4. **IMMUNOMODULATING DRUGS**

==========

CYTOTOXIC AGENTS

DR SHAMS SULEMAN

LEARNING OBJECTIVES

- Describe mechanism of action of cytotoxic agents used as immunosuppressant
- Describe clinical uses and adverse effects of cytotoxic agents used as immunosuppressant

CYTOTOXIC AGENTS

- Azathioprine (AZT)
- 6 Mercaptopurine (6 MP)
- Cyclophosphamide
- Hydroxychloroquine
- Methotrexate
- Thalidomide

- *(Immunomodulatory derivatives of thalidomide* ***(IMIDS)***
  - Lenalidomide
  - Pomalidomide (CC4047)

CYTOTOXIC AGENTS

- Sulphasalazine
- Cytosine Arabinoside (Cytarabine)
- Dactinomycin
- Leflunomide
- Vincristine
- Vinblastine
- Pentostatin
- D Penicillamine (cysteine analogue)
- Capcitabine
- Gemcitabine

Antimetabolites

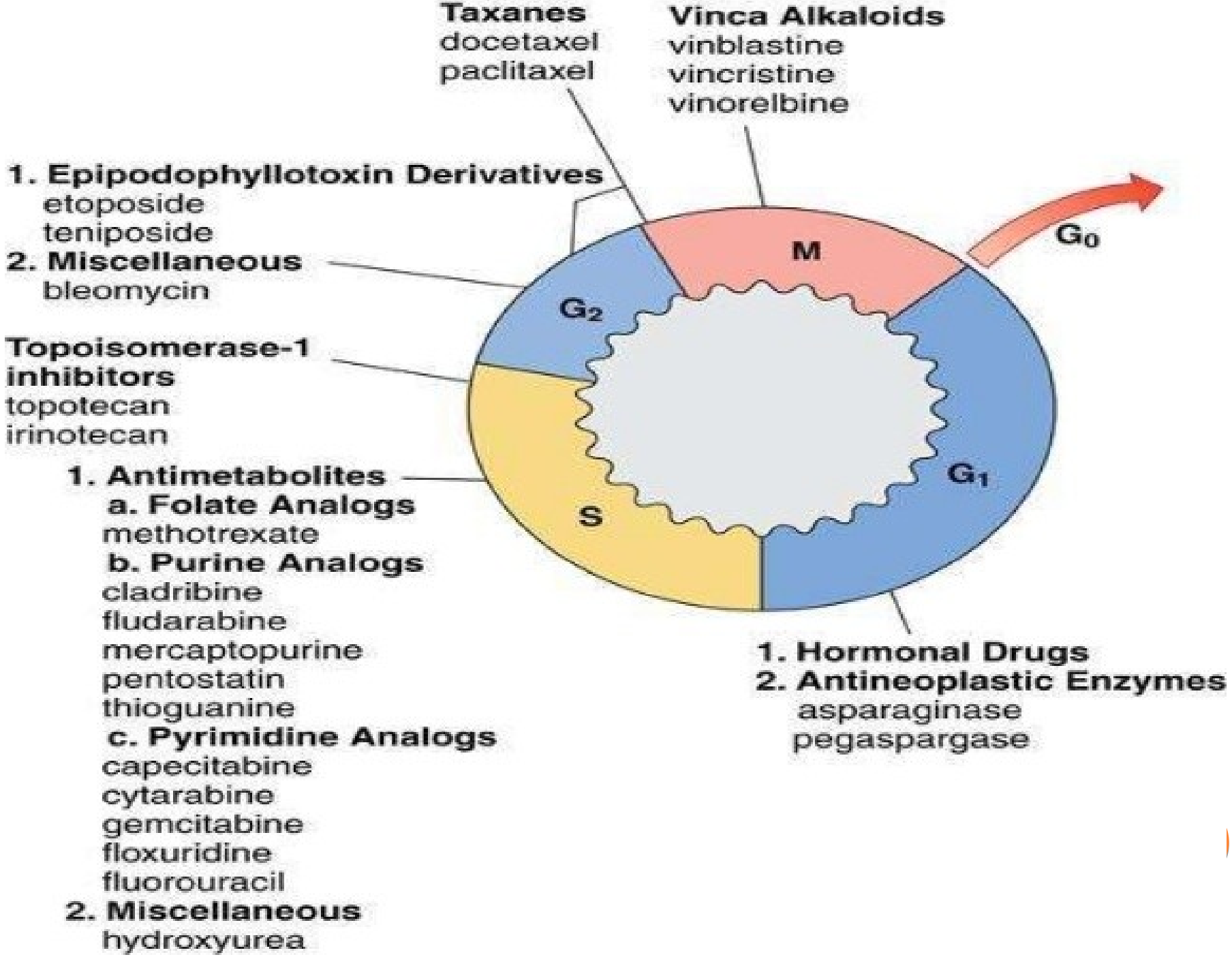
- Antimetabolites are structurally related to normal compounds that exist within the cell.
- Antimetabolites generally **interfere with the availability of normal purine or pyrimidine nucleotide precursors**, either by inhibiting their synthesis or by competing with them in DNA or RNA synthesis.
- Their **maximal cytotoxic effects are in S-phase** (and are, therefore, cell-cycle specific).

Antimetabolites

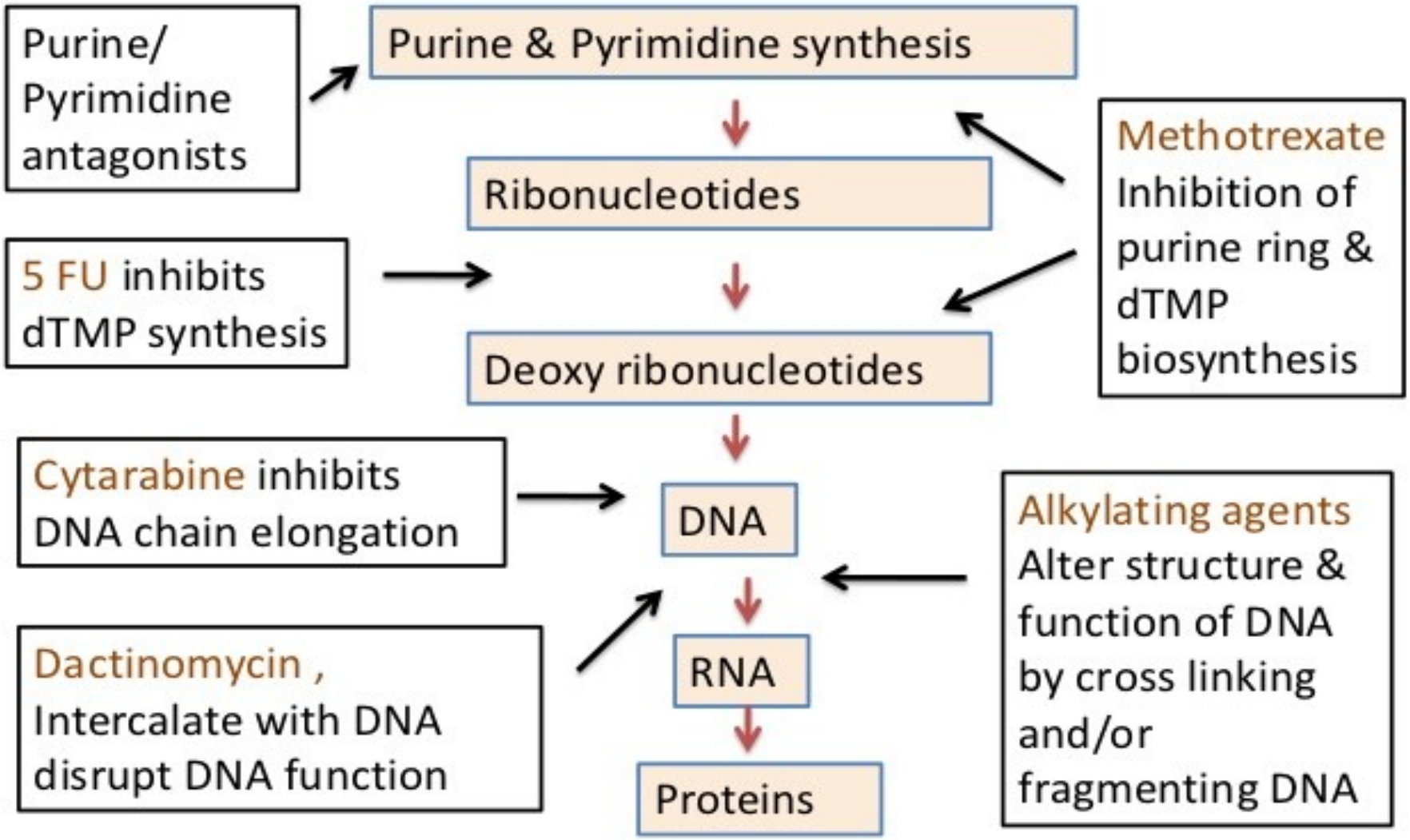
Folate antagonist:	Methotrexate (Mtx).
Purine antagonist:	6-Mercaptopurine (6-MP), 6-Thioguanine (6-TG), Azathioprine, Fludarabine.
Pyrimidine antagonist:	5-Fluorouracil (5-FU), Capecitabine Cytarabine (cytosine arabinoside).

Purine Analog Antimetabolites Drugs

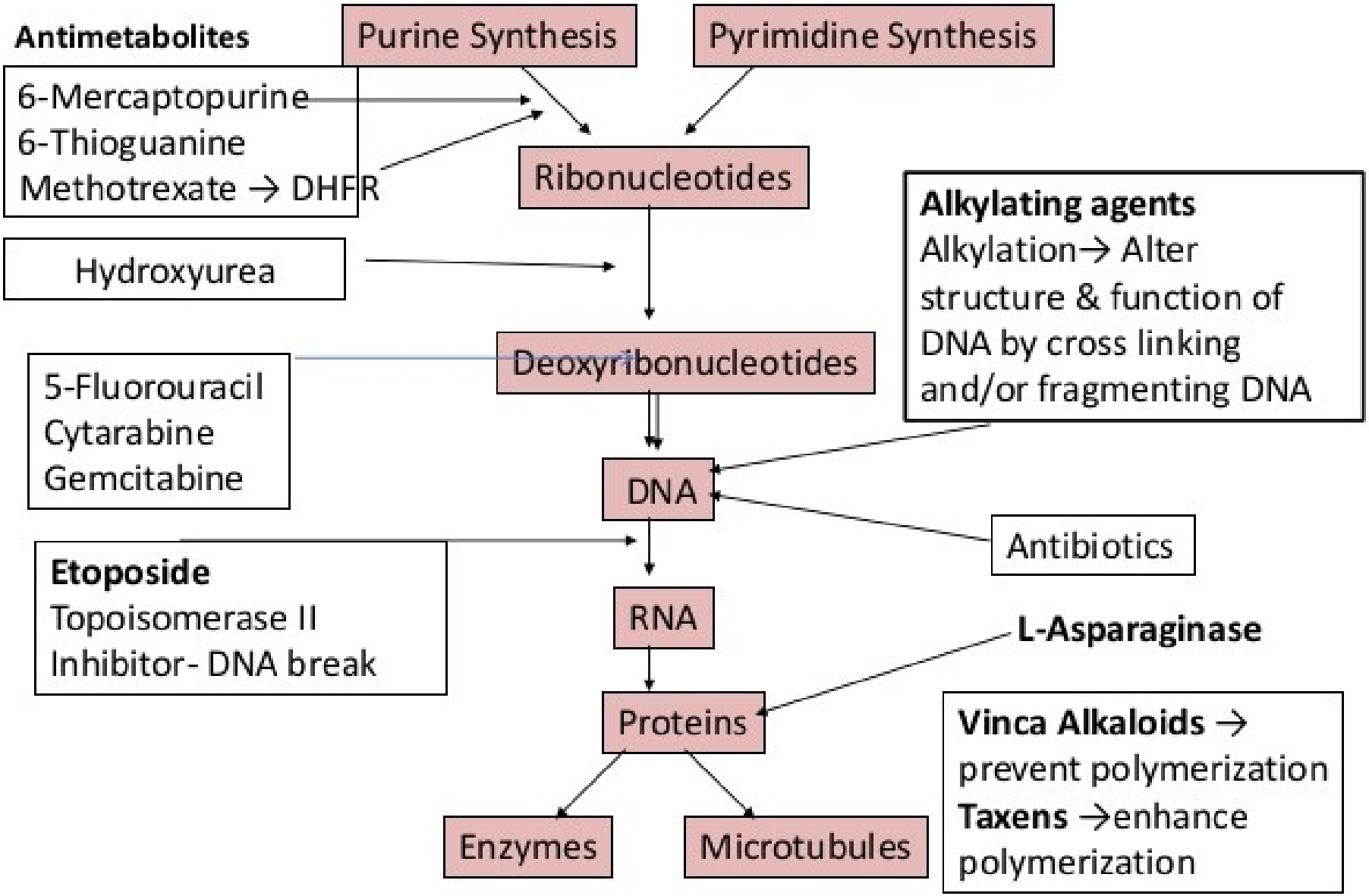
1. Azathioprine (Imuran)
2. Mercaptopurine (Purixan)
3. Thioguanine (Tabloid)
4. Fludarabine (Fludara)
5. Pentostatin (Nipent)
6. Cladribine


Taxanes
docetaxel
paclitaxel
Vinca Alkaloids
vinblastine
vincristine
vinorelbine
1. Epipodophyllotoxin Derivatives
etoposide
teniposide
2. Miscellaneous
bleomycin
Topoisomerase-1 inhibitors
topotecan
irinotecan
1. Antimetabolites
a. Folate Analogs
methotrexate
b. Purine Analogs
cladribine
fludarabine
mercaptopurine
pentostatin
thioguanine
c. Pyrimidine Analogs
capecitabine
cytarabine
gemcitabine
floxuridine
fluorouracil
2. Miscellaneous
hydroxyurea
M
G0
G2
G1
S
1. Hormonal Drugs
2. Antineoplastic Enzymes
asparaginase
pegaspargase

MOA of some anticancer drugs



MOA of some anticancer drugs



AZATHIOPRINE/6 MERCAPTOPURINE

- Prodrug of 6-mercaptopurine (6 MP)
- Antimetabolite
- More widely used than mercaptopurine for immunosuppression in humans.
- Anti proliferative in the immune response.

AZATHIOPRINE/6 MERCAPTOPURINE

PHARMACOKINETICS

- Well absorbed from the gastrointestinal tract
- **Xanthine oxidase** splits much of the active material to 6-thiouric acid prior to excretion in the urine.
- Drug interactions:

_____Toxicity in patients using Allopurinol: Xanthine oxidase inhibitor

Patients receiving allopurinol for control of hyperuricemia should have the dose of azathioprine reduced to one-fourth to one-third the usual amount to prevent excessive toxicity

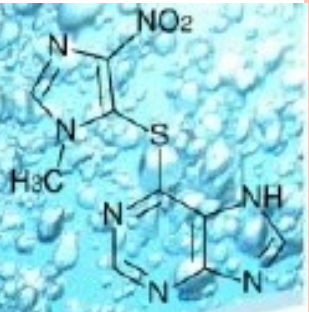
AZATHIOPRINE/6 MERCAPTOPURINE

MOA

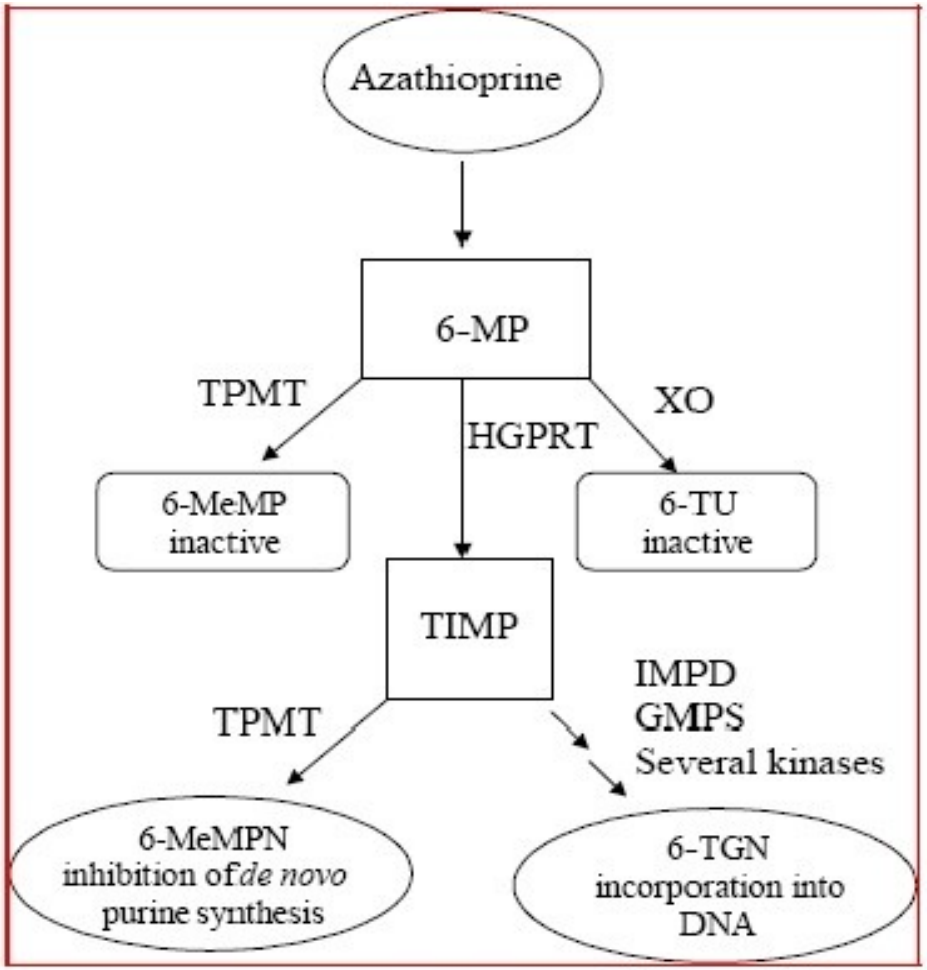
- Interfering with purine nucleic acid metabolism at steps that are required for the wave of lymphoid cell proliferation that follows antigenic stimulation.

- Cellular immunity as well as primary and secondary serum antibody responses can be blocked

Azathioprine



- Imuran: immunosuppressive antimetabolite, is available in tablet form for oral administration and 100-mg vials for intravenous injection
- Description
  - Prodrug that releases 6-mercaptopurine
  - the first immunosuppressive agent to achieve widespread use in organ transplantation
- Mechanism
  - Purine synthesis inhibitor, inhibiting the proliferation of cells, especially leukocyte
  - Converts 6-mercaptopurine to tissue inhibitor of metalloproteinase, which is converted to thioguanine nucleotides that interfere with DNA synthesis; thioguanine derivatives may inhibit purine synthesis
  - prevents mitosis and proliferation of rapidly dividing cells, such as activated B and T lymphocytes



•Azathioprine is metabolized to 6-mercaptopurine (6-MP), which is either inactivated by two enzymes, thiopurine s-methyltransferase (TPMT) and xanthine oxidase (XO), or is further metabolized to thioinosine monophosphate (TIMP).
•Genetic polymorphism involving the inactivating enzyme TPMT
•Allopurinol inhibits XO, elevate azathioprine bioavailability by fivefold

USES

- Prescribed for
  - FDA :
    - **Renal Homotransplantation:** Experience with over 16,000 transplants shows a 5-year patient survival of 35% to 55%, but this is dependent on donor, match for HLA antigens, anti-donor or anti-B-cell alloantigen antibody, and other variables.
    - **Rheumatoid Arthritis: treatment of active rheumatoid arthritis (RA) to reduce signs and** symptoms
    - Juvenile Rheumatoid Arthritis
  - Non-FDA : Multiple Sclerosis, Crohn's disease, myasthenia gravis, chronic ulcerative colitis, and autoimmune hepatitis
- Side effect
  - Leukopenia, bone marrow depression, macrocytosis, liver toxicity (uncommon); blood-count monitoring required

AZATHIOPRINE/6 MERCAPTOPURINE

ADVERSE EFFECTS

- Bone marrow suppression

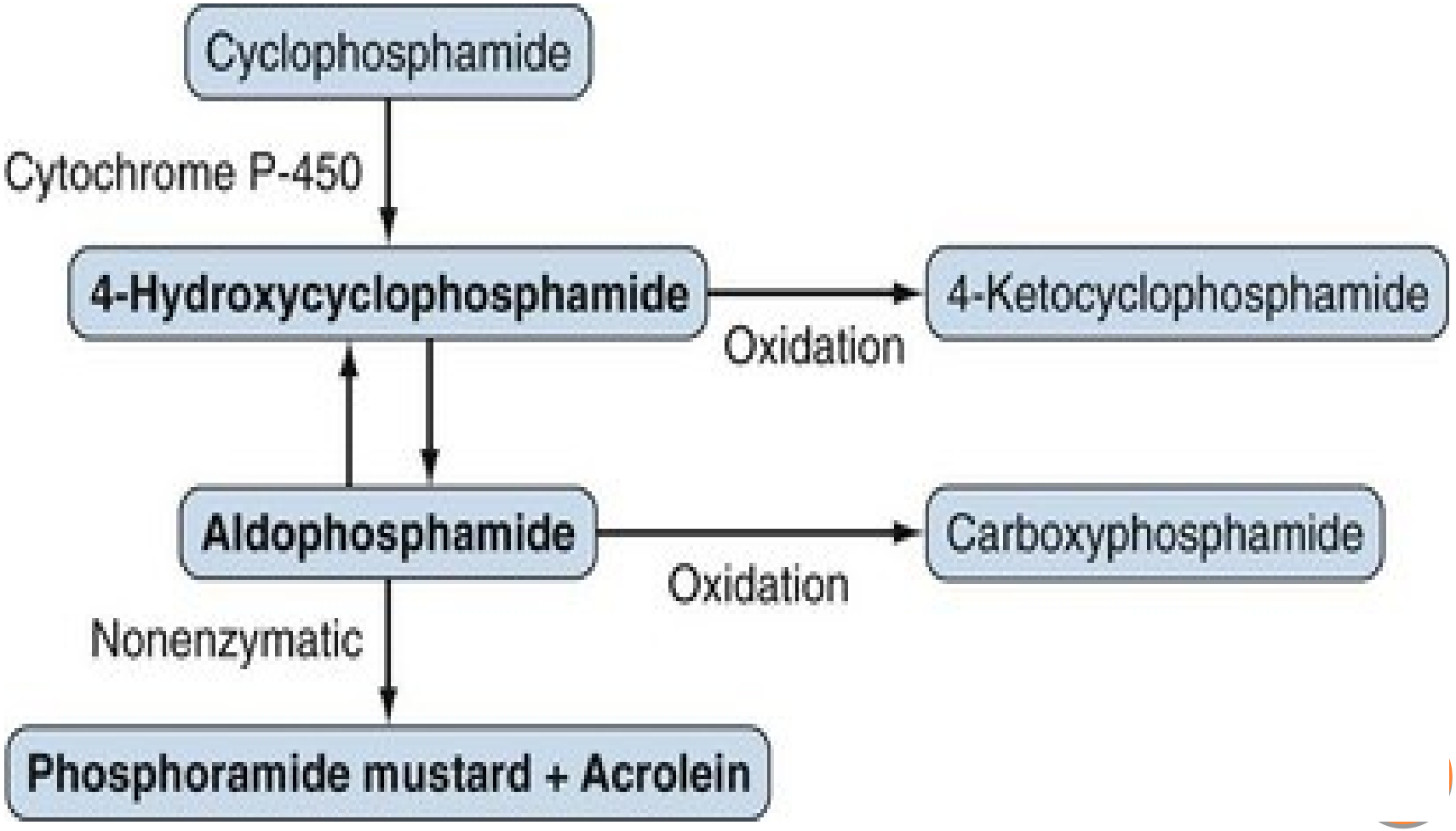
  manifested as leukopenia, anaemia and thrombocytopenia.
- Skin rashes
- Fever
- Nausea and vomiting, and diarrhoea,
- Hepatic dysfunction

  manifested by very high serum alkaline phosphatase levels and mild jaundice, particularly in pre-existing hepatic dysfunction

CYCLOPHOSPHAMIDE

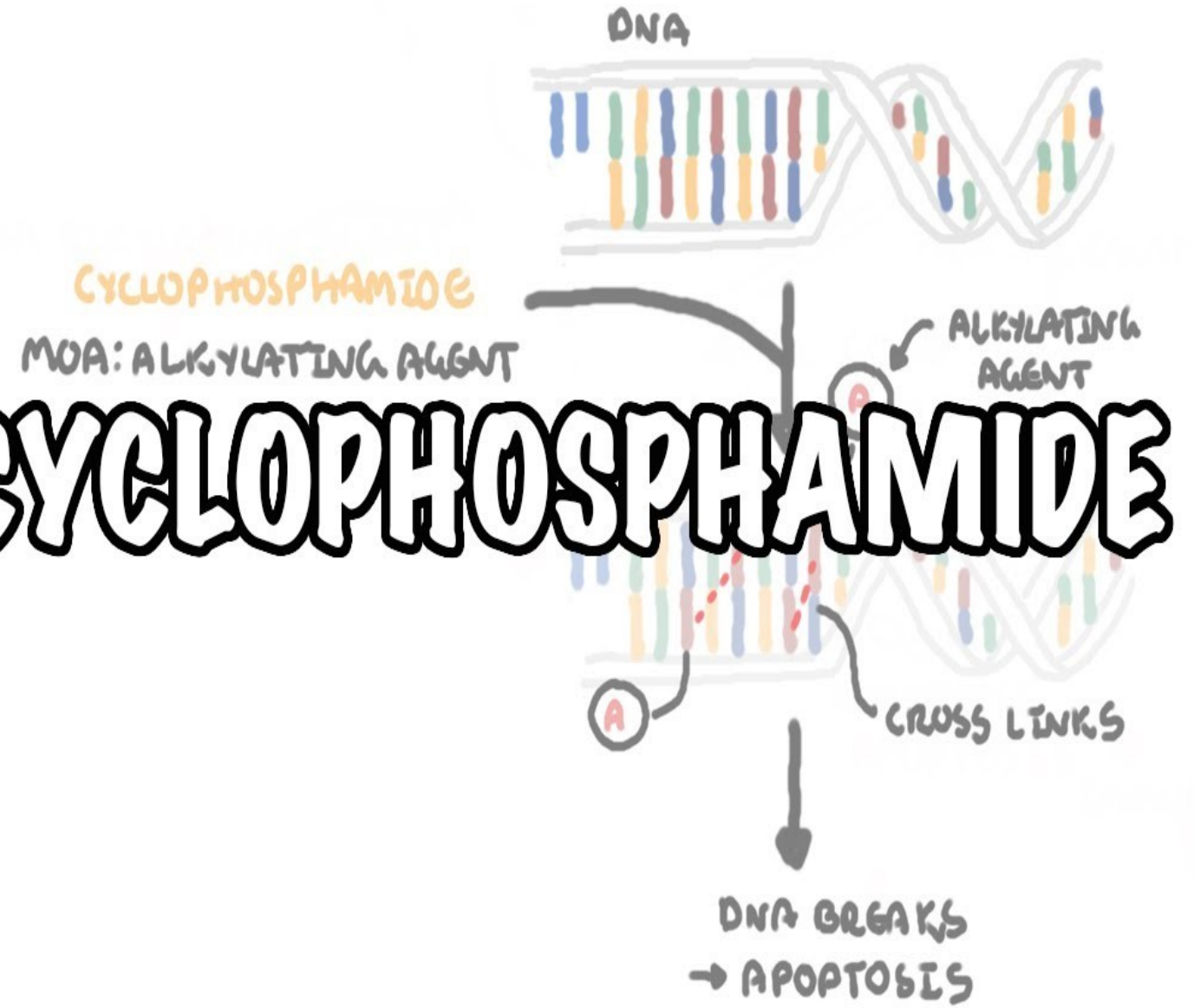
- Alkylating agent.
- Lymphoid cells anti-proliferative
- Alkylates DNA and other molecules resting cells.
- Very large doses (e.g. > 120 mg/kg intravenously over several days) may induce an apparent specific tolerance to a new antigen if the drug is administered simultaneously with or shortly after the antigen.

CYCLOPHOSPHAMIDE: PHARMACOKINETICS



PHARMACOLOGY

CYCLOPHOSPHAMIDE



CYCLOPHOSPHAMIDE

✓ Mechanism of Action

- the main effect of cyclophosphamide is due to its metabolite Phosphoramide mustard
- Phosphoramide mustard forms DNA crosslinks between (interstrand crosslinkages) and within (intrastrand crosslinkages) DNA strands at guanine N-7 position, this eventually leads to cell death

Uses of cyclophosphamide

- Neoplastic conditions
  - Hodgkins and non hodgkins lymphoma
  - ALL, CLL, Multiple myeloma
  - Burkits lymphoma
  - Neuroblastoma , retinoblastoma
  - Ca breast , adenocarcinoma of ovaries
- Non neoplastic conditions
  - Control of graft versus host reaction
  - Rheumatoid arthritis
  - Nephrotic syndrome

OTHER NON NEOPLASTIC USES

- Systemic lupus erythematosus = smaller doses
- Acquired factor XIII antibodies and bleeding syndromes,
- Autoimmune haemolytic anaemia,
- Antibody-induced pure red cell aplasia
- Wegener's granulomatosis/polyateritis nodosa
- Necrotizing scleritis associated with relapsing polychondritis

Cyclophosphamide

- Adverse effects:
  - Hemorrhagic cystitis,
  - alopecia,
  - nausea & vomiting,
  - SIADH
  - hepatic damage
- Dose: 2-3 mg/kg/day oral

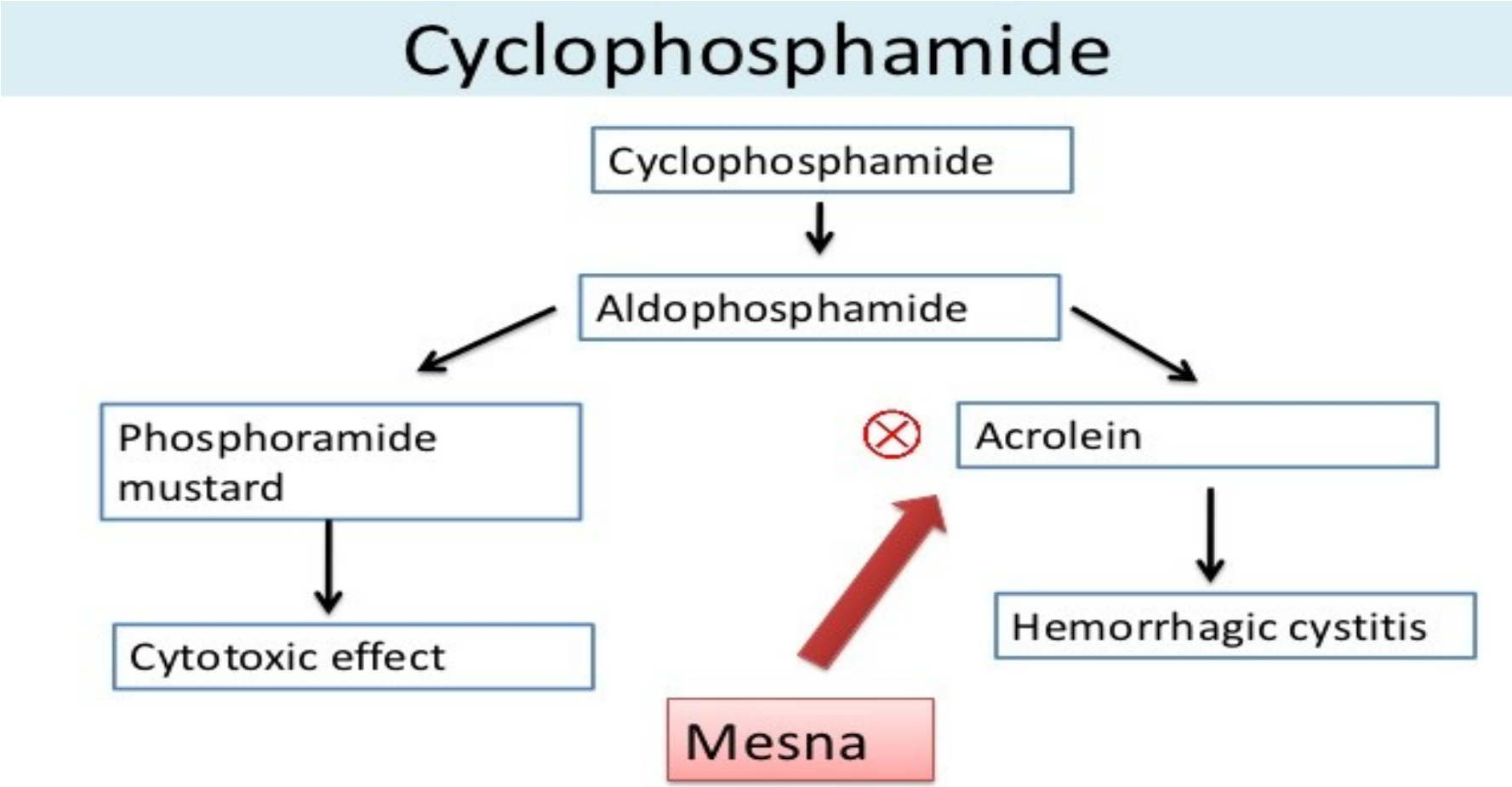
  10-15 mg/kg IV every 7-10 days
- It can be administered IV, IM, IP, intrapleurally, Intraarterialy, directly into tumor

CYCLOPHOSPHAMIDE

OTHER ADVERSE EFFECTS

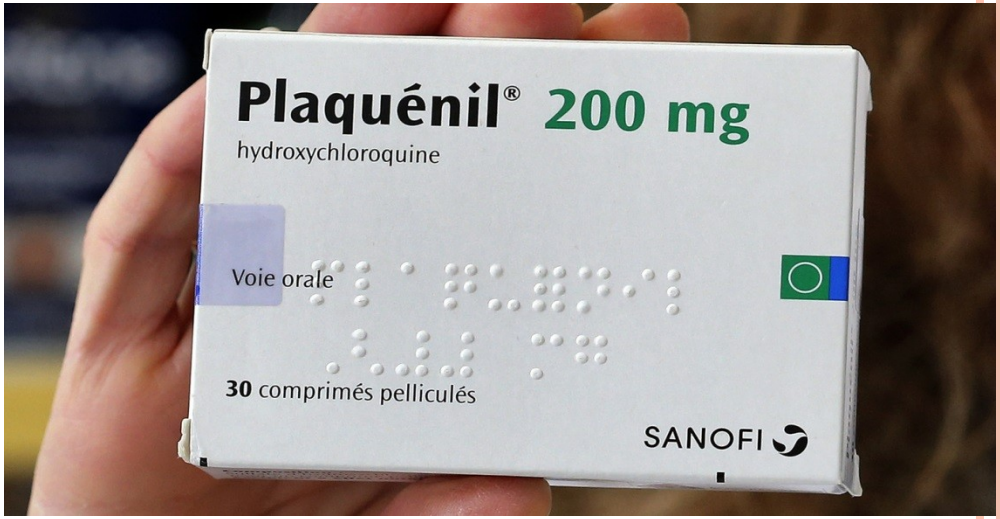
- Pancytopenia
- Subsequent graft-versus-host disease syndrome
- Cardiac toxicity
- Electrolyte disturbances.

CYCLOPHOSPHAMIDE TOXICITY: MANAGEMENT



HYDROXYCHLOROQUINE

- Antimalarial agent with immunosuppressant properties.
- Decreased T-cell activation: due to
  - Increased PH of lysosomal and endosomal compartments
  - Suppress intracellular antigen processing
  - Decreased loading of peptides onto MHC class II molecules



HYDROXYCHLOROQUINE

USES

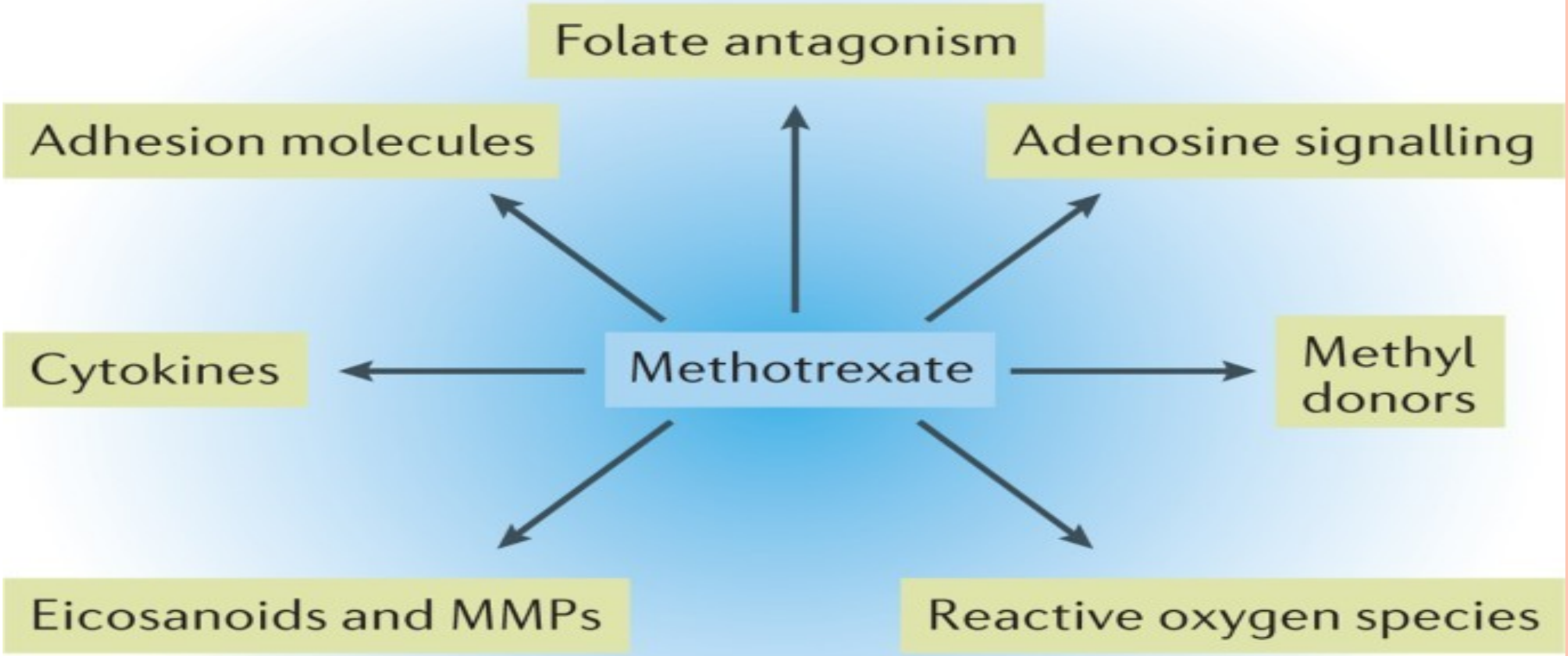
- Rheumatoid arthritis
- Systemic lupus erythematosus.
- Graft -versus-host disease in allogenic stem cell transplantation: both for treatment and prevention
- COVID-19 infection : Controversial data

METHOTREXATE

IMMUNOSUPPRESSIVE ROLE

- Rheumatoid arthritis
- Treatment of graft versus host disease

The use of methotrexate (orally) appears reasonable in patients with idiosyncratic reactions to purine antagonists



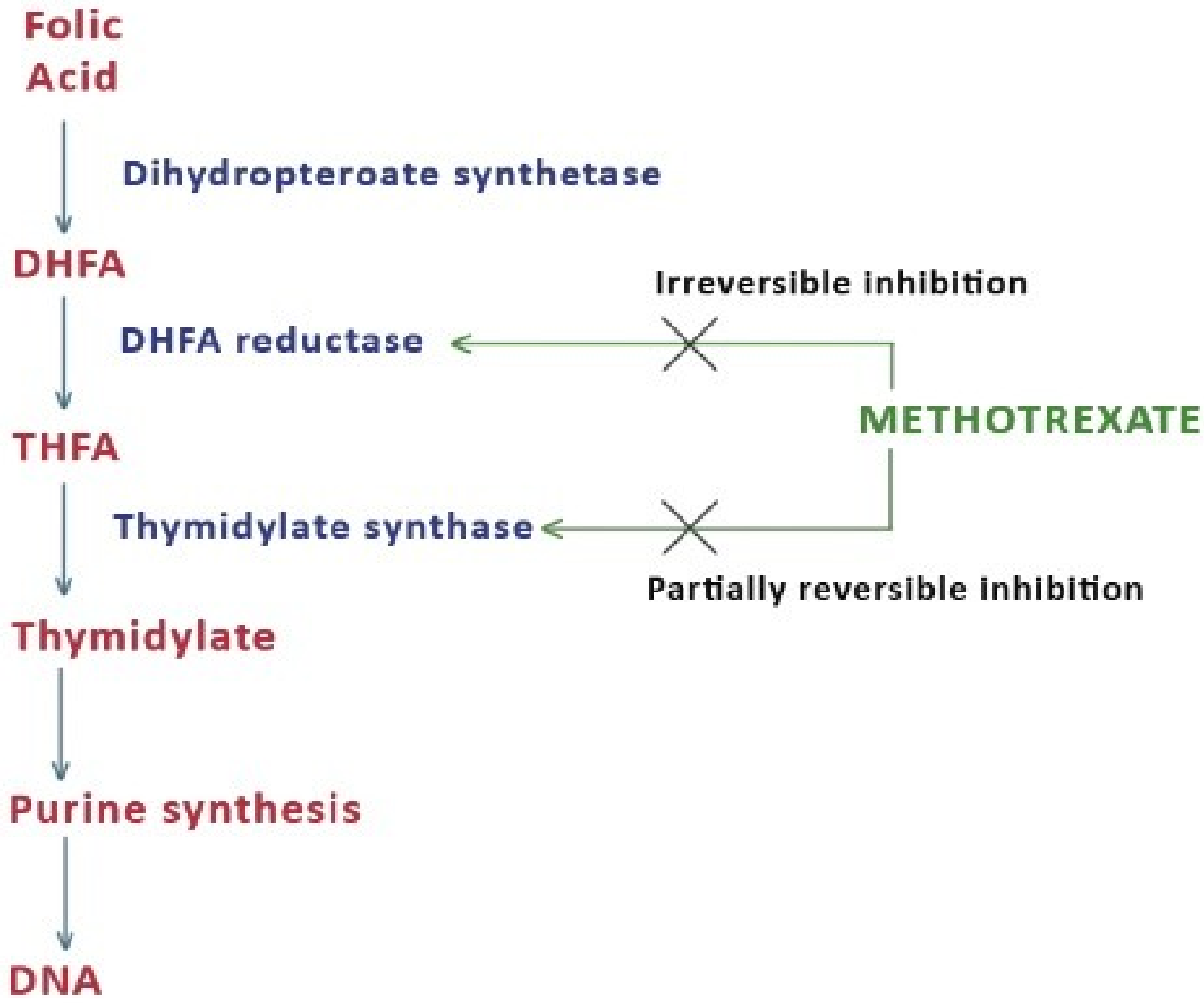
Nature Reviews | Rheumatology

Folate antagonist: Methotrexate

Therapeutic uses:

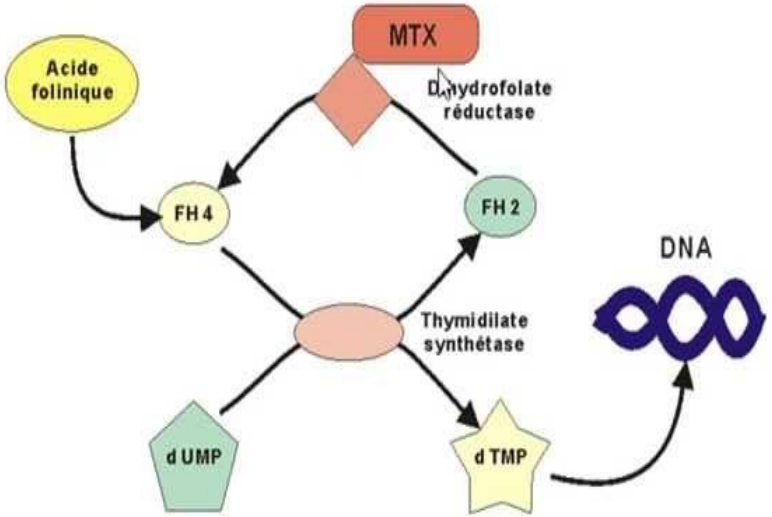
- *MTX*, usually in combination with other drugs, is effective against acute lymphocytic leukemia, choriocarcinoma, Burkitt lymphoma in children, breast cancer, and head and neck carcinomas. In addition, **low-dose *MTX*** is effective as a single agent against certain inflammatory diseases, such as **severe psoriasis and rheumatoid arthritis as well as Crohn disease**.

MECHANISM OF ACTION



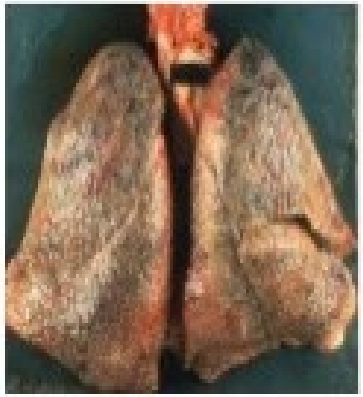
Methotrexate Mechanism of Action

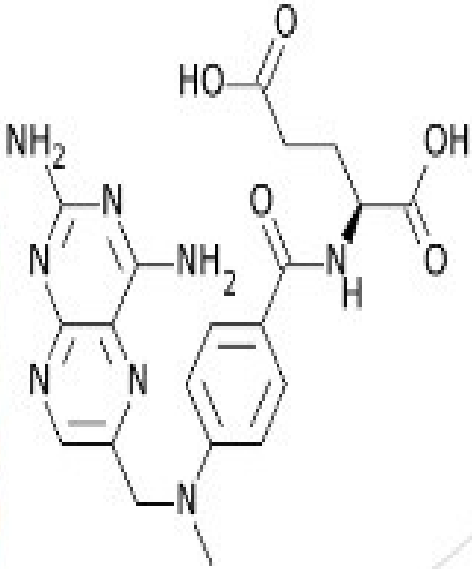
- MTX acts as a competitive analog of folate blocking dihydrofolate reductase preventing the formation of THF and blocking purine biosynthesis
- This drug much like AZA induces the apoptosis of activated lymphocytes



Methotrexate

- a folic acid antagonist
- *Mechanism of Action:*
- Inhibits dihydrofolate reductase required for folic acid activation (tetrahydrofolic)
- Inhibition of DNA, RNA &protein synthesis
- Interferes with T cell replication.
- Rheumatoid arthritis & psoriasis and Crohn disease
- Adverse effects
- Nausea-vomiting-diarrhea
- Alopecia
- Bone marrow depression
- Pulmonary fibrosis
- Renal & hepatic disorders





Folate antagonist: Methotrexate

Adverse effects:

- *MTX* causes stomatitis, myelosuppression, erythema, rash, urticaria, and alopecia.
- Most frequent toxicities: nausea, vomiting, and diarrhea.
- **Adverse effects can be prevented or reversed by administering *leucovorin*.**
- **Hepatic function:** Long-term use of *MTX* may lead to cirrhosis.
- **Renal function:** Variable
- **Neurologic toxicities:** subacute meningeal irritation, stiff neck, headache, and fever. Rarely, seizures, encephalopathy or paraplegia occur.
- **Contraindications:** Because *MTX* is teratogenic in experimental animals and is an abortifacient, it should be avoided in pregnancy.

THALIDOMIDE : MOA

- Inhibits angiogenesis and has anti-inflammatory and immunomodulatory effects
- Inhibits tumor necrosis factor-alpha (TNF-α), reduces phagocytosis by neutrophils
- Increases production of IL-10
- Alters adhesion molecule expression
- Enhances cell-mediated immunity via interactions with t cells

THALIDOMIDE : USES

- **Multiple myeloma**

  at initial diagnosis and for relapsed-refractory disease.

  Signs of response within 2–3 months of starting the drug, with response rates from 20% to 70%.

  When combined with dexamethasone, the response rates in myeloma are 90%.

- Myelodysplastic syndrome
- Acute myelogenous leukaemia
- Graft -versus-host disease

THALIDOMIDE : USES…..

- Solid tumours
  - Carcinoma colon
  - Renal cell carcinoma
  - Malignant melanoma
  - Carcinoma prostate
- Erythema nodosum leprosum
- Cutaneous manifestations of systemic lupus erythematosus.

ADVERSE EFFECTS

- Teratogenicity
- Peripheral neuropathy
- Constipation
- Rash
- Fatigue
- Hypothyroidism
- Deep vein thrombosis

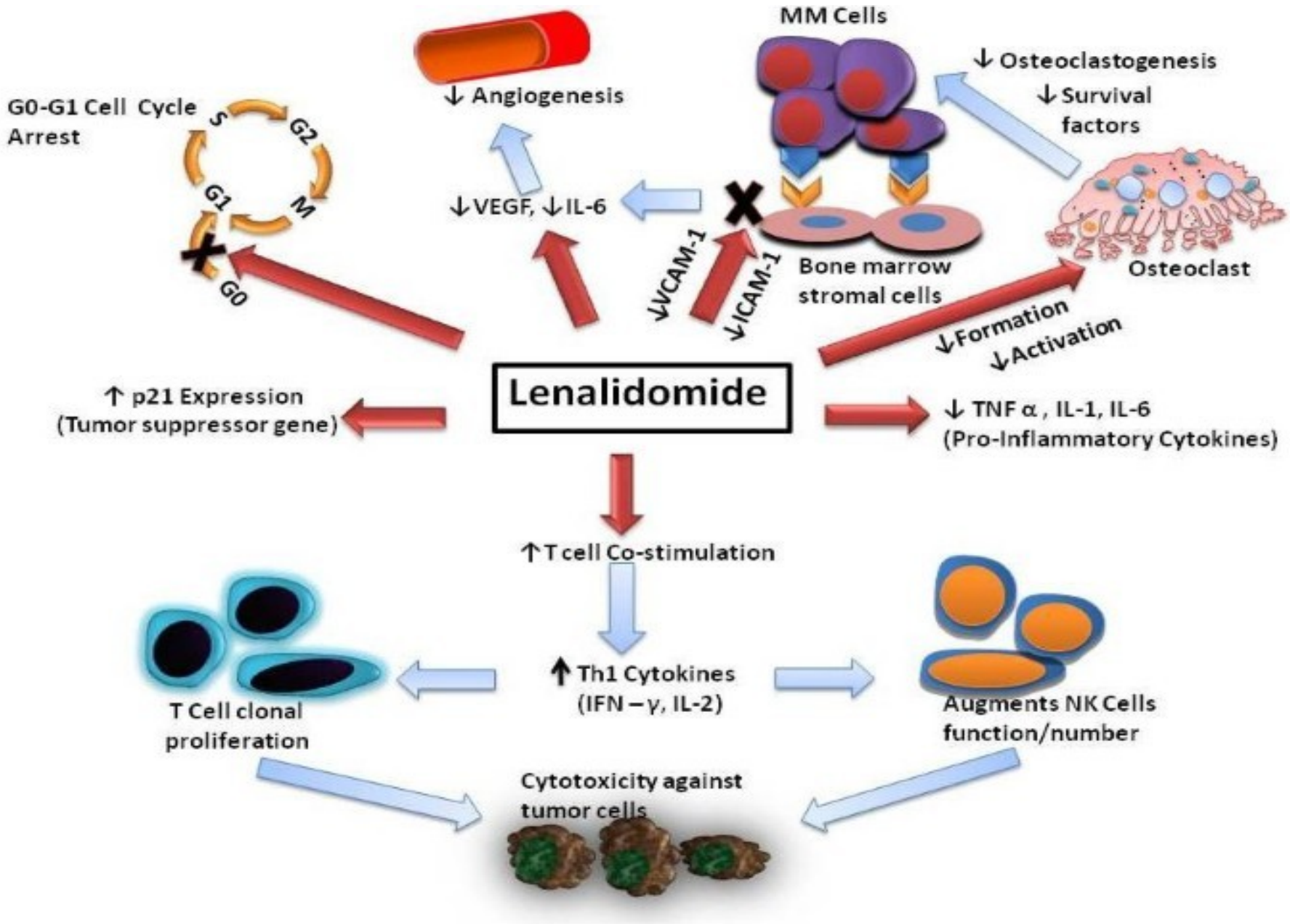
Anticoagulants may be needed when thalidomide treatment is initiated for haematological malignancies

IMMUNOMODULATORY DERIVATIVES OF THALIDOMIDE(IMIDS)

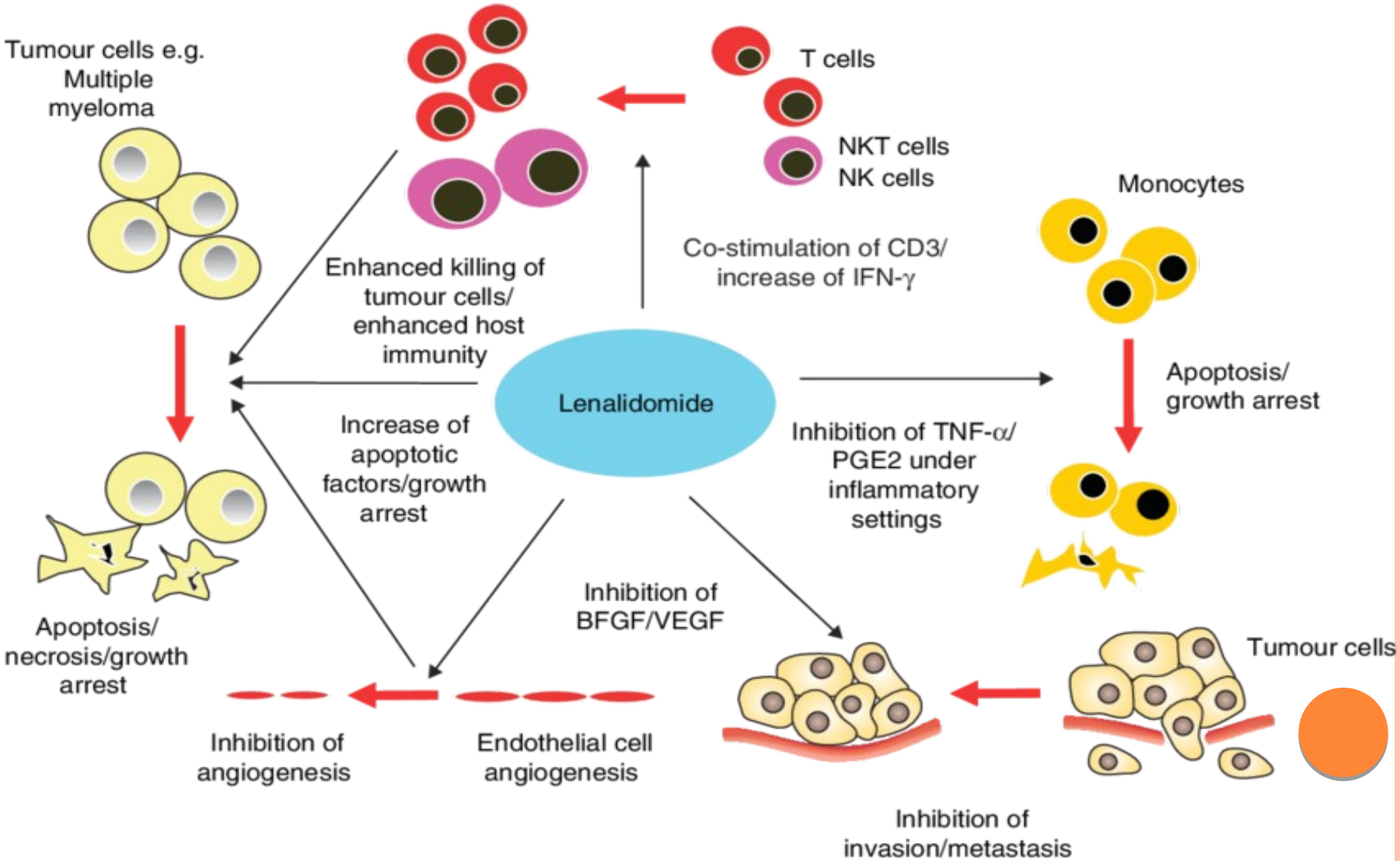
- Immunomodulatory derivatives of thalidomide are termed **IMiDs.**
- Some IMiDs are much more potent than thalidomide in regulating cytokines and affecting T-cell proliferation

LENALIDOMIDE

- An IMID
- Similar to thalidomide in action
- But with less teratogenicity and fewer thromboembolic events


MM Cells
↓ Osteoclastogenesis
↓ Survival factors
↓ Angiogenesis
G0-G1 Cell Cycle Arrest
S
G2
G1
M
G0
↓VEGF, ↓IL-6
↓VCAM-1
↓ICAM-1
Bone marrow stromal cells
Osteoclast
↓Formation
↓Activation
↑ p21 Expression
(Tumor suppressor gene)
Lenalidomide
↓ TNF α , IL-1, IL-6
(Pro-Inflammatory Cytokines)
↑T cell Co-stimulation
↑Th1 Cytokines
(IFN – γ, IL-2)
T Cell clonal proliferation
Augments NK Cells function/number
Cytotoxicity against tumor cells

LENALIDOMIDE IN MULTIPLE MYELOMA



LENALIDOMIDE USES

- Myelodysplastic syndrome.
- Multiple myeloma:

newly diagnosed /relapsed or refractory

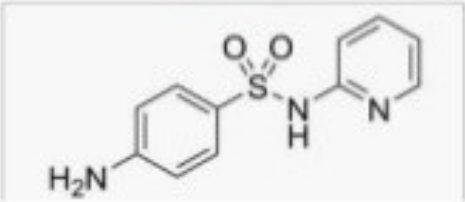
POMALIDOMIDE (CC4047)

- Oral IMiD
- USES
  - Treatment of multiple myeloma
  - Treatment of myelodysplasia.
- Not as immunosuppressant in transplant rejection

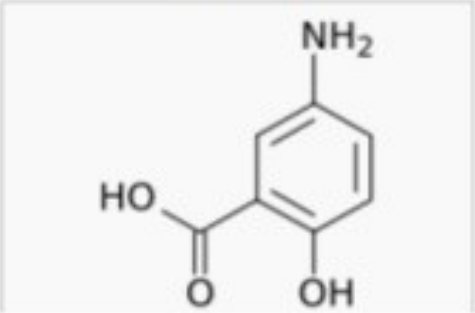
SULFASALAZINE

- Compound of Sulfapyridine and 5-amino salicylic acid
- Actual mechanism of action is not known
- Sulfapyridine split off by bacteria and absorbed systemically appears to be the active moiety
- Suppresses the generation of superoxide radicals and cytokine elaboration by inflammatory cells

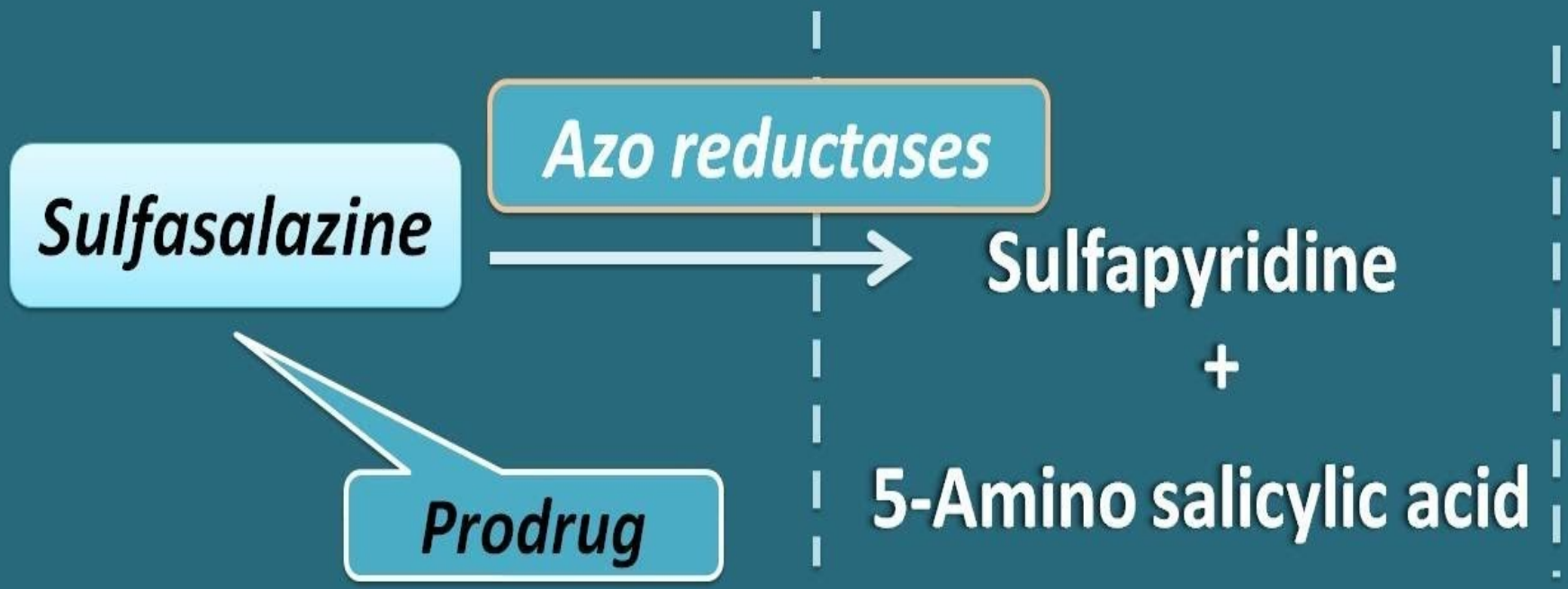
Sulfapyridine

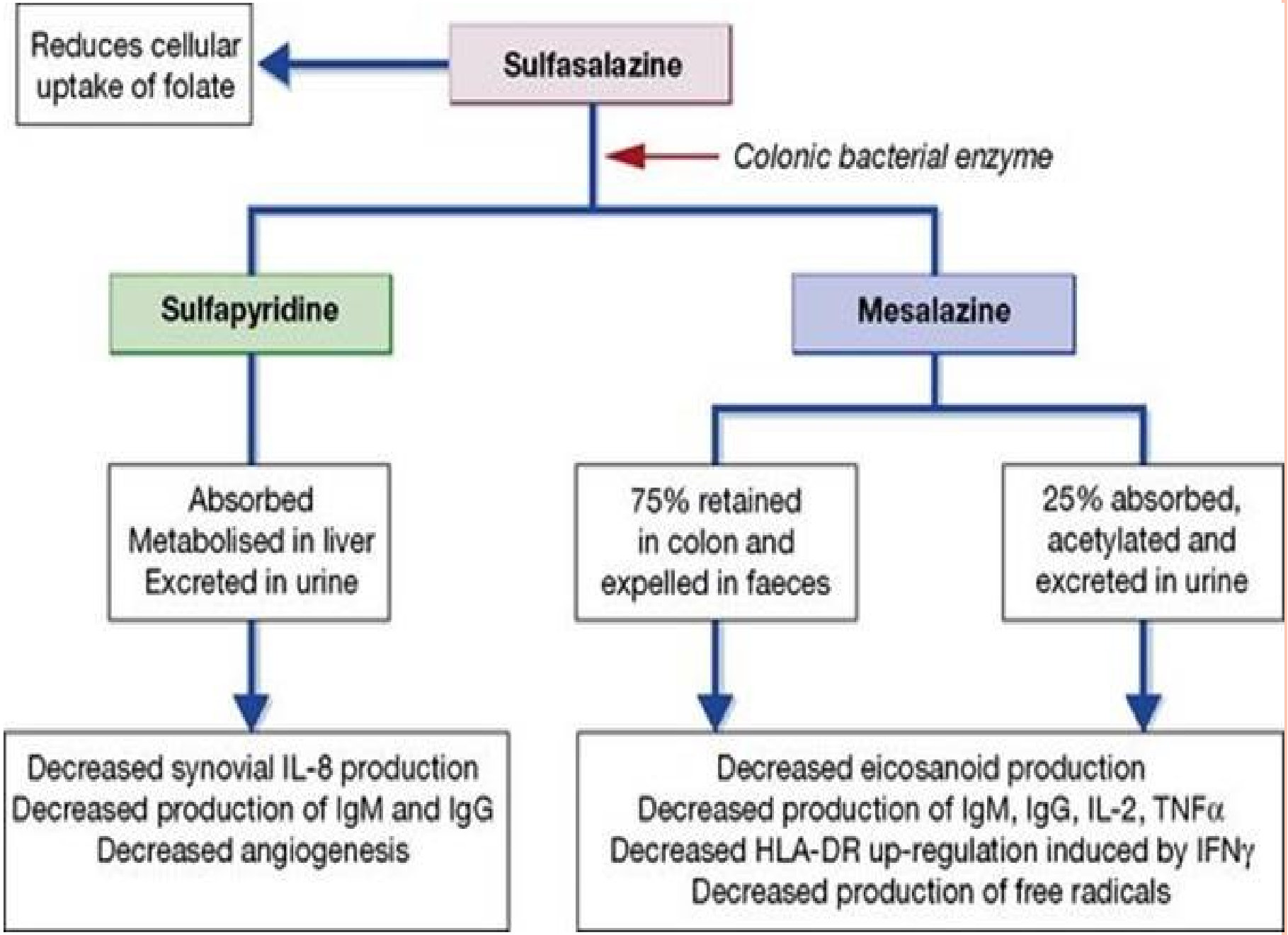


Mesalazine



How SULFLASALAZINE Acts...?




Reduces cellular uptake of folate
Sulfasalazine
Colonic bacterial enzyme
Sulfapyridine
Mesalazine
Absorbed
Metabolised in liver
Excreted in urine
75% retained in colon and expelled in faeces
25% absorbed, acetylated and excreted in urine
Decreased synovial IL-8 production
Decreased production of IgM and IgG
Decreased angiogenesis
Decreased eicosanoid production
Decreased production of IgM, IgG, IL-2, TNFα
Decreased HLA-DR up-regulation induced by IFNγ
Decreased production of free radicals

Sulfasalazine

Mechanism of Action

- Poorly understood
- Prodrug
- Broken down in gut into **5-aminosalicylic acid (5-ASA)** and sulfapyridine
- 5-ASA has anti-inflammatory activity

Dosing and Administration

- Start at 0.5 – 1 g po daily
- Increase weekly to 1 g bid
- Max: 3 g per day

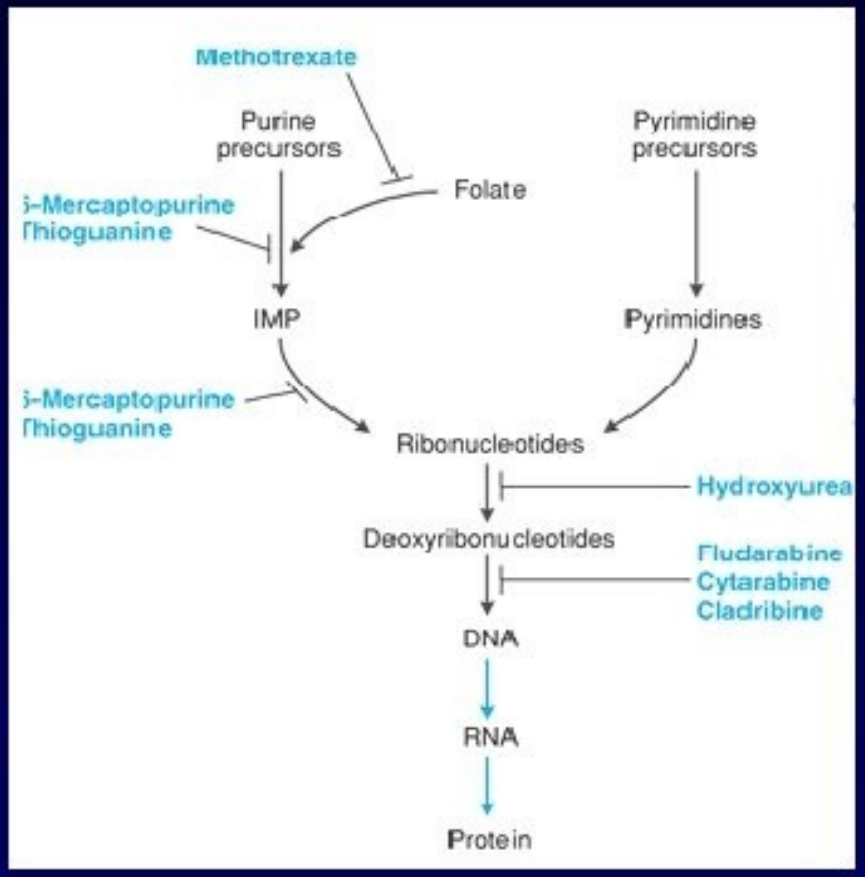
Clinically significant interaction with warfarin

Sulfasalazine

- Uses:
  - ✓ Rheumatoid arthritis:
    - Second line drug (for milder cases)
    - Combined with Mtx
  - ✓ Inflammatory bowel disease
- Adverse effects:
  - ✓ Neutropenia, thrombocytopenia
  - ✓ Hepatitis

Cytarabine (Ara-C)

- Cytarabine arabinoside is a pyrimidine antimetabolite
- Inhibits conversion of cytidine to deoxycytidine
- The drug is activated by kinases to AraCTP
  - This acts as an inhibitor of DNA polymerase
- Side effect: At high doses cause neurotoxicity (cerebellar dysfunction and peripheral neuritis)
  - Hand-foot syndrome



Cytosine arabinoside

- Used in treatment of leukemia
- Short half-life and cell cycle sepcificity
  - Schedule dependent cytotoxicity
  - 3 +7 regimen for acute myelogenous leukemia induction: 3 days of intravenous administration of an anthracycline and 7 days of continuous infusion of cytosine arabinoside (168 hours)
- High dose cytosine arabinoside therapy
  - Myelosuppression, cerebellar toxicity, conjunctivitis
- Approved for intrathecal administration

CYTARABINE

- ✓ Cytarabine is converted to its activated <u>Triphosphate form</u>
- ✓ Upon incorporation into DNA, the Cytidine analog interferes with chain elongation and promotes abnormal fragment ligation of newly synthesized DNA
- ✓ If araCTP ends up being incorporated into DNA, it leads to Apoptosis

Dactinomycin

Mechanism of Action

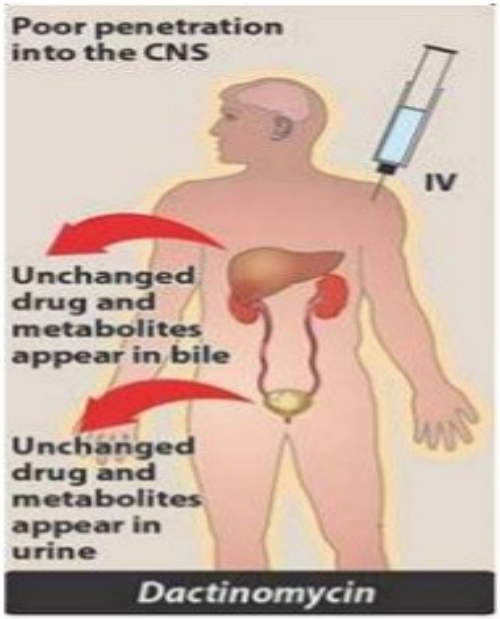
- Binds to double stranded DNA through intercalation between adjacent guanine-cytosine base pairs
- Inhibits all forms of DNA-dependent RNA synthesis

DACTINOMYCIN

- Immune modulation == to prevent impending renal transplant rejection.

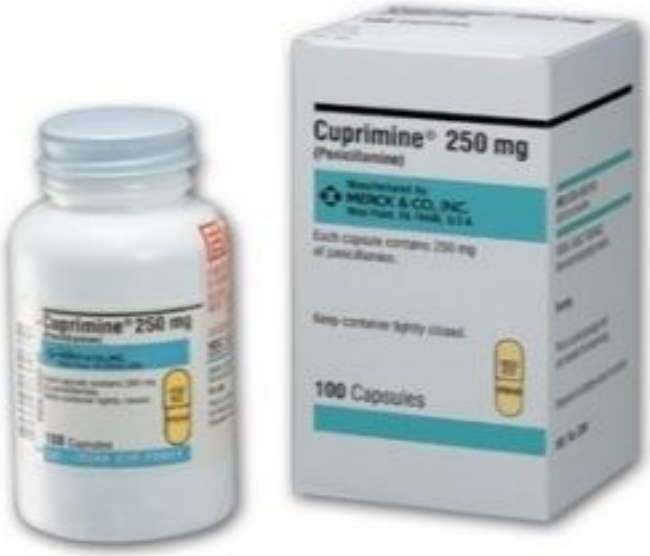
Dactinomycin

- Uses:
  - Wilms tumor,
  - gestational choriocarcinoma
- Adverse effects
  - bone marrow supression
  - Irritant like meclorethamine
  - sensitizes to radiation, and inflammation at sites of prior radiation therapy may occur
  - Gastrointestinal adverse effects



Penicillamine

(Vistamin, Cuprimine)



Mechanism of action

- Penicillamine chelates several metals including copper, lead, iron, and mercury, forming stable water soluble complexes that are renally excreted.
- It also combines chemically with cystine to form a stable, soluble, readily excreted complex.
- It may also have antifibrotic effects as it inhibits lysyl oxidase, an enzyme necessary for collagen production.

D-penicillamine

Therapeutic indications

a) Severe, active rheumatoid arthritis, including juvenile forms

b) Wilson's disease (hepatolenticular degeneration) in adults and children (0 to 18 years)

c) Cystinuria-dissolution and prevention of cystine stones in adults and children (0 to 18 years)

d) Lead poisoning in adults and children (0 to 18 years)

e) Chronic active hepatitis in adults

VINCRISTINE

- Idiopathic thrombocytopenic purpura refractory to prednisone.

Comparison between

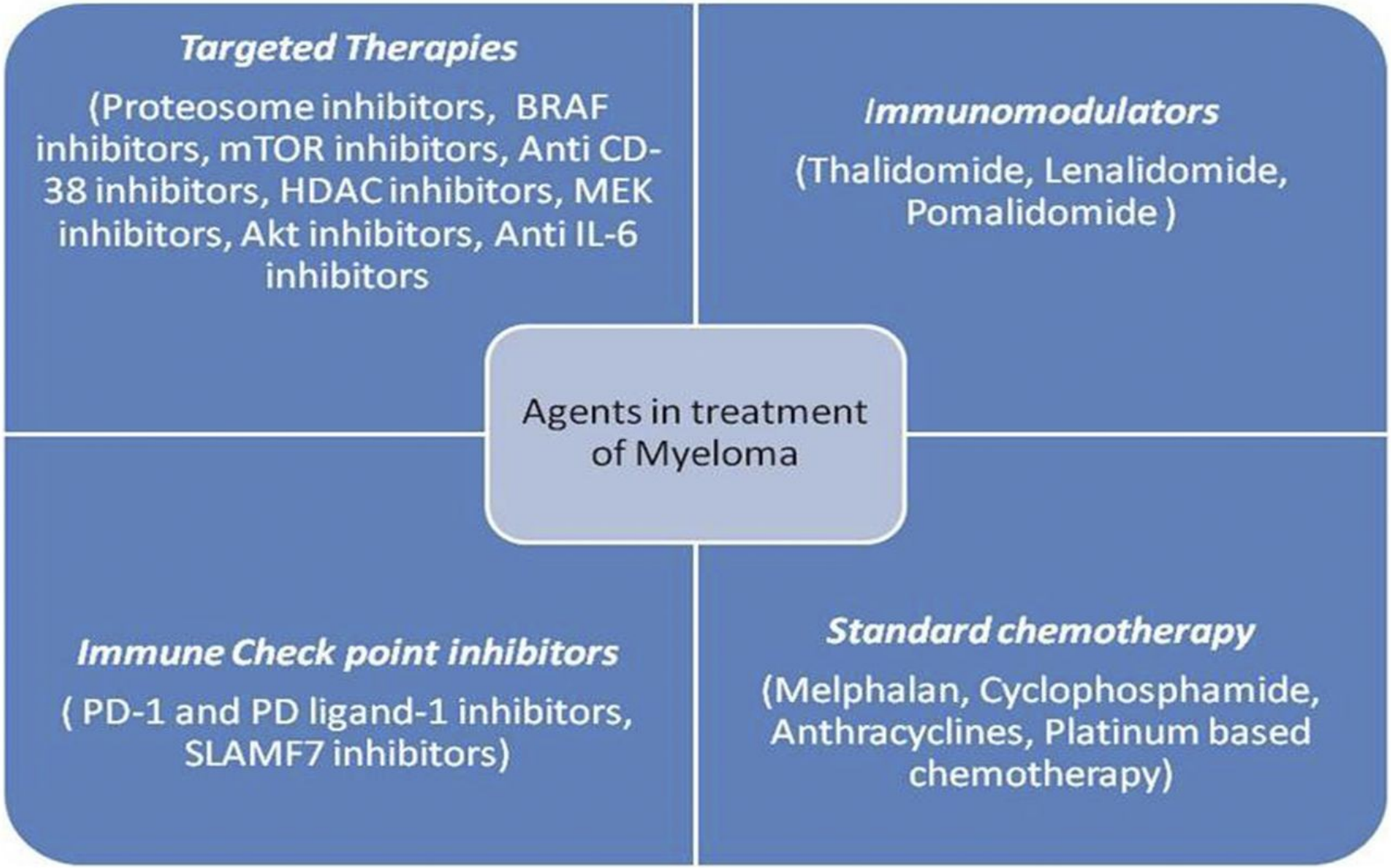
Vincristine	Vinblastine
• Marrow sparing effect	• Bone marrow supression
• Alopecia more common	• Less common
• Peripheral & autonomic neuropathy & muscle weakness (CNS)	• Less common, temp. mental depresssion
• Constipation	• Nausea, vomiting, diarrhoea
• Uses: (Childhood cancers)	• uses
– ALL , Hodgkins, lymphosarcoma, Wilms tumor, Ewings sarcoma	– Hodgkins disease & other lymphomas , breast cancer, testicular cancer

VINBLASTINE

- Prevents mast cell degranulation by binding to microtubule units within the cell
- Prevents release of histamine and other vasoactive compounds.

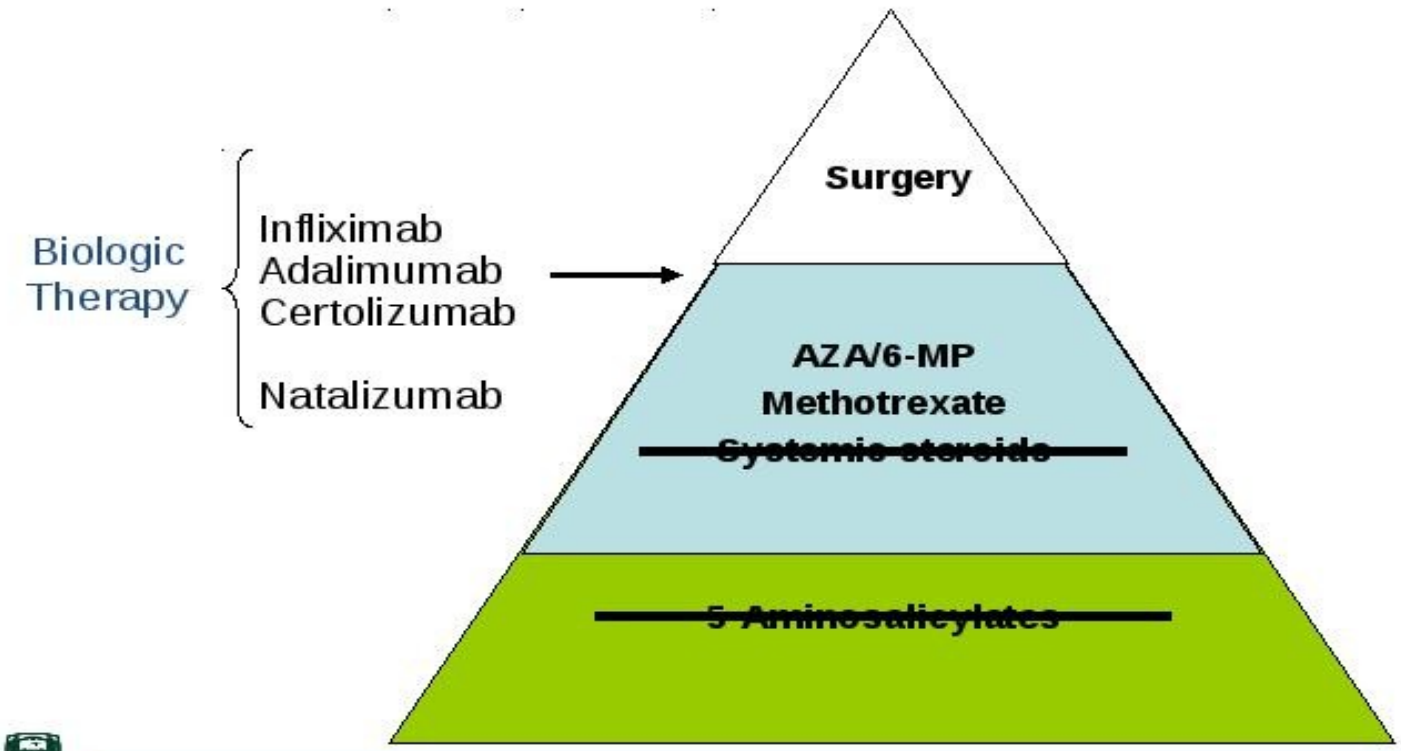
- **Uses :**
- **Vinblastine is used for treatment of Hodgkin's disease (Pseudoleukemia or Lymphatic anaemia) and carcinoma resistant to other therapy.**
- **Vincristine has a cytotoxic effect .It is useful in the treatment of leukemia in children, small cell lung cancer, cervical and vaginal cancers.**
- **Mechanism:**
- Both alkaloids are Antimetabolites interfere with the syntheses of Desoxyribonucleic acids.

CLINICAL PHARMACOLOGY



CLINICAL PHARMACOLOGY

Therapeutic Pyramid for Crohn's disease



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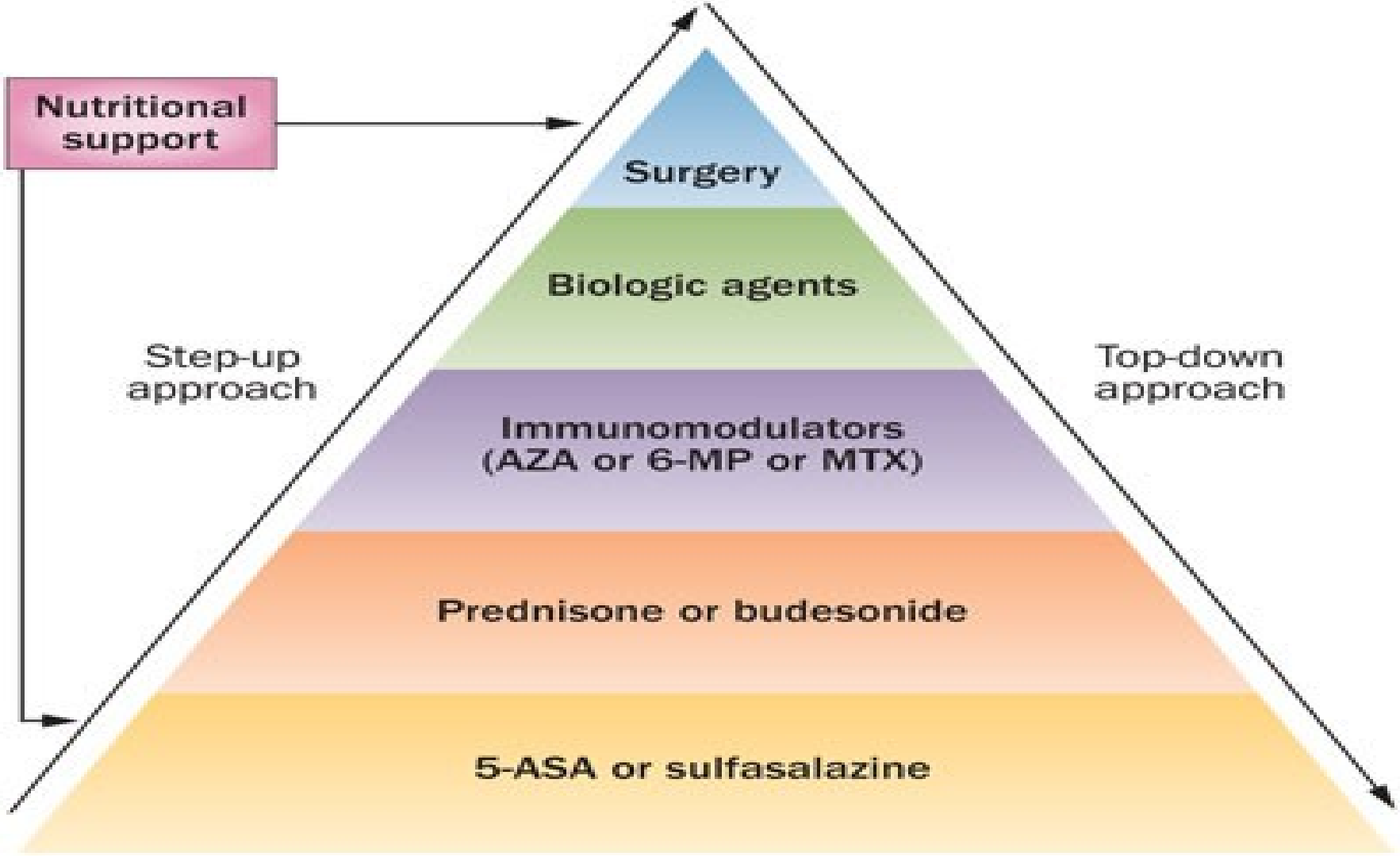

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