodies
- They possess a small radioactive particle attached to them where the drug along with radioactive agents are delivered directly to the target cells, causing the traditions to affect the target and the neighboring cells to some extent.
- For example, ibritumomab tiuxetan (Zevalin) acts against CD20 antigen found on the B-lymphocytes.
- They deliver the radioactivity directly to the cancer cells.
- Ibritumomab tiuxetan drugs are made up of mAb drugs known as rituximab and a radioactive agent called Yttrium-90.
- Radiolabelled monoclonal antibody treatment is known as radioimmunotherapy (RIT).
    - Chemolabeled antibodies
- These mAbs have powerful chemotherapy (or other) drugs attached to them. Examples include:
- Brentuximab vedotin (Adcetris), an antibody that targets the CD30 antigen (found on lymphocytes), attached to a chemo drug called MMAE.
- Ado-trastuzumab emtansine (Kadcyla, also called TDM-1), an antibody that targets the HER2 protein, attached to a chemo drug called DM1.

C. Bispecific monoclonal antibodies

- These are monoclonal antibodies drugs that are made up of two different monoclonal antibodies attached to each other.
- For example, a leukemic drug referred to as blinatumomab (Blincyto) has one part attached to the CD19 protein found on leukemia and lymphoma cells and another part attaches to the CD3 protein found on immune T-cells.
- This drug brings together the target cancer cell and the immune cells enabling the immune system to attack the cancer cell promptly.

PRODUCTION OF MONOCLONAL ANTIBODIES

- The production of monoclonal antibodies is an in vitro process by the use of the tissue-culture techniques.
- Producing monoclonal antibodies (mAbs) is initially done by identifying a specific antigen, immunizing an animal with the antigen multiple times. The most commonly used animal models are laboratory mice.
- The B-cells of the immunized animals are removed from the spleen and then fused with cancer B-cells known as the myeloma cells.
- The fusion of adjacent plasma membranes of the myeloma cells is done using polyethylene glycol, however, it has a low success rate, and therefore the selective medium must have the fusion activity as well to enhance cell growth.
- Myeloma cancer cells have an immortal characteristic of continuously proliferating, unlike the normal B cells which proliferate for a period of 6-8 hours, and they normally have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the degradative synthesis of nucleic acids.
- The myeloma cells are placed in a selective medium known as the Hypoxanthine Aminopetrin Thymidine (HAT)-which is made up of hypoxanthine, aminopterin, and thymidine, where it allows the growth and yield of fused hybridoma cells, and the infused myeloma cells do not grow and infused B-cells die off.
- Hybridoma cells have the ability to grow continuously in culture as they produce antibodies. They are then screened for the desired or specific monoclonal antibodies, and those producing the desired mAbs are then transferred and grown in tissue culture.
- Harvesting is done periodically and the monoclonal antibodies are then purified from the medium.
- Growing and harvesting of these monoclonal antibodies are done for several weeks in large media quantities in order to produce enough mAbs that can be used for experimentation or to treat at least a single patient.
- The monoclonal antibodies produced are in millions of numbers and they are specific for the antigen that was initially injected into the animal model.

Hybridoma technology for production of monoclonal antibodies:

Monoclonal antibodies are produced by hybridoma technology. The term hybridoma is used to fused cells resulting due to fusion of following two types of cells-a lymphocytes and tumor cell. An antibody producing B- lymphocytes (eg. Spleen cell of mouse immunized with RBCs from sheep). A single myeloma cell (eg. Bone marrow tumor cell) that can adopted to grow for infinite time in culture The fused product derived the ability of two different types of cells. ie. Ability to produce large amount of pure antibodies as lymphocytes and ability to grow or multiply indefinitely like tumor cell.

Steps in production of monoclonal antibodies:

Step I: Immunization of rabbit or rat and extraction of B-lymphocytes

- In order to isolate B-lymphocyte producing certain antibodies, rabbit or lab rat is immunized through repeated injection of specific antigen (sheep RBCs)
- A sample of B-cells is extracted from spleen of rabbit or rat

Step II: fusion of myeloma cell with B-lymphocytes:

- The extracted B-lymphocytes is added to a culture of myeloma cell from bone marrow.
- The intended result is the formation of hybridoma cells formed by fusion of B-cell and myeloma cell.
- The fusion is done by using Polyethylene glycol (PEG) or by electrophoration or by using phages.

Step III: selection of hybridoma cell

- The next step is selection of hybridoma cells.
- The B-lymphocytes contains HPRT1 gene which codes for enzyme Hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The enzyme HGPRT involved in synthesis of nucleotides from Hypoxanthine present in culture medium. Therefore B- cells can grow in medium containing Hypoxanthine amonopterin thymine (HAT media).
- But myeloma cell lack HPRT1 gene so, it does not produce HGPTR enzyme and it does not grow in HAT medium.
- The myeloma cell fused with another myeloma cell or those do not fused at all die in HAT medium since they do not utilize Hypoxanthine.
- Similarly, B- cell that fuse with another B- cell or those do not fuse at all die eventually because they do not have capacity to divide indefinitely,

- So, only hybridoma cell ie. Fused cell between myeloma and B-cell can survive and divide in HAT medium.
- Screening is done to select hybridoma cells which are the desired cell for monoclonal antibodies production.

Step IV: culture of Hybridoma cell:

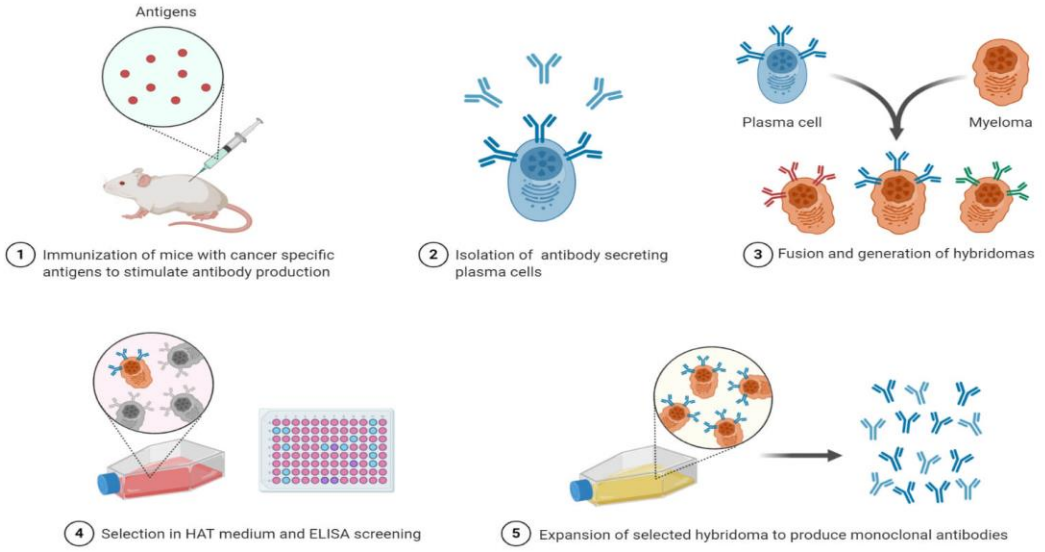
- The selected hybridoma cells are cultured in suitable culture medium, often supplemented with insulin, transferon, ethanol, amine and other additional hormones.
- Some commonly used culture media for hybridoma cell for production of monoclonal antibodies are:
- DMEM (Dulbecco's modified eagle medium)
- IMDM (Iscove's Modified Dulbecco's Medium)
- Ham's F12
- RPMI 1640 medium (Roswell Park Memorial Institute 1640 medium)

Step V: Inoculation of hybridoma cell into suitable host

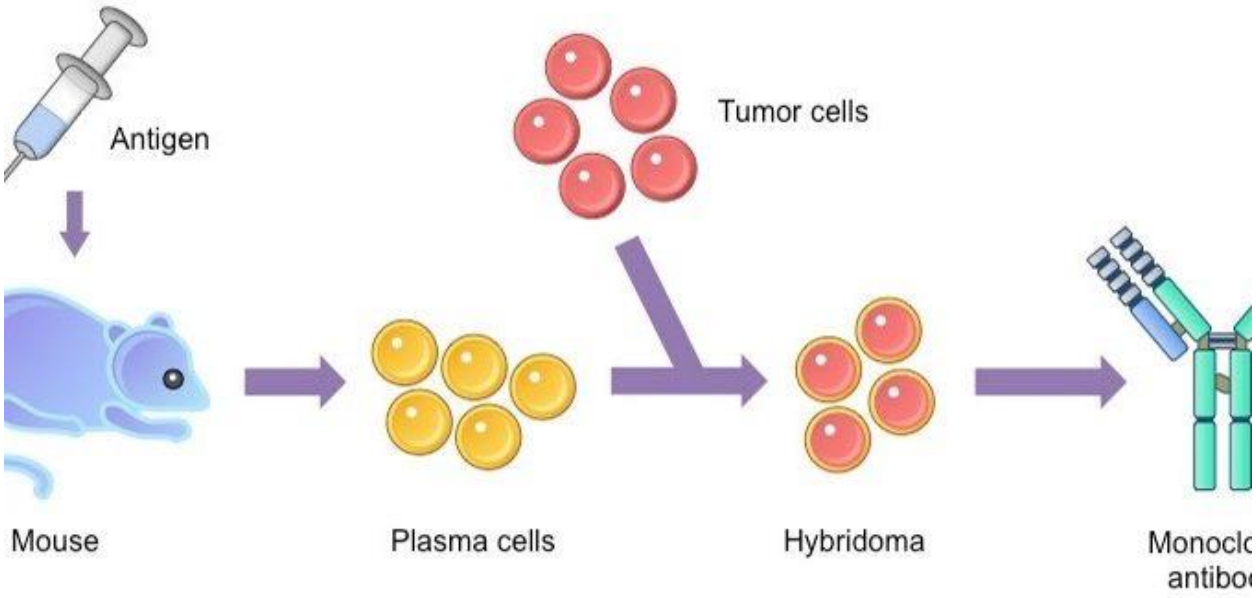
- These hybridoma cells are then injected into lab animal so that they starts to produce monoclonal antibodies.
- These hybridoma cells may be frozen and store for future use.

Step VI: extraction and purification of Monoclonal antibodies:

- Monoclonal antibodies from host animal is extracted and purified by one of the following methods;
- Ion exchange chromatography
- Antigen affinity chromatography
- Radial immunoassay
- Immune precipitation



Production of MAbs



FUNCTIONS AND APPLICATIONS OF MONOCLONAL ANTIBODIES

- ✓ Monoclonal antibodies are used in the treatment of several diseases and disorders and their application is known as immunotherapy.
- ✓ Some diseases and disorders treated using mAbs include: Cancers, Rheumatoid arthritis, Multiple sclerosis, Systemic Lupus erythematous, Cardiovascular diseases, Crohn's disease, Ulcerative colitis, Psoriasis, and Rejections associated with transplantation

- ✓ Monoclonal antibodies are widely used in therapies, laboratory technique studies, and research for potential treatments for certain infections, disorders, and cancers
- ✓ Commonly, monoclonal antibodies were initially studied in cancer treatment where they are currently used in the treatment of some types of cancer.

Some of the specific applications include:

1. Some monoclonal antibodies are designed to target specific tumor antigens. They have been used to stimulate the production of anti-idiotypic antibodies stimulating a strong antitumor immune response when they are inoculated in patients with B-cell lymphoma. However, anti-idiotypic antibodies are developed in animal models, which hiders productions of monoclonal antibodies in human. Though, humanized antiHer2 monoclonal antibodies Herceptin has proved effective in patients with chemotherapy-resistant breast cancer.
2. Monoclonal antibodies are used to effectively bind the Tumor Necrotic Factor-alpha (TNF-alpha), which is a cytokine that helps in the progression of Rheumatoid arthritis (RA). Hence monoclonal antibodies are used as a therapeutic means for Rheumatoid Arthritis.
3. Monoclonal antibodies have been generated against Tumor-specific Transplantation Antigens (TSTAs). These are antigens that result from gene mutations that cause altered proteins that are expressed by tumor cells. Practically, the patient tumor cells are tagged with the monoclonal antibodies that have toxins or radioactive materials. This delivers a direct ‘magic-bullet’ therapeutic effect to the tumor and spares the healthy cells.
4. They are used as an identification tool for several cancers and to also deliver drug therapies to target cancer cells and initiate immune responses against the cancer cells.
5. Monoclonal antibodies are used in the diagnosis of several diseases by detecting specific antigens circulating in the body tissues and detecting them by the use of immunoassay techniques.
6. Currently, monoclonal antibodies are being studied by the COVID-19 Prevention Network. for the treatment of COVID-19. Some trials have been rallied out in the US to understand the role of monoclonal antibodies in providing short-term protection against SARS-CoV-2 the causative agent of COVID-19.

Side effects of Monoclonal antibodies

- The use of its man-made monoclonal antibodies can cause side effects and these effects vary from individual to individual. these effects depend on what the patient is being treated for, the progression of the disease/disorder and the type of monoclonal being administered, and its dosage.

Reactions depend on several factors including:

- Needle site reaction associated with Pain, swelling, soreness, redness, itchiness, rash
- Flu-like symptoms associated with chills, fatigue, nausea, vomit, fever, diarrhea, muscle ache, pain

Effects of monoclonal antibodies associated:

- Mouth and skin sores that can lead to serious infections,
- High blood pressure,
- Congestive heart failure,
- Heart attacks, and Inflammatory lung disease,
- Mild to a severe allergic reaction when receiving the drug, and can even cause death.
- Capillary leak syndrome causes fluid and protein leak out of tiny blood vessels and flowing into the surrounding tissues leading to extremely low pressure and can lead to multiple organ failure and shock.
- Cytokine release syndrome due to elevated levels in their production which is associated with monoclonal antibodies activating various mechanisms in the body. The syndrome is associated with symptoms such as fever, nausea, headache, rash, rapid heartbeat, low blood pressure, and breathing difficulties.

List of Types of monoclonal antibodies approved for therapeutic application

Abciximab

- Trade name: Reopro
- Used after angioplasty to prevent blood clot in coronary artery

Adalimumab

- Trade name: Humira, Amjevita
- Used to treat rheumatoid arthritis

Alefacept

- Trade name: Amevive

- Used as immunosuppressive drug

Alemtuzumab

- Trade name: Campath
- Used for the treatment of chronic lymphocytic leukemia, cutaneous T-cell lymphoma and T-cell lymphoma

Basiliximab

- Trade name: Simulect
- Used as immunosuppressive drug

Belimumab

- Trade name: Benlysta
- Used as immunosuppressive. It inhibits B cell activation.

Bezlotoxumab

- Trade name: Zinplava
- Used to treatment of recurrence of *Clostridium difficile* infections

Canakinumab

- Trade name: (Ilaris
- Used to neutralize Interlukin I beta

Certolizumab pegol

- Trade name: Cimzia
- Used for the treatment of Crohn's disease, rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis

Cetuximab

- Trade name: Erbitux)
- Used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer.

Daclizumab

- Trade name: Zenapax, Zinbryta
- Used for the treatment of adults with relapsing forms of multiple sclerosis

Denosumab

- Trade name: Prolia, Xgeva

- Used for the treatment of osteoporosis, treatment-induced bone loss, metastases to bone, and giant cell tumor of bone.

Efalizumab

- Trade name: Raptiva
- Used for treatment of autoimmune disease

Golimumab

- Trade name: Simponi, Simponi Aria
- Used to treat rheumatoid arthritis

Inflectra

- Trade name: Remicade
- Used to treat rheumatoid arthritis

Ipilimumab

- Trade name: Yervoy
- Used to activate immune system

Ixekizumab

- Trade name; Taltz
- Used to treat autoimmune disease

Natalizumab

- Trade name: Tysabri
- Used for the treatment of multiple sclerosis and Crohn's disease.

Nivolumab

- Trade name: Opdivo)
- Used to treat cancer

Olaratumab

- Trade name: Lartruvo
- Used to treat tumor

Omalizumab

- Trade name: Xolair)
- Used in treatment of allergies

Palivizumab

- Trade name: Synagis)

- Used for treatment of Respiratory syncytial virus

Panitumumab

- Trade name: Vectibix
- Used for colorectal cancer

Pembrolizumab

- Trade name: Keytruda
- Used in cancer immunotherapy

Rituximab

- Trade name: Rituxan
- Used to treat autoimmune disease

Tocilizumab

- Trade name: Actemra
- Used as Immunosupressive drugs

Trastuzumab

- Trade name: Herceptin
- Used in treatment of breast cancer

Secukinumab

- Trade name: Cosentyx
- Used for treatment of psoriasis, ankylosing spondylitis, and psoriatic arthritis.

Ustekinumab

- Trade name: Stelara
- Used for treatment of Psoriasis and Crohn's disease

BIOSIMILAR

A biosimilar is a biologic medical product (also known as biologic) highly similar to another already approved biological medicine (the 'reference medicine'). Biosimilars are approved according to the same standards of pharmaceutical quality, safety and efficacy that apply to all biological medicines. Biosimilars are officially approved versions of original "innovator" products and can be manufactured when the original product's patent expires.[2] Reference to the innovator product is an integral component of the approval.

Unlike with generic drugs of the more common small-molecule type, biologics generally

exhibit high molecular complexity and may be quite sensitive to changes in manufacturing processes. Despite that heterogeneity, all biopharmaceuticals, including biosimilars, must maintain consistent quality and clinical performance throughout their lifecycle. A biosimilar is not regarded as a generic of a biological medicine. This is mostly because the natural variability and more complex manufacturing of biological medicines do not allow an exact replication of the molecular micro-heterogeneity. Drug-related authorities such as the EU's European Medicines Agency (EMA), the US's Food and Drug Administration (FDA), and the Health Products and Food Branch of Health Canada hold their own guidance on requirements for demonstration of the similar nature of two biological products in terms of safety and efficacy. According to them, analytical studies demonstrate that the biological product is highly similar to the reference product, despite minor differences in clinically inactive components, animal studies (including the assessment of toxicity), and a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics). They are sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which

The reference product is licensed and is intended to be used and for which licensure is sought for the biological product.

The World Health Organization (WHO) published its "Guidelines for the evaluation of similar biotherapeutic products (SBPs)" in 2009. The purpose of this guideline is to provide an international norm for evaluating biosimilars with a high degree of similarity with an already licensed, reference biotherapeutic medicine.

Europe was the first region in the world to develop a legal, regulatory, and scientific framework for approving biosimilar medicines. The EMA has granted a marketing authorisation for more than 50 biosimilars since 2006 (first approved biosimilar Somatropin(Growth hormone)). The first monoclonal antibody that was approved in 2013, was infliximab, putting the EU at the forefront of biologics regulatory science. Meanwhile, on March 6, 2015, the FDA approved the United States's first biosimilar product, the biosimilar of filgrastim called filgrastim-sndz (trade name Zarxio) by Sandoz.

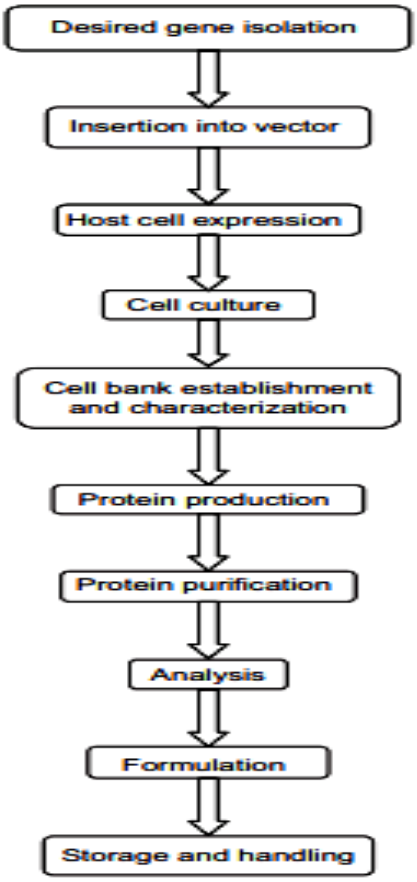
Regulation aspects of biosimilars

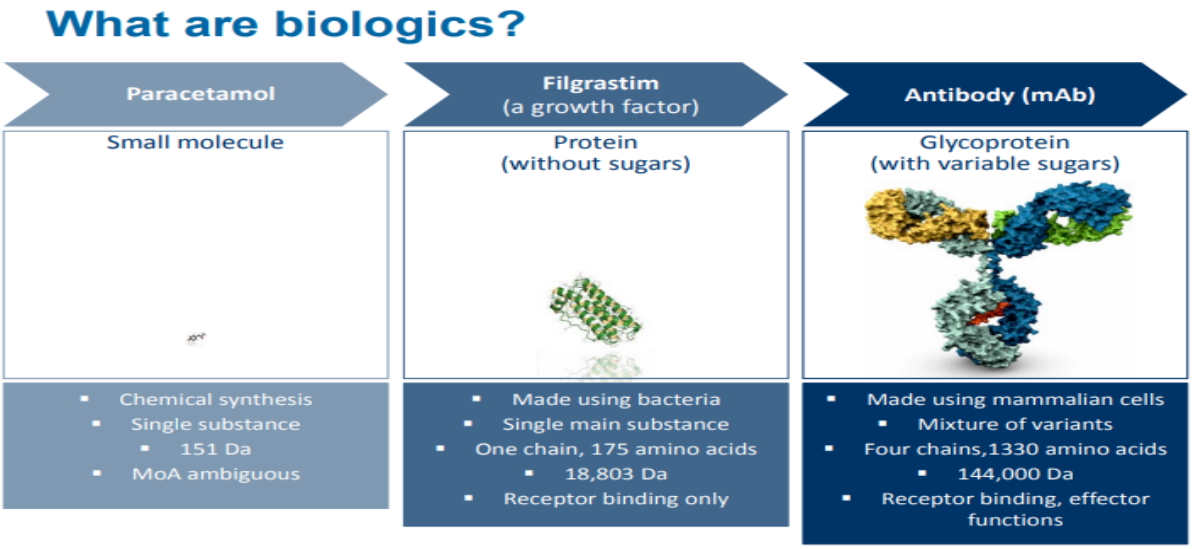
A generic drug is a much less expensive copy of an innovator drug product. Generics can be produced when the patent on a drug has expired, for drugs which have never held patent, in countries where a patent(s) is/are not in force, and where the generic companies certify that the branded companies' patents are either invalid, unenforceable, or will not be infringed. Generic drug manufacturers apply for marketing approval of generic drugs under the Abbreviated New Drug Application (ANDA) pathway established by FDA. Moreover, generic drug applications are termed "abbreviated" because they are generally not required to include preclinical and clinical data to establish safety and effectiveness. The generic manufacturer needs to demonstrate only pharmaceutical equivalence and bioequivalence between the generic and innovator products, in order to gain approval for their generic product. This approach cannot be extrapolated to biosimilars, however, because the active substance of a biopharmaceutical is a collection of large protein isoforms and not a single molecular entity, as is generally true for conventional smallmolecule drugs. Thus the active substances in two products are highly unlikely to be identical and, therefore, unlike generics, biosimilars are only similar and not identical to the innovator products. These differences imply that biosimilars should not be approved and regulated in the same way as conventional generic drugs. The regulatory pathway for approval of biosimilars is more complex than for the generic innovator product because the design of a scientifically valid study to demonstrate the similarity of a highly process-dependent product is not easy. Further, the analytical tests currently available are not sophisticated enough to detect the slight but important structural differences between innovator and biosimilar products. Modest differences may have clinical implications and pose a significant risk to patient safety. Therefore, it is considered necessary that biosimilars must be assessed for clinical efficacy and safety by valid preclinical and clinical studies before marketing approval.

Biosimilars in clinical practice

Despite the comparability of biosimilars to the innovator product, clinicians and health care workers should be aware of some of the issues that have emerged during the development and approval of these products, which highlight the challenges of biosimilars.65 The use of biosimilars is essentially a change in clinical management.90 By taking a leading role in educating patients and medical professionals about the risks and benefits of biosimilars, the

Pan American and Health Education Foundation is actively involved in improving patient safety.





Very Short Answer (2 MARKS)

1. Define androgens and anabolic steroids.
2. What are the uses of immunosuppressant?
3. Write MOA of methotrexate.
4. Define malignancy.
5. Write the MOA of cisplatin
6. Define nitroso ureas.
7. What are UTI infections?
8. What are corticosteroids?
9. What is AUGMENTIN?
10. What are anti neoplastic agents?
11. Define protein drugs.
12. Write the MOA of tacrolimus
13. Define monoclonal antibodies.
14. What are the various target drugs to antigen?
15. Define biosimilars.

Short Answers Questions (5 MARKS)

1. Write a note on drugs used in treatment of syphilis.
2. Discuss vinca alkaloids as anti neoplastics.
3. Write the therapeutics uses of immunosupressants and immunostimulants.
4. Discuss the pharmacological actions of 5-Flourouracil.
5. Write note on drugs used in the management of urinary tract infections.
6. Write note on drugs used in STDs.
7. Write the mechanism of alkalyting agents in cancer.
8. Discuss in detail about immunostimulants.

Long Answer Questions (10 MARKS)

9. Classify the drugs used for sexually transmitted diseases with their pharmacology.
10. Classify immunosupressants. Discuss mechanism, uses and side effects of cyclosporins.
11. Discuss in detail about the mechanism, uses and side effects of anti neoplastic agents.