ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

ΠΜΣ «Εφαρμογές Μοριακής Βιολογίας – Γενετική.
Διαγνωστικοί δείκτες

ΑΝΤΙΚΑΡΚΙΝΙΚΑ ΦΑΡΜΑΚΑ

Νικόλαος Μπαλατσός

Overview

Introduction

- **Malignant disease accounts for a high proportion of deaths in industrialised countries.**
- **The treatment of anticancer drug is to give palliation, induce remission and, if possible, cure.**

Overview

Introduction

- **Cancer occurs after normal cells have been transformed into neoplastic cells through alteration of their genetic material and the abnormal expression of certain genes.**
- **Neoplastic cells usually exhibit *chromosomal abnormalities* and the *loss of their differentiated properties*. These changes lead to uncontrolled cell division and many result in the invasion of previously unaffected organs, a process called metastasis.**

Advances in Cancer Chemotherapy

Treatment options of cancer:

- **Surgery: before 1955**
- **Radiotherapy: 1955~1965**
- **Chemotherapy: after 1965**
- **Immunotherapy and Gene therapy**

Leading New Cancer Cases and Deaths – 2012 Estimates

Estimated New Cases*		Estimated Deaths	
Male	**Female**	**Male**	**Female**
Prostate 241,740 (29%)	Breast 226,870 (29%)	Lung & bronchus 87,750 (29%)	Lung & bronchus 72,590 (26%)
Lung & bronchus 116,470 (14%)	Lung & bronchus 109,690 (14%)	Prostate 28,170 (9%)	Breast 39,510 (14%)
Colon & rectum 73,420 (9%)	Colon & rectum 70,040 (9%)	Colon & rectum 26,470 (9%)	Colon & rectum 25,220 (9%)
Urinary bladder 55,600 (7%)	Uterine corpus 47,130 (6%)	Pancreas 18,850 (6%)	Pancreas 18,540 (7%)
Melanoma of the skin 44,250 (5%)	Thyroid 43,210 (5%)	Liver & intrahepatic bile duct 13,980 (5%)	Ovary 15,500 (6%)
Kidney & renal pelvis 40,250 (5%)	Melanoma of the skin 32,000 (4%)	Leukemia 13,500 (4%)	Leukemia 10,040 (4%)
Non-Hodgkin lymphoma 38,160 (4%)	Non-Hodgkin lymphoma 31,970 (4%)	Esophagus 12,040 (4%)	Non-Hodgkin lymphoma 8,620 (3%)
Oral cavity & pharynx 28,540 (3%)	Kidney & renal pelvis 24,520 (3%)	Urinary bladder 10,510 (3%)	Uterine corpus 8,010 (3%)
Leukemia 26,830 (3%)	Ovary 22,280 (3%)	Non-Hodgkin lymphoma 10,320 (3%)	Liver & intrahepatic bile duct 6,570 (2%)
Pancreas 22,090 (3%)	Pancreas 21,830 (3%)	Kidney & renal pelvis 8,650 (3%)	Brain & other nervous system 5,980 (2%)
All sites 848,170 (100%)	All sites 790,740 (100%)	All sites 301,820 (100%)	All sites 275,370 (100%)

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Advances in Cancer Chemotherapy

The treatment of a patient with cancer may aim to:

- **give palliation, for example prompt relief of unpleasant symptoms such as superior vena cava obstruction from a mediastinal tumor**
- **induce ‘remission’ so that all macroscopic and microscopic features of the cancer disappear, though disease is known to persist**
- **cure, for which all the cells of the clone must be destroyed.**

Cancer Chemotherapy

Disease Name	5 Years Survival Rate
• Small Cell Lung Cancer (Limited Stage)	10~20%
• (Extensive Stage)	0~5%
• Non-Hodgkin' s lymphoma*	40~65%
• Ovarian Cancer	40~60%
• Children Solid Tumor (Nephroblastoma, Rhabdomyosarcoma, Lymphoma, Osteosarcoma)*	60~90%
• Trophoblastoma (Chorion Epithelioma)	80~90%
• Seminoma of Testis	60~90%
• Embryonic Carcinoma of Testis	60~80%

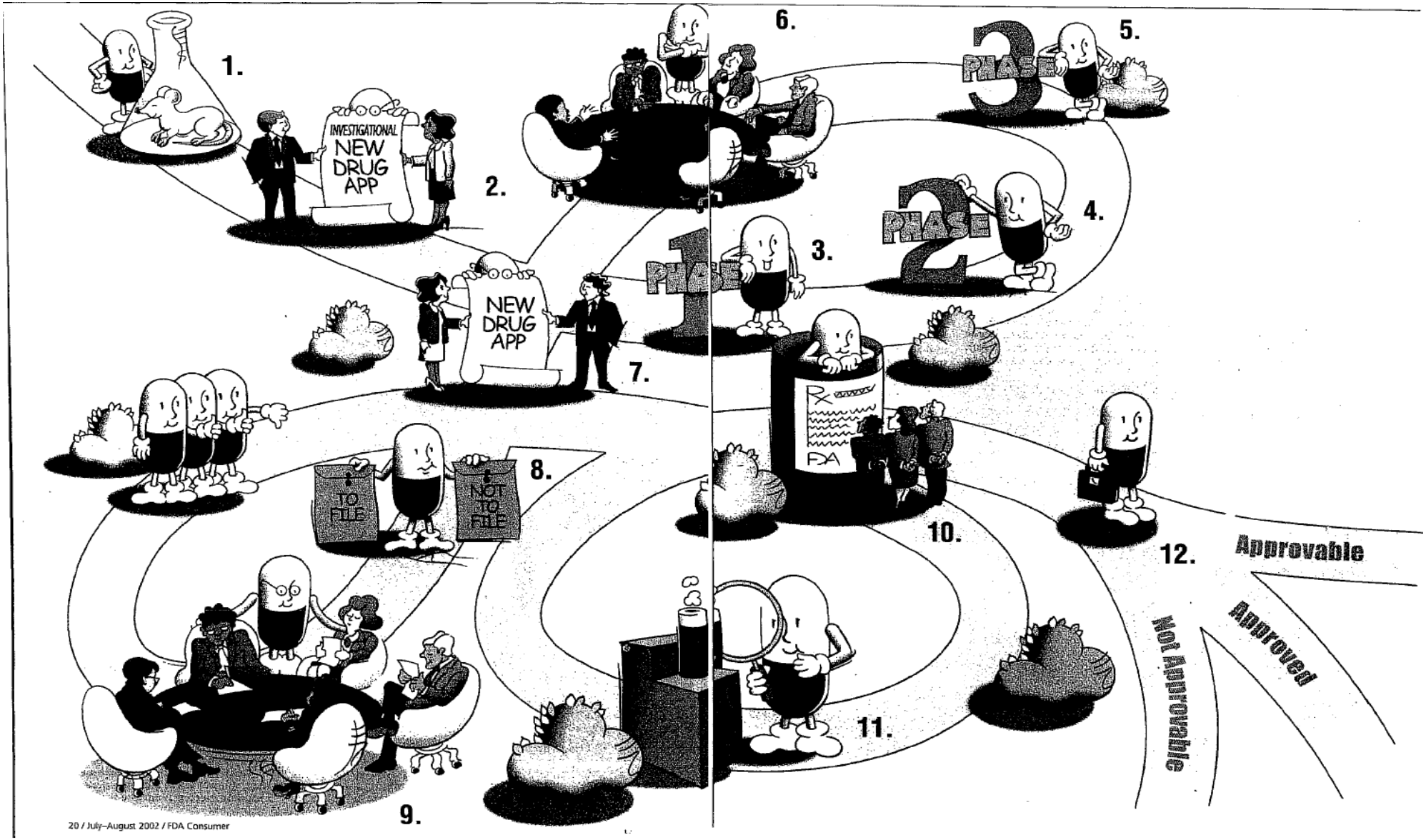
* *Combination with other therapeutics*

Interfere Protein Synthesis

- ***Antitubulin:*** **vinca alkaloids and taxanes;**
- ***Interfere the function of ribosome:*** **harringtonines;**
- ***Influence amino acid supply:*** **L-asparaginase**

Bind tubulin, destroy spindle to produce mitotic arrest.

The Long Road of a New Medicine



The Main Step of Anticancer Drug Research

- **Non-clinical Research：**

1.Anticancer Drug Screen:

***in vitro*：** **tumor cell culture, tumor inhibitor/kill test**

***in vivo*：** **animal xenograft model**

e.g. Ehrlich ascites tumor,

S180 lymphosarcoma

2. Pharmacodynamics, pharmacokinetics and toxicology test

The Main Step of Anticancer Drug Research

- **Clinical Research:**
  - Phase 1 clinical trial
  - Phase 2 clinical trial
  - Phase 3 clinical trial
  - Phase 4 clinical trial

The Main Step of Anticancer Drug Research

Phase 1 clinical trial

In Phase 1 clinical trials, researchers test a new drug or treatment in a small group of people (20-80) for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.

- **TOLERANCE**
- **PHARMACOKINETICS**

The Main Step of Anticancer Drug Research

Phase 2 clinical trial

In Phase 2 clinical trials, the study drug or treatment is given to a larger group of people (40-100) to see if it is effective and to further evaluate its safety.

The Main Step of Anticancer Drug Research

Phase 3 clinical trial

In Phase 3 studies, the study drug or treatment is given to large groups of people (more than 200) to further determine its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.

The Main Step of Anticancer Drug Research

Phase 4 clinical trial

Phase 4 studies are done after the drug or treatment has been marketed. These studies continue testing the study drug or treatment to collect information about their effect in various populations and any side effects associated with long-term use.

The Classification of Anticancer Drugs

1. According to chemical structure and resource of the drug;
2. According to biochemistry mechanisms of anticancer action;
3. According to the cycle or phase specificity of the drug

The Classification of Anticancer Drugs

- **According to chemical structure and resource of the drug:**

1. **Alkylating Agents**
2. **Antimetabolite**
3. **Antibiotics**
4. **Plant Extracts**
5. **Hormones**
6. **Others**

The Classification of Anticancer Drugs

- **According to biochemistry mechanisms of anticancer action:**

1. **Block nucleic acid biosynthesis**
2. **Direct influence the structure and function of DNA**
3. **Interfere transcription and block RNA synthesis**
4. **Interfere protein synthesis and function**
5. **Influence hormone homeostasis**
6. **Others**

Κατάταξη αντικαρκινικών φαρμάκων

- **According to the cycle or phase specificity of the drug:**

1. **cell cycle nonspecific agents (CCNSA)**
2. **cell cycle specific agents (CCSA)**

Κατάταξη αντικαρκινικών φαρμάκων

- **Σύμφωνα με δομή και προέλευση φαρμάκου**

1. **Παράγοντες Αλκυλίωσης, Alkylating Agents**
2. **Αντιμεταβολίτες, Antimetabolite**
3. **Αντιβιοτικά, Antibiotics**
4. **Φυτικά εκχυλίσματα, Plant Extracts**
5. **Ορμόνες, Hormones**
6. **Αλλα, Others** (cis-platinum, carboplatin, lobaplatin)

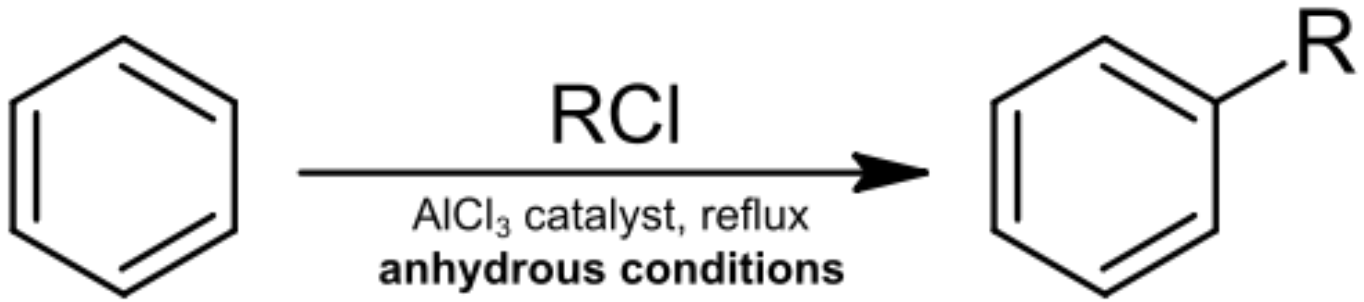
Alkylating Agents

- **One of the frightening developments of World War I was the introduction of chemical warfare. These compounds were known as the nitrogen mustard gases. The nitrogen mustards were observed to inhibit cell growth, especially of bone marrow. Shortly after the war, these compounds were investigated and shown to inhibit the growth of cancer cells.**

Alkylating Agents

Mechanism of Action

- **Nitrogen mustards inhibit cell reproduction by binding irreversibly with the nucleic acids (DNA). The specific type of chemical bonding involved is *alkylation*. After alkylation, DNA is unable to replicate and therefore can no longer synthesize proteins and other essential cell metabolites. Consequently, cell reproduction is inhibited and the cell eventually dies from the inability to maintain its metabolic functions.**



Mechanism of action of alkylating agents

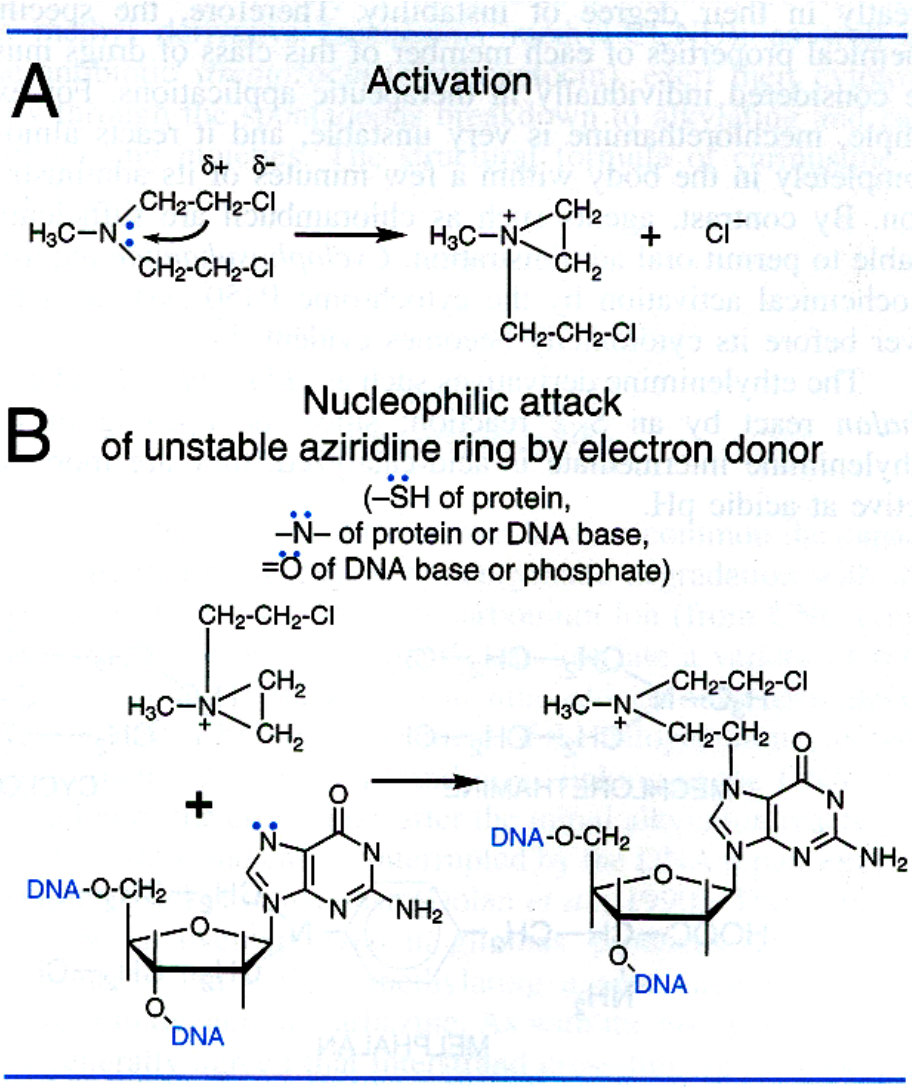


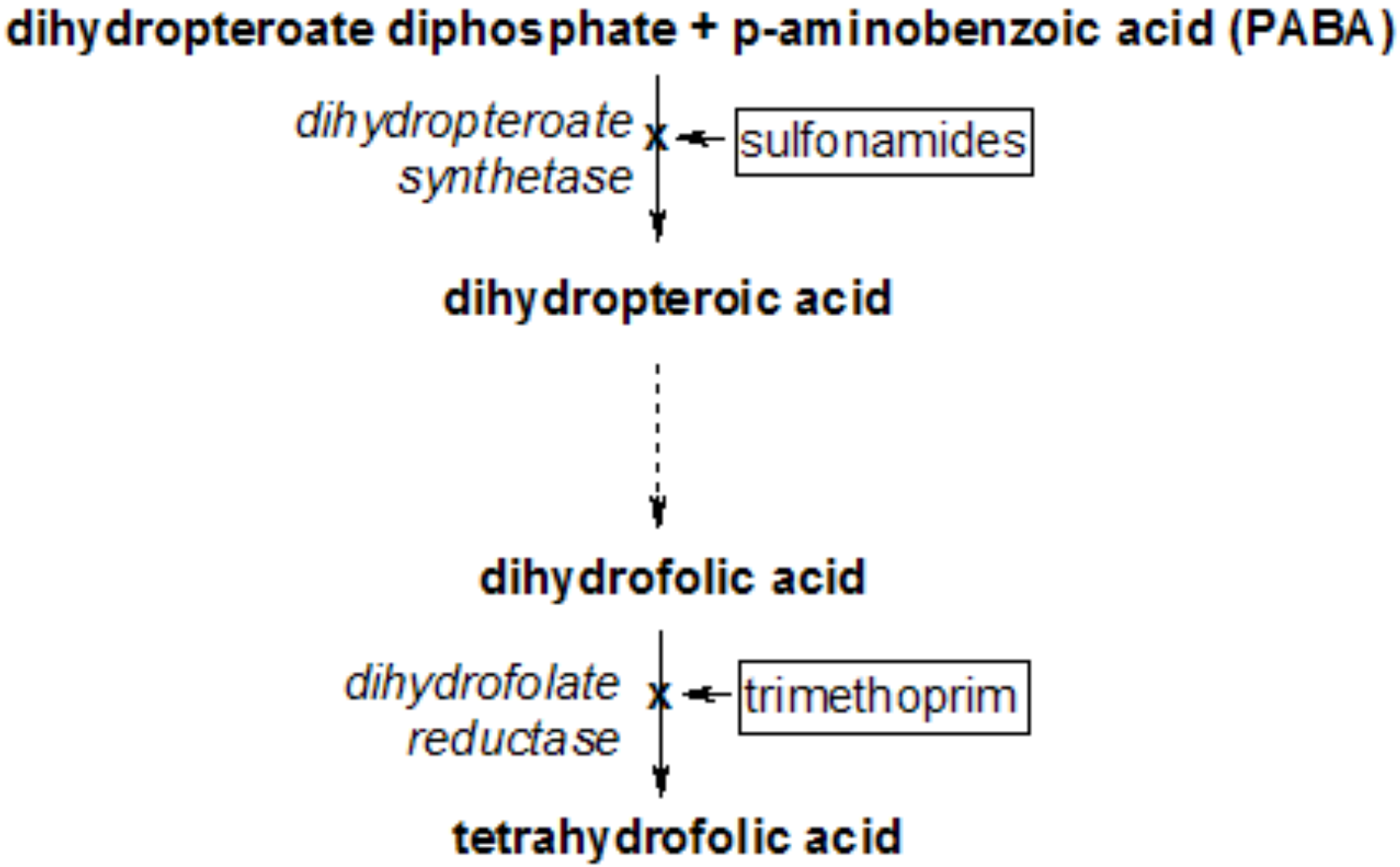
Figure 52–1. Mechanism of action of alkylating agents.

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed).
The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

Chemistry. The chemotherapeutic alkylating agents have in common the property of becoming strong electrophiles through the formation of carbonium ion intermediates or of transition complexes with the target molecules. These reactions result in the formation of covalent linkages by alkylation of various nucleophilic moieties such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. The chemotherapeutic and cytotoxic effects are directly related to the alkylation of DNA. The 7 nitrogen atom of guanine is particularly susceptible to the formation of a covalent bond with bifunctional alkylating agents and may well represent the key target that determines their biological effects. It must be appreciated, however, that other atoms in the purine and pyrimidine bases of DNA—particularly, the 1 and 3 nitrogens of adenine, the 3 nitrogen of cytosine, and the 6 oxygen of guanine—also may be alkylated, as will be the phosphate atoms of the DNA chains and amino and sulfhydryl groups of proteins.

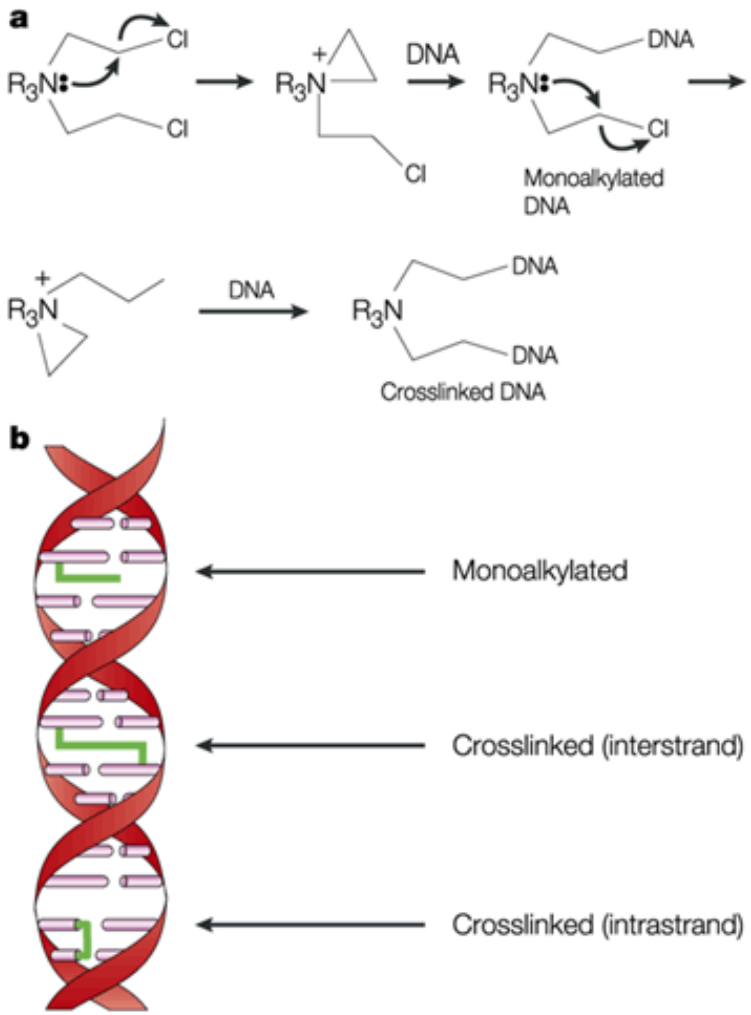
To illustrate the actions of alkylating agents, possible consequences of the reaction of *mechlorethamine* (nitrogen mustard) with guanine residues in DNA chains are shown in Figure 52–1. First, one 2-chloroethyl side chain undergoes a first-order (S_N1) intramolecular cyclization, with release of Cl^- and formation of a highly reactive ethyleniminium intermediate (Figure 52–*1A*). By this reaction, the tertiary amine is converted to an unstable quaternary ammonium compound, which can react avidly, through formation of a carbonium ion or transition complex intermediate, with a variety of sites that possess high electron density. This reaction proceeds as a second-order (S_N2) nucleophilic substitution. Alkylation of the 7 nitrogen of guanine residues in DNA (Figure 52–*1B*), a highly favored

Tetrahydrofolate synthesis



http://en.wikipedia.org/wiki/Dihydrofolate_reductase_inhibitor

Alkylation of DNA



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In alkylation chemistry (see **a**), chlorine is a good leaving group that facilitates nucleophilic attack of nitrogen to form an imminium ion (R_3N) in a strained ring system. This readily undergoes alkylation at N7 of guanine to form a monoalkylation adduct (see b). This process can then be repeated to form the crosslinked DNA. Crosslinking can occur either between two complementary strands of DNA (interstrand), as generated by chlorambucil and melphalan, or within a strand of DNA (intrastrand), as generated by cisplatin (see b).

Hurley LH. *Nature Reviews Cancer* ***2, 188-200***

Κατάταξη παραγόντων αλκυλίωσης

- **Δις χλωροαίθυλ αμίνες (Bis Chloroethyl Amines):**

  Cyclophosphamide,
  Chlormethine,
  Chlorambucil,
  Sarcolysine

- **Νιτροζουρίες (Nitrosoureas):**

Carmustine，Lomustine

- **Αιθυλεναμμώνιο, ή Αριζιδίνες,**

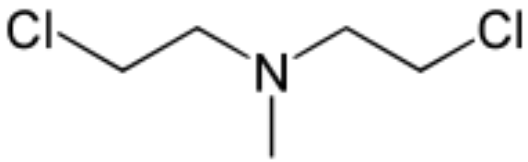
  (Ethyeneammonium or Aziridines):

  Thiotepa
  triethylene melamine

- **Αλκυλοσουλφονικά (Alkysulfonates): Busulfan**

Alkylating Agents——Mustine

- **Mustine must be injected intravenously because it is highly reactive. It disappears very rapidly from the blood, the activity of Mustine lasts only a few minutes.**
- **The main indication for Mustine is in treatment of Hodgkins disease and lymphomas, but it may also be useful in other malignancies.**



Alkylating Agents—— Cyclophosphamide

Cyclophosphamide can also be given orally.

Indications：

- **It is used in the treatment of chronic lymphocyctic leukemia, non-Hodgkin's lymphomas, breast and ovarian cancer, and a variety of other cancers.**
- **It is also a potent immunosuppressant, it is used in the management of rheumatoid disorders and autoimmune nephritis.**

Adverse Effects:

- **Alopecia, nausea, vomiting, myelosuppression, and hemorrhagic cystitis.**

Alkylating Agents——Nitrosoureas

Carmustine, Lomustine, Semustine

Pharmacokinetics:

- **Nitrosoureas are highly lipophilic and reach cerebrospinal fluid concentrations that are about 30% of plasma concentrations.**

Indications:

- **Because of their excellent CNS penetration, carmustine and lomustine have been used to treat brain tumors.**

Alkylating Agents
Phenylalanine Nitrogen Mustard

- **Melphalan is a nitrogen mustard that is primarily used to treat multiple myeloma (plasma cell myeloma), breast cancer, and ovarian cancer.**

Alkylating Agents——Alkysulfonates

Busulfan [Myleran]

Indications:

- **Busulfan is administered orally to treat chroic granulocytic leukemia and other myeloproliferative disorders.**

Adverse Effects:

- **Busulfan produces advers effects related to myelosuppression. It only occasionally produces nausea and vomitting. In high doses, it produces a rare but sometimes fatal pulmonary fibrosis, ”busulfan lung”.**

Alkylating Agents——Thiotepa

Thiotepa is converted rapidly by liver mixed-function oxidases to its active metabolite triethylenephosphoramide (TEPA); it is active in bladder cancer.

Resistance of Alkylating Agents

Resistance to alkylating agents has several causes:

- **Membrane transport may be decreased.**
- **The drug may be bound by glutathione (GSH) via GSH-S-transferase or metallothioneins in the cytoplasm and inactivated.**
- **The drug may be metabolized to inactive species.**

Δυσμενείς επιπτώσεις παραγόντων αλκυλίωσης

- **Μυελοκαταστολή**

  Myelosuppression is the dose-limiting adverse effect for alkylating agents**.**

- **Ναυτία**

  Nausea and vomiting are common as are **teratogenesis** and **gonadal atrophy,** although in the latter cases these are variable, according to the drug, its schedule, and route of administration.

- **Καρκινογενέσεις, λευχαιμιογένεση,**

  Treatment also carries a major risk of **leukemogenesis** and **carcinogenesis.**

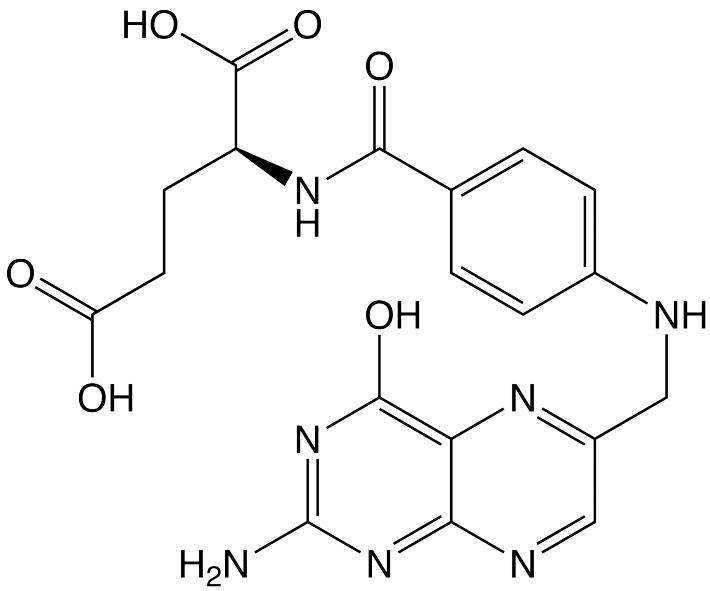
Αντιμεταβολίτες

Χαρακτηριστικά

- **Φάρμακα ειδικά για την S-φάση του κύκλου. Είναι δομικά ανάλογα απαραίτητων μεταβολιτών που εμπλέκονται στη σύνθεση του DNA.**
- **Η μυελοκαταστολή είναι ο περιορισμός της δόσης της κατηγορίας**

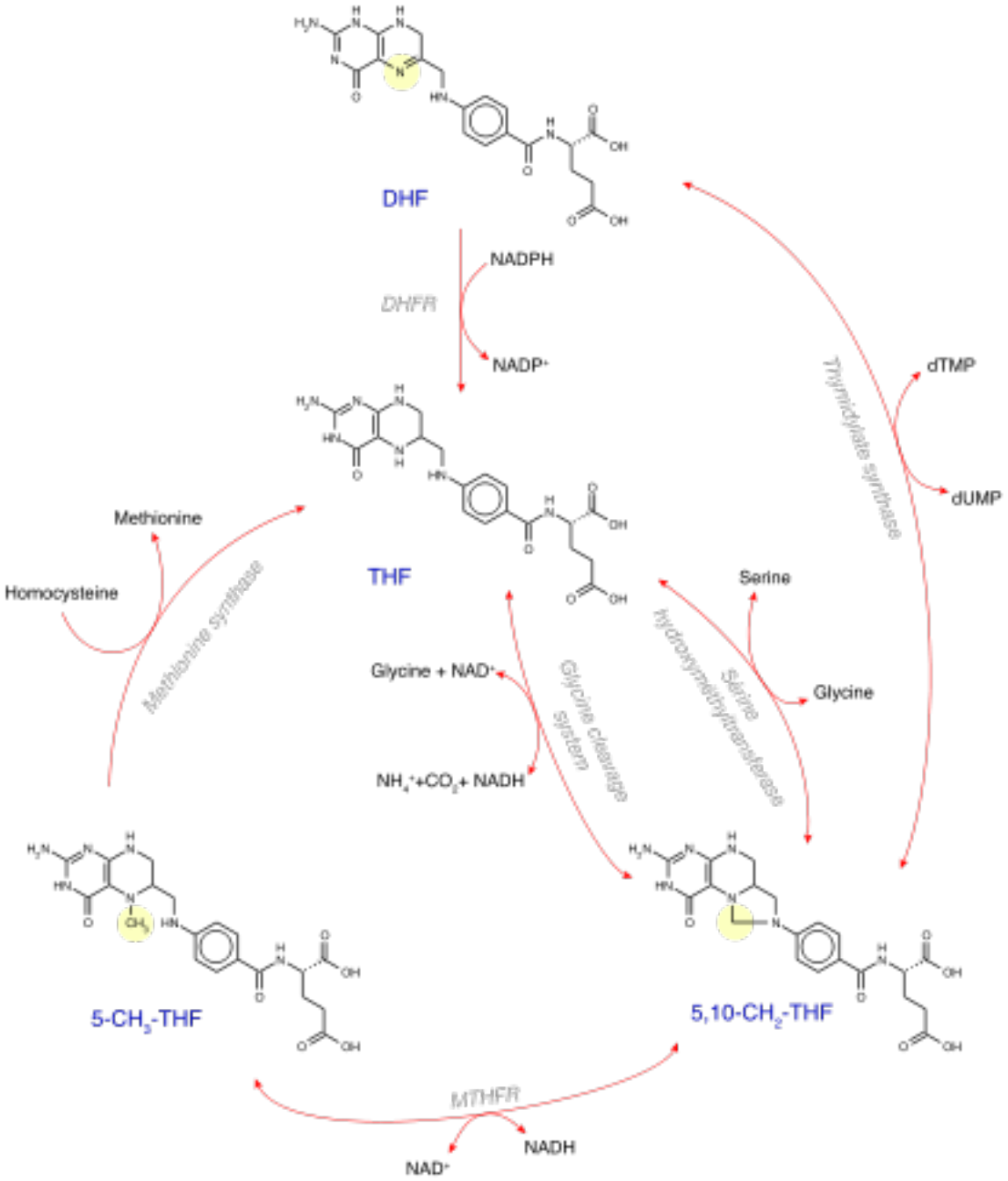
Κατάταξη αντιμεταβολιτών

- **Ανταγωνιστές φυλλικού οξέος :** **MTX**
- **Ανταγωνιστές πουρινών:** **6MP**
  6TG
- **Ανταγωνιστές πυριμιδιών:** **5FU**
  araC
  HU



Folic acid

Folate metabolism



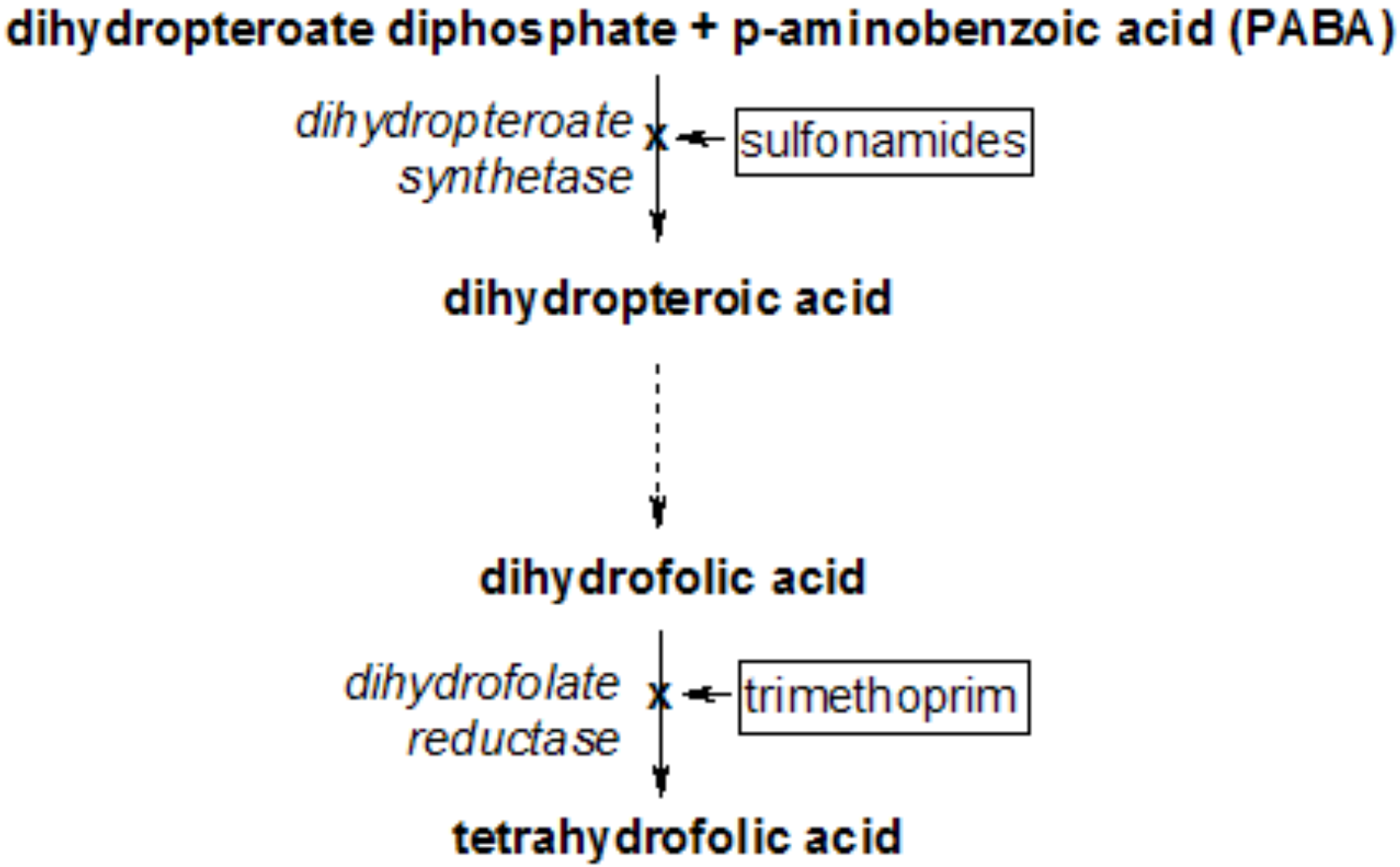
Antimetabolites——
Folic Acid Antagonist

Methotrexate （MTX）

Mechanism of Action：

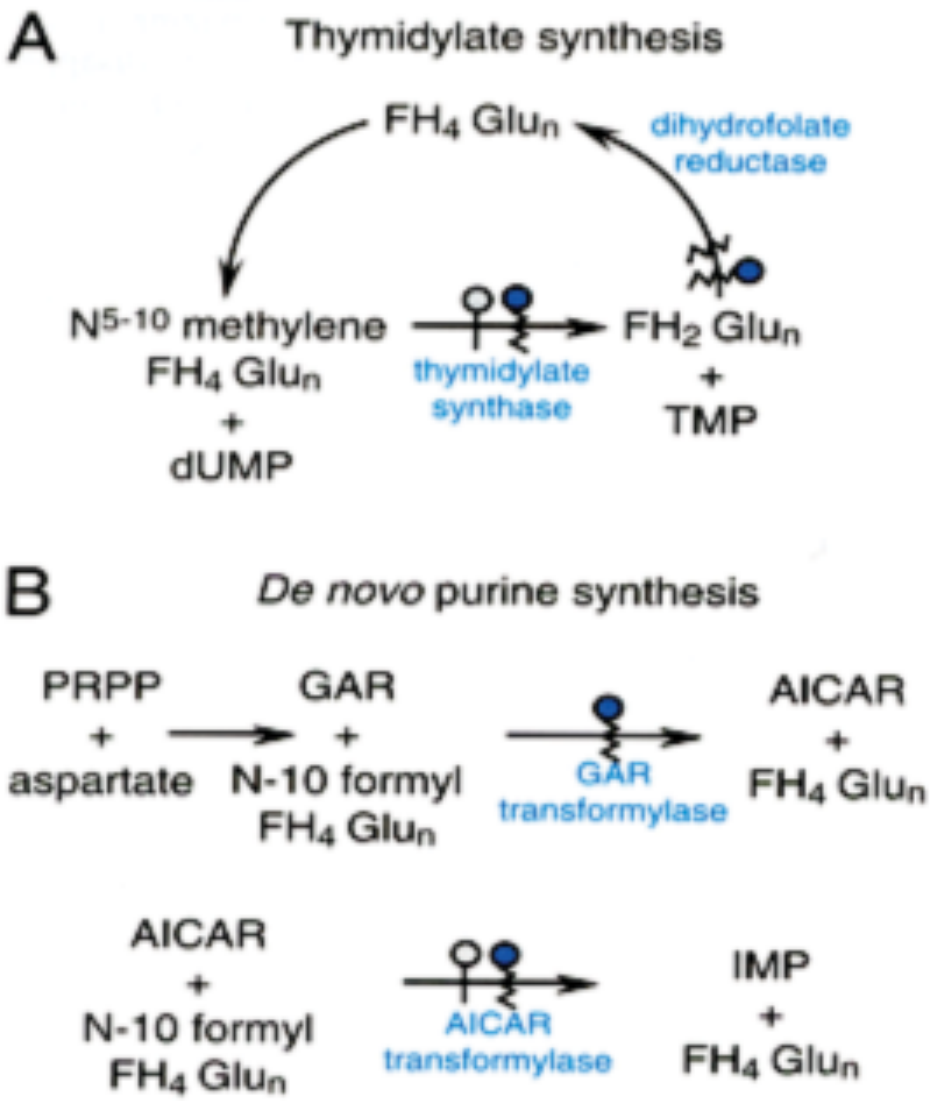
- **The structures of MTX and folic acid are similar. MTX is actively transported into mammalian cells and inhibits dihydrofolate reductase, the enzyme that normally converts dietary folate to the tetrahydrofolate form required for thymidine and purine synthesis.**

Tetrahydrofolate synthesis



http://en.wikipedia.org/wiki/Dihydrofolate_reductase_inhibitor

MTX, Mechanism of action



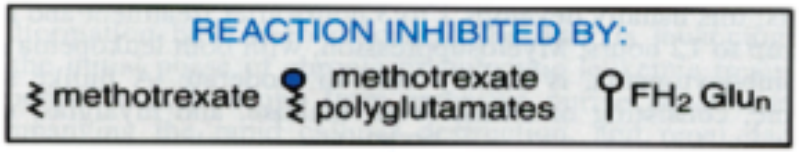


Figure 52–5. Sites of action of methotrexate and its polyglutamates.

AICAR, aminoimidazole carboxamide; TMP, thymidine monophosphate; dUMP, deoxyuridine monophosphate; FH_2Glu_n, dihydrofolate polyglutamate; FH_4Glu_n, tetrahydrofolate polyglutamate; GAR, glycinamide ribonucleotide; IMP, inosine monophosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate.

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed).
The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

Methotrexate

Antifolates occupy a special place in antineoplastic chemotherapy, in that they produced the first striking, although temporary, remissions in leukemia (Farber *et al.*, 1948) and the first cure of a solid tumor, choriocarcinoma (Hertz, 1963). The consistent cure of choriocarcinoma by methotrexate provided great impetus to investigations into the chemotherapy of cancer. Interest in folate antagonists further increased with the introduction of high-dose regimens with "rescue" of host toxicity by the reduced folate, *leucovorin* (folinic acid, citrovorum factor). These methods extend the usefulness of methotrexate to tumors such as osteogenic sarcoma that do not respond to lower doses.

Recognition that methotrexate, an inhibitor of dihydrofolate reductase, also directly inhibits the folate-dependent enzymes of *de novo* purine and thymidylate synthesis focused attention on the development of antifolate analogs that specifically target these other folate-dependent enzyme targets of methotrexate (*see* Figure

Structure–Activity Relationship. Folic acid is an essential dietary factor from which is derived a series of tetrahydrofolate cofactors that provide single carbon groups for the synthesis of precursors of DNA (thymidylate and purines) and RNA (purines). A detailed description of the biological functions and therapeutic applications of folic acid appears in Chapter 54.

The enzyme dihydrofolate reductase (DHFR) is the primary site of action of most folate analogs studied to date (*see* Figures 52–5 and 52–6). Inhibition of DHFR leads to toxic effects through partial depletion of the tetrahydrofolate cofactors that are required for the synthesis of purines and thymidylate (Messmann and Allegra, 2001) and through direct inhibition of the folate-dependent enzymes of purine and thymidylate metabolism by the polyglutamates of methotrexate and the dihydrofolate polyglutamates that accumulate with DHFR inhibition (Figure 52–5) (Allegra *et al.*, 1986, 1987b). Inhibitors of DHFR differ in their relative potency for blocking enzyme from different species. Agents have been identified that have little effect on the human enzyme but have strong activity against bacterial and parasitic infections (*see* discussions of trimethoprim, Chapter 44; pyrimethamine, Chapter 40). By contrast, methotrexate is an effective inhibitor of DHFR in all species investigated. Crystallographic studies have revealed the atomic basis for the high affinity of methotrexate for DHFR (Kraut and Matthews, 1987; Schweitzer *et al.*, 1989; Bystroff and Kraut, 1991; Blakley and Sorrentino, 1998) and the species specificity of the various DHFR inhibitors (Matthews *et al.*, 1985; Stone and Morrison, 1986).

Antimetabolites——
Folic Acid Antagonist

Methotrexate （MTX）

Indications：

- **The use of MTX in the treatment of choriocarinoma, a trophoblastic tumor, was the first demonstration of curative chemotherapy.**
- **It is especially effective for treating acute lymphocytic leukemia and for treating the meningeal metastases of a wide range of tumors.**

Αντιμεταβολίτες
Ανταγωνιστές φυλλικού οξέος

Methotrexate （MTX）

Δυσμενείς συνέπειες

- **Το MTX είναι μυελοκατασταλτικό, προκαλεί οξεία λευκοπενία, απλασία του μυελού, θρομβοπενία**
- **Γαστρεντερολογικές διαταραχές.**
- **Τοξικότητα σε νεφρούς λόγω κατακρήμνισης (crystalluria) του μεταβολίτη 7-OH του MTX.**

Μηχανισμοί αντίστασης στο MTX

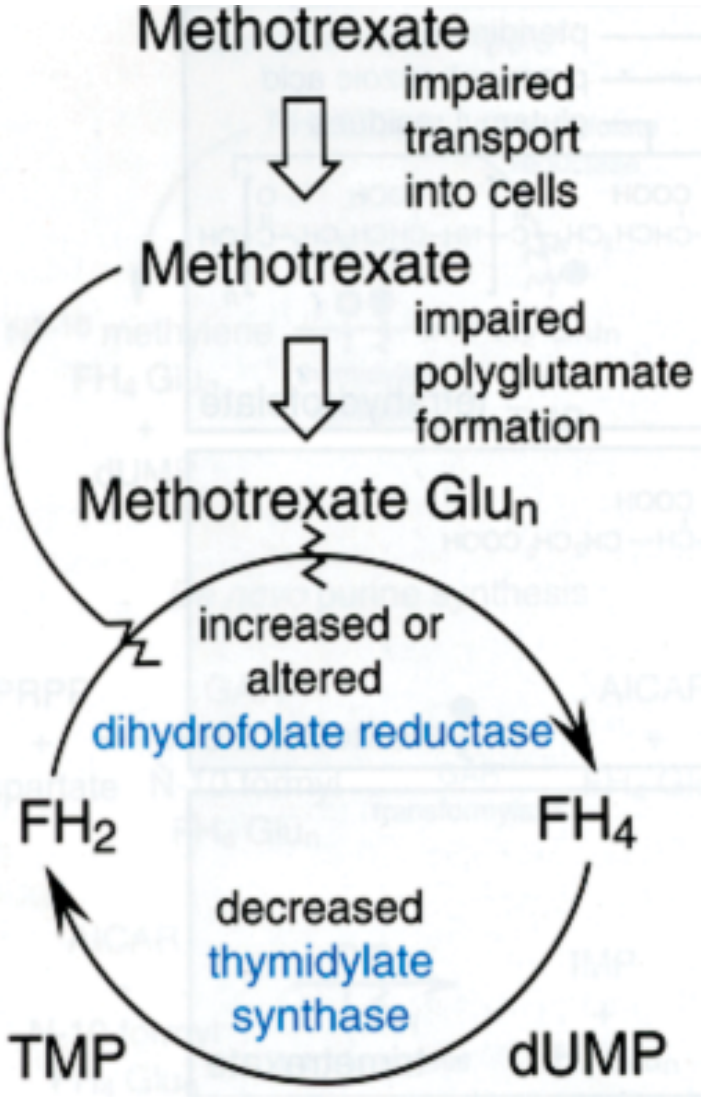


Figure 52–7. Mechanisms of tumor cell resistance to methotrexate.

TMP, thymidine monophosphate; dUMP, deoxyuridine monophosphate; FH_2, dihydrofolate; FH_4, tetrahydrofolate; Glu_n, polyglutamate.

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed).
The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

Mechanisms of Resistance to Antifolates. In experimental systems, a vast array of biochemical mechanisms of acquired resistance to methotrexate have been demonstrated (Figure 52–7) affecting each known step in methotrexate action, including: (1) impaired transport of methotrexate into cells (Assaraf and Schimke, 1987; Trippett *et al.,* 1992); (2) production of altered forms of DHFR that have decreased affinity for the inhibitor (Srimatkandada *et al.,* 1989); (3) increased concentrations of intracellular DHFR through gene amplification or altered gene regulation (Pauletti *et al.,* 1990; Matherley *et al.,* 1997); (4) decreased ability to synthesize methotrexate polyglutamates (Li *et al.,* 1992); and (5) decreased thymidylate synthase activity (Curt *et al.,* 1985). DHFR levels in leukemic cells increase within 24 hours after treatment of patients with methotrexate; this likely reflects induction of new enzyme synthesis. Recent investigations have demonstrated that the intracellular level of DHFR protein is controlled at the level of mRNA translational efficiency through an autoregulatory mechanism whereby the DHFR protein may bind to and control the translational efficiency of its own messenger RNA (Chu *et al.,* 1993). Over longer periods of treatment, tumor cell populations emerge that contain markedly increased levels of DHFR. These cells contain multiple gene copies of DHFR either in mitotically unstable double-minute chromosomes or in stable, homogeneously staining regions or amplisomes of the tumor cell chromosomes. First identified as an explanation for resistance to methotrexate (Schimke *et al.,* 1978), gene amplification has since been implicated in the resistance to many antitumor agents, including fluorouracil and pentostatin (2′-deoxycoformycin) (Stark and Wahl, 1984). Evidence supports the conclusion that DHFR gene amplification is clinically significant in patients with lung cancer (Curt *et al.,* 1983) and leukemia (Goker *et al.,* 1995).

To overcome resistance, high doses of methotrexate with leucovorin rescue may permit entry of drug into transport-defective cells and may permit the intracellular accumulation of methotrexate in concentrations that inactivate high levels of DHFR.

Αντιμεταβολίτες
Ανταγωνιστές πουρινών

6-Mercapapurine（6-MP）

The drugs are believed to act similarly to inhibit purine base synthesis, although their exact mechanisms of action are still uncertain.

Indications:

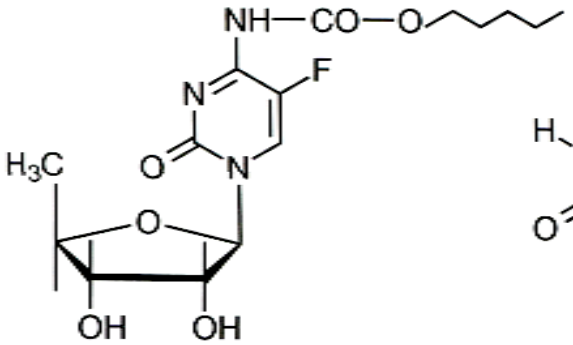
- **Mercaptopurine is used primarily for the maintenance of remission in patients with acute lymphocytic leukemia and is given in combination with MTX for this purpose.**

Adverse Effects:

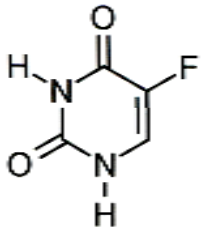
- **Well tolerate.**
- **Myelosuppression is generally mild with thioguanine.Long-term mercaptopurine use may cause hepatotoxicity.**

Αντιμεταβολίτες, ανταγωνιστές πυριμιδινών

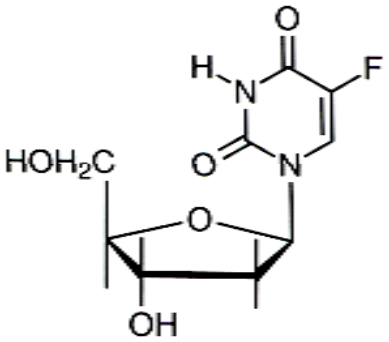
FLUOROPYRIMIDINE ANALOGS



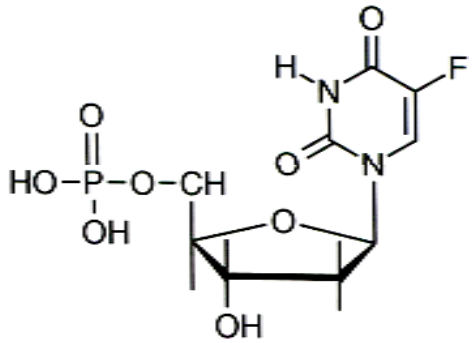
Capecitabine



5-Fluorouracil
(5-FU)

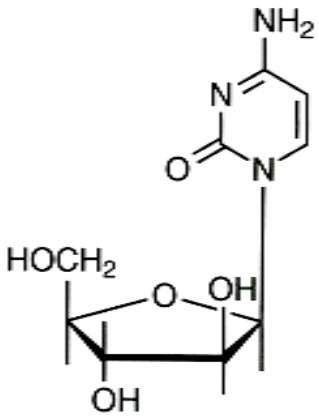


5-Fluorodeoxyuridine
(floxuridine)

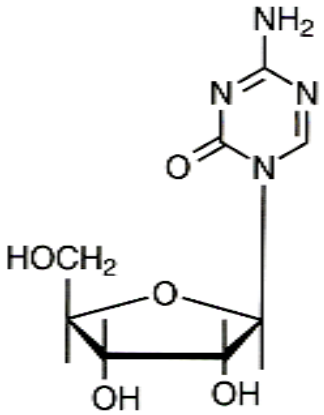


5-Fluorodeoxyuridine
monophosphate
(active metabolite)

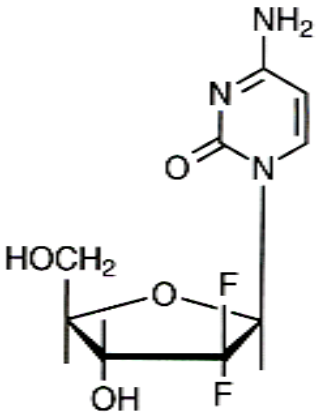
CYTIDINE ANALOGS



Cytosine arabinoside
(cytarabine; AraC)



5-Azacytidine



2', 2'-Difluorodeoxycytidine
(gemcitabine)

Figure 52–8. Structures of available pyrimidine analogs.

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The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

Antimetabolites——
Pyrimidine Antagonists

5-Fluorouracil (5-FU)

Mechanism of Action：

- **Fluorouracil is an analogue of thymine in which the methyl group is replaced by a fluorine atom.**
- **It has two active metabolites: 5-FdUMP and 5-FdUTP.**
- **5-FdUMP inhibits thymidylate synthetases and prevents the synthesis of thymidine, a major building block of DNA.**
- **5-FdUTP is incorporated into RNA by RNA polymerase and interferes with RNA function.**

5FU, mechanism of action

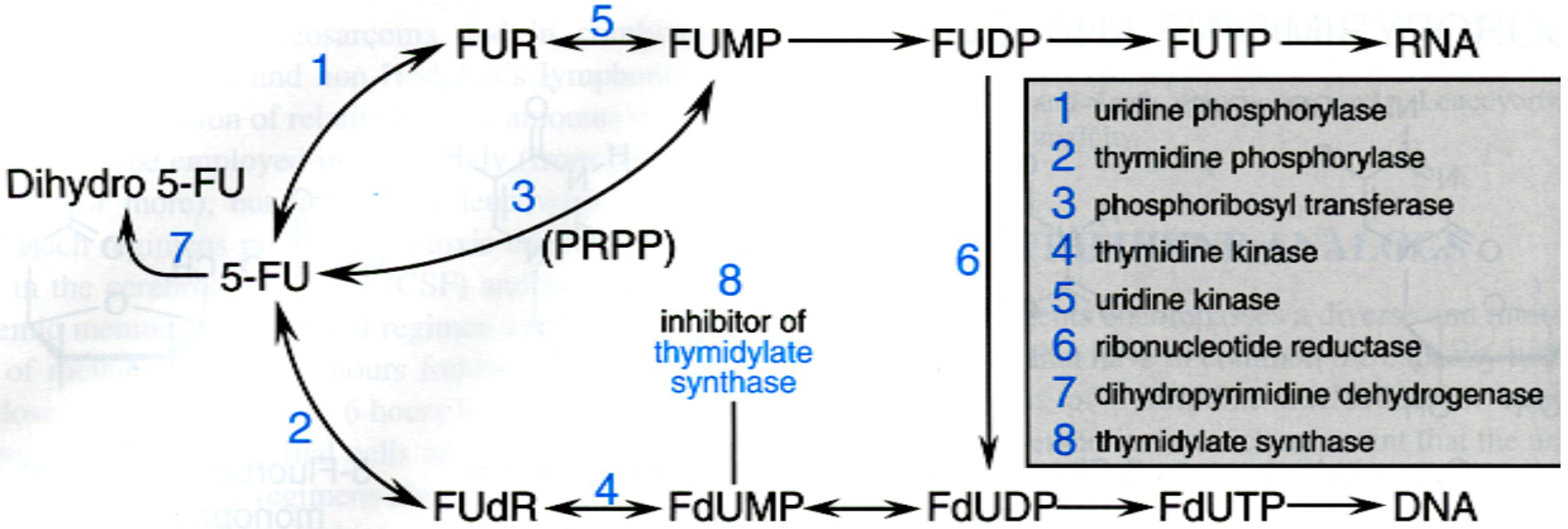

Figure 52–9. Activation pathways for 5-fluorouracil (5-FU) and 5-floxuridine (FUR).

FUDP, floxuridine diphosphate; FUMP, floxuridine monophosphate; FUTP, floxuridine triphosphate; FUdR, fluorodeoxyuridine; FdUDP, fluorodeoxyuridine diphosphate; FdUMP, fluorodeoxyuridine monophosphate; FdUTP, fluorodeoxyuridine triphosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate.

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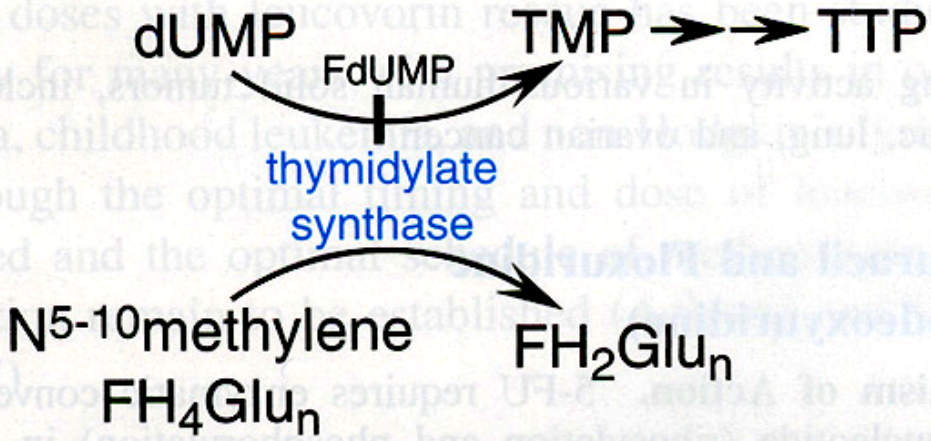
5FU, mechanism of action



Other Actions of 5-FU nucleotides:

- Inhibition of RNA processing
- Incorporation into DNA

Figure 52–10. Site of action of 5-fluoro-2′-deoxyuridine-5′-phosphate (5-FdUMP).

5-FU, 5-fluorouracil; dUMP, deoxyuridine monophosphate; TMP, thymidine monophosphate; TTP, thymidine triphosphate; FdUMP, fluorodeoxyuridine monophosphate; FH_2Glu_n, dihydrofolate polyglutamate; FH_4Glu_n, tetrahydrofolate polyglutamate

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed). The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

Antimetabolites——
Pyrimidine Antagonists

5-Fluorouracil (5-FU)

Indications：

- **Fluorouracil is exclusively used to treat solid tumors, especially breast, colorectal, and gastric tumors and squamous cell tumors of the head and neck.**

Αντιμεταβολίτες
Ανταγωνιστές πυριμιδινών

5-Fluorouracil (5-FU)

Δυσμενείς επιπτώσεις

- **ναυτία και εμέσεις (ήπιες),**
- **μυελοκαταστολή,**
- **έλκη.**

Modulators of 5FU cell toxicity

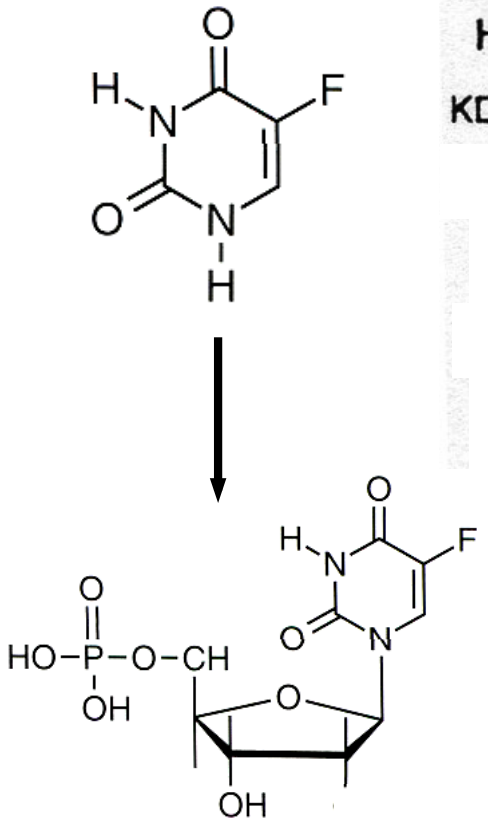
Table 52–2
Modulators of Cytotoxic Activity of 5-Fluorouracil (5-FU)

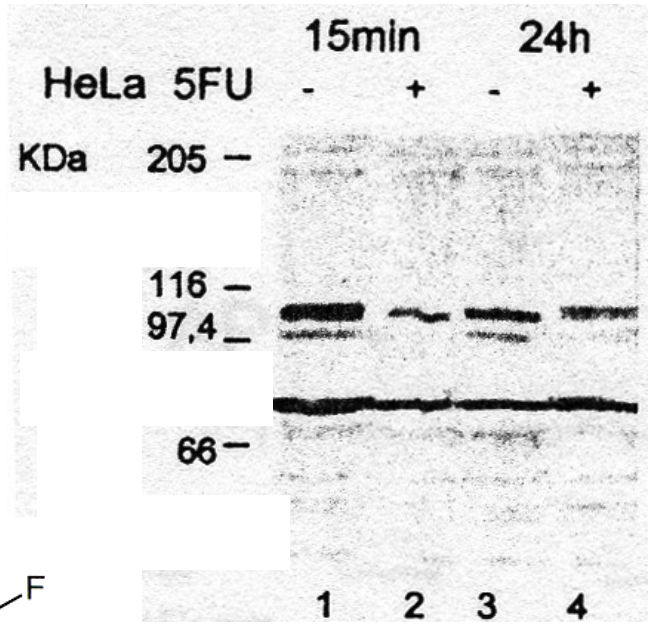
MODULATOR	PURPORTED MECHANISM(S) OF INTERACTION
Cisplatin	Enhanced DNA strand breaks secondary to decreased repair Enhanced thymidylate synthase inhibition
Interferon	Enhanced 5-FU anabolism Decreased "rebound" synthesis of thymidylate synthase
Leucovorin	Enhanced thymidylate synthase inhibition
Methotrexate	Enhanced 5-FU anabolism Enhanced RNA incorporation
PALA*	Enhanced 5-FU anabolism Enhanced RNA incorporation
Uridine	Diminished RNA incorporation (? selective rescue for normal cells)

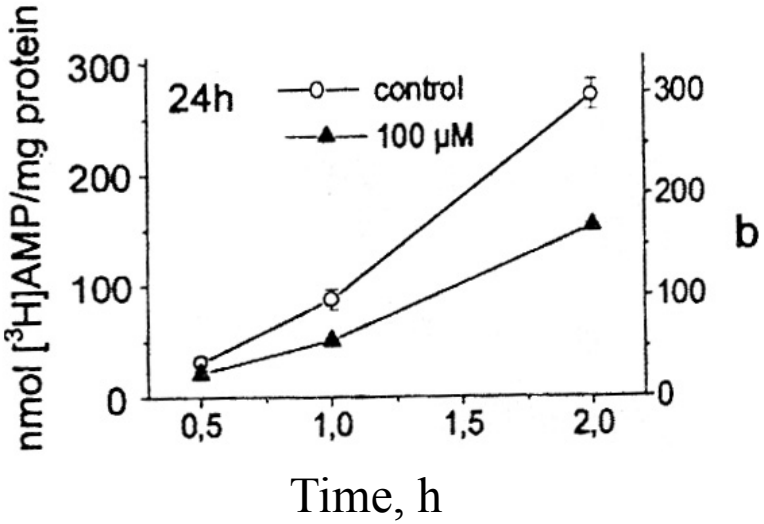
*PALA, *N*-phosphonoacetyl-L-aspartate.

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed). The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

PAP activity: Drug action, 5-FU







Balatsos et al, *Int J Biol Mark*. 2000; 15: 294

PAP as a biological marker for breast cancer

[CANCER RESEARCH 60, 5427–5433, October 1, 2000]

Polyadenylate Polymerase Enzymatic Activity in Mammary Tumor Cytosols: A New Independent Prognostic Marker in Primary Breast Cancer[1]

Andreas Scorilas,[2] Maroulio Talieri, Alexandros Ardavanis, Nelly Courtis, Euthymios Dimitriadis, Julia Yotis, Chris Milton Tsiapalis, and Theoni Trangas[3]

G. Papanikolaou Research Center of Oncology, Athens 11522, Greece [A. S., M. T., N. C., E. D., C. M. T., T. T.], and 1st Medical Oncology Department and Hormone Receptor Unit, St. Savvas Hospital, Athens 11522, Greece [A. A., J. Y.]

PYRIMIDINE ANALOGS

This class of agents encompasses a diverse and interesting group of drugs that have in common the capacity to inhibit the biosynthesis of pyrimidine nucleotides or to mimic these natural metabolites to such an extent that the analogs interfere with the synthesis or function of nucleic acids. Analogs of deoxycytidine and thymidine have been synthesized as inhibitors of DNA synthesis, and an analog of uracil, 5-fluorouracil, effectively inhibits both RNA function and/or processing and synthesis of thymidylate (*see* Figure 52–8). Drugs in this group have been employed in the treatment of diverse afflictions, including neoplastic diseases, psoriasis, and infections caused by fungi and DNA-containing viruses. The pathways for metabolic activation and degradation of these compounds during systemic administration present opportunities for the development of synergistic combination therapies with other clinically effective drugs.

General Mechanism of Action. The best-characterized agents in this class are the halogenated pyrimidines, a group that includes *fluorouracil* (5-fluorouracil, or 5-FU), *floxuridine* (5-fluoro-2′-deoxyuridine, or 5-FUdR), and *idoxuridine* (5-iododeoxyuridine; *see* Chapter 50). If one compares the van der Waals radii of the various 5-position substituents, the dimension of the fluorine atom resembles that of hydrogen, whereas the bromine and iodine atoms are larger and close in size to the methyl group. Thus, idoxuridine behaves as an analog of thymidine, and its primary biological action results from its phosphorylation and ultimate incorporation into DNA in place of thymidylate. In 5-FU, the smaller fluorine at position 5 allows the molecule to mimic uracil biochemically. However, the fluorine–carbon bond is much tighter than that of C—H and prevents the methylation of the 5 position of 5-FU by thymidylate synthase. Instead, in the presence of the physiological cofactor 5,10-methylene tetrahydrofolate, the fluoropyrimidine locks the enzyme in an inhibited state. Thus, substitution of a halogen atom of the correct dimensions can produce a molecule that sufficiently resembles a natural pyrimidine to interact with enzymes of pyrimidine metabolism but at the same time interferes drastically with certain other aspects of pyrimidine action.

A number of 5-FU analogs have reached the clinic. The most important of these is *capecitabine* (N4-pentoxycarbonyl-5′-deoxy-5-fluorocytidine), a drug with proven activity against colon and breast cancers. This orally administered agent is converted to 5′-deoxy-5-fluorocytidine by carboxylesterase activity in liver and other normal and malignant tissues. From that point, it is converted to 5′-deoxy-fluorodeoxyuridine by cytidine deaminase. The final step in its activation occurs when thymidine phosphorylase cleaves off the 5′-deoxy sugar, leaving intracellular 5-FU. Tumors with elevated thymidine phosphorylase activity seem particularly susceptible to this drug (Ishikawa *et al.,* 1998).

Nucleotides in RNA and DNA contain ribose and 2′-deoxyribose, respectively. Among the various modifications of the sugar moiety that have been attempted, the replacement of the ribose of cytidine with arabinose has yielded a useful chemotherapeutic agent, *cytarabine* (AraC). As may be seen in Figure 52–8, the hydroxyl group in this molecule is attached to the 2′-carbon in the β, or upward, configuration, as compared with the α, or downward, position of the 2′-hydroxyl in ribose. The arabinose analog is recognized enzymatically as a 2′-deoxyriboside; it is phosphorylated to a nucleoside triphosphate that competes with dCTP for incorporation into DNA (Chabner *et al.,* 2001), where it blocks elongation of the DNA strand and its template function.

Two other cytidine analogs have received extensive clinical evaluation. 5-*Azacytidine,* an inhibitor of DNA methylation as well as a cytidine antimetabolite, becomes incorporated predominantly into RNA and has antileukemic as well as differentiating actions *in vitro*. A newer analog, 2′,2′-difluorodeoxycytidine (*gemcitabine*), becomes incorporated into DNA and inhibits the elongation of nascent DNA strands (*see* Figure 52–8). It has promising activity in various human solid tumors, including pancreatic, lung, and ovarian cancer.

Fluorouracil and Floxuridine (Fluorodeoxyuridine)

Mechanism of Action. 5-FU requires enzymatic conversion to the nucleotide (ribosylation and phosphorylation) in order to exert its cytotoxic activity (Figure 52–9). Several routes are available for the formation of the 5′-monophosphate nucleotide (F-UMP) in animal cells. 5-FU may be converted to fluorouridine by uridine phosphorylase and then to F-UMP by uridine kinase, or it may react directly with 5-phosphoribosyl-1-pyrophosphate (PRPP), in a reaction catalyzed by the enzyme orotate phosphoribosyl transferase, to form F-UMP. Many metabolic pathways are available to F-UMP, including incorporation into RNA. A reaction sequence crucial for antineoplastic activity involves reduction of the diphosphate nucleotide by the enzyme ribonucleotide diphosphate reductase to the deoxynucleotide level and the eventual formation of 5-fluoro-2′-deoxyuridine-5′-phosphate (F-dUMP). 5-FU also may be converted directly to the deoxyriboside 5-FUdR by the enzyme thymidine phosphorylase and further to F-dUMP, a potent inhibitor of thymidylate synthesis, by thymidine kinase. This complex metabolic pathway for the generation of F-dUMP may be bypassed through use of the deoxyribonucleoside of fluoro–uracil—floxuridine (fluorodeoxyuridine, FUdR)—which is converted directly to F-dUMP by thymidine kinase.

The interaction between F-dUMP and the enzyme thymidylate synthase leads to deletion of TTP, a necessary constituent of DNA (Figure 52–10). The folate cofactor, 5,10-methylenetetrahydrofolate, and F-dUMP form a covalently bound ternary complex with the enzyme. This inhibitory complex resembles the transition state formed during the normal enzymatic reaction when dUMP is converted to thymidylate. Although the physiological complex progresses to the synthesis of thymidylate by transfer of the methylene group and two hydrogen atoms from folate to dUMP, this reaction is blocked in the inhibitory complex by the stability of the fluorine carbon bond on F-dUMP; sustained inhibition of the enzyme results (Santi *et al.,* 1974).

5-FU also is incorporated into both RNA and DNA. In 5-FU–treated cells, both F-dUTP and dUTP (the substrate that accumulates behind the blocked thymidylate synthase reaction) incorporate into DNA in place of the depleted physiological TTP. The significance of the incorporation of F-dUTP and dUTP into DNA is unclear (Canman *et al.,* 1993). Presumably, the incorporation of deoxyuridylate and/or fluorodeoxyuridylate into DNA would call into action the excision–repair process. This process may result in DNA strand breakage because DNA repair requires TTP, but this substrate is lacking as a result of thymidylate synthase inhibition (Mauro *et al.,* 1993). 5-FU incorporation into RNA also causes toxicity as the result of major effects on both the processing and functions of RNA (Armstrong, 1989; Danenberg *et al.,* 1990).

A number of biochemical mechanisms have been identified that are associated with resistance to the cytotoxic effects of 5-FU or floxuridine. These mechanisms include loss or decreased activity of the enzymes necessary for activation of 5-FU, decreased pyrimidine monophosphate kinase (which decreases incorporation into RNA), amplification of thymidylate synthase (Washtein, 1982), and altered thymidylate synthase that is not inhibited by F-dUMP (Barbour *et al.,* 1990). Both experimental studies and clinical trials support the position that the response to 5-FU correlates significantly with low levels of the degradative enzymes, dihydrouracil dehydrogenase and thymidine phosphorylase, and a low level of expression of the target enzyme, thymidylate synthase (van Triest *et al.,* 2000). Recent investigations have demonstrated that the level of thymidylate synthase is finely controlled by an autoregulatory feedback mechanism wherein the thymidylate synthase protein interacts with and controls the translational efficiency of its own messenger RNA. This mechanism provides for the rapid modulation of the level of thymidylate synthase necessary for cellular division and also

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed). The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

PYRIMIDINE ANALOGS (συνέχεια)

Table 52–2
Modulators of Cytotoxic Activity of 5-Fluorouracil (5-FU)

MODULATOR	PURPORTED MECHANISM(S) OF INTERACTION
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PALA*	Enhanced 5-FU anabolism Enhanced RNA incorporation
Uridine	Diminished RNA incorporation (? selective rescue for normal cells)

*PALA, *N*-phosphonoacetyl-L-aspartate.

may be an important mechanism by which malignant cells become rapidly insensitive to the effects of 5-fluorouracil (Chu *et al.,* 1991; Swain *et al.,* 1989). Some malignant cells appear to have insufficient concentrations of 5,10-methylene tetrahydrofolate and, thus, cannot form maximal levels of the inhibited ternary complex with thymidylate synthase. Addition of exogenous folate in the form of 5-formyl-tetrahydrofolate (leucovorin) increases formation of the complex in both laboratory and clinical experiments and has enhanced responses to 5-FU in clinical trials (Ullman *et al.,* 1978; Grogan *et al.,* 1993). Except for inadequate intracellular folate pools, it is not established which (if any) of the other mechanisms is associated with clinical resistance to 5-FU and its derivatives (Grem *et al.,* 1987).

In addition to leucovorin, a number of other agents have been combined with 5-FU in attempts to enhance the cytotoxic activity through biochemical modulation. These agents, along with their proposed mechanisms of interaction, are shown in Table 52–2. The most clinically interesting combinations with 5-FU include methotrexate, interferon, leucovorin, or cisplatin, all of which are currently under investigation to define their ultimate clinical roles. Agents that inhibit early steps in pyrimidine biosynthesis, such as PALA (*N*-phosphonoacetyl-L-aspartate), an inhibitor of aspartate transcarbamylase, provide synergistic interaction with 5-FU in experimental systems, but these combinations have no proven clinical value (Grem *et al.,* 1988). Methotrexate, by inhibiting purine synthesis and increasing cellular pools of PRPP, enhances the activation of 5-FU and increases antitumor activity of 5-FU when given prior to but not following 5-FU. In clinical trials, the combination of cisplatin and 5-FU has yielded impressive responses in tumors of the upper aerodigestive tract, but the molecular basis of their interaction is not well understood (Grem, 2001).

Absorption, Fate, and Excretion. 5-FU and floxuridine are [illegible] primarily by [illegible] absorption after ingestion of the drugs is unpredictable and incomplete. Metabolic degradation occurs in many tissues, particularly the liver. Floxuridine is converted by thymidine or deoxyuridine phosphorylases into 5-FU. 5-FU is inactivated by reduction of the pyrimidine ring; this reaction is carried out by dihydropyrimidine dehydrogenase (DPD), which is found in liver, intestinal mucosa, tumor cells, and other tissues. Inherited deficiency of this enzyme leads to greatly increased sensitivity to the drug (Lu *et al.,* 1993; Milano *et al.,* 1999). The rare individual who totally lacks this enzyme may experience profound drug toxicity following conventional doses of the drug. DPD deficiency can be detected either by enzymatic or molecular assays using peripheral white blood cells, or by determining the plasma ratio of 5-FU to its metabolite, 5-fluoro-5,6-dihydrouracil, which is ultimately degraded to α-fluoro-β-alanine (Heidelberger, 1975; Zhang *et al.,* 1992).

Rapid intravenous administration of 5-FU produces plasma concentrations of 0.1 to 1.0 mM; plasma clearance is rapid ($t_{1/2}$ 10 to 20 minutes). Urinary excretion of a single dose of 5-FU given intravenously amounts to only 5% to 10% in 24 hours. Although the liver contains high concentrations of DPD, dosage does not have to be modified in patients with hepatic dysfunction, presumably because of degradation of the drug at extrahepatic sites or by vast excess of this enzyme in the liver. Given by continuous intravenous infusion for 24 to 120 hours, 5-FU achieves plasma concentrations in the range of 0.5 to 8.0 μM. 5-FU readily enters the CSF, and concentrations greater than 0.01 μM are sustained for up to 12 hours following conventional doses (Grem, 2001).

Capecitabine is well absorbed orally, yielding high plasma concentrations of 5′-deoxy-fluorodeoxyuridine (5′-dFdU), which disappears with a half-life of about 1 hour. 5-FU levels are less than 10% of those of 5′-dFdU. Liver dysfunction delays the conversion of the parent compound to 5′-dFdU and 5-FU, but there is no consistent effect on toxicity (Twelves *et al.,* 1999).

Therapeutic Uses. ***5-Fluorouracil.*** Accumulated experience with 5-FU (ADRUCIL) indicates that the drug produces partial responses in 10% to 20% of patients with metastatic carcinomas of the breast and the gastrointestinal tract; beneficial effects also have been reported in carcinoma of the ovary, cervix, urinary bladder, prostate, pancreas, and oropharyngeal areas. For average-risk patients in good nutritional status with adequate hematopoietic function, the weekly dosage regimen employs 750 mg/m^2 alone or 500 to 600 mg/m^2 with leucovorin once each week for 6 of 8 weeks. Other regimens use daily doses of 500 mg/m^2 for 5 days, repeated in monthly cycles. When used with leucovorin, daily doses of 5-FU must be reduced to 375 to 425 mg/m^2 for 5 days because of mucositis and diarrhea. It also has been given as a continuous infusion for up to 21 days (300 mg/m^2 per day), or as a biweekly 48-hour continuous infusion (de Gramont *et al.,* 1998).

Floxuridine (FUdR). FUdR (fluorodeoxyuridine; FUDR) is used primarily by continuous infusion into the hepatic artery for treatment of metastatic carcinoma of the colon

Antimetabolites——
Pyrimidine Antagonists

Cytarabine

Indications：

- **Cytarabine has a narrow clinical spectrum and is primarily used in combination with daunorubicin or thioguanine for the treatment of acute nonlymphocytic leukemia.**

Adverse Effects:

- **High doses of cytarabine can damage the liver, heart, and other organs.**

Αντιβιοτικά

Κατάταξη αντιβιοτικών

- **Αντριαμυκίνη** Adriamycin (Anthracyaline Antibiotics)
- **Μιτομυκίνη C**
- **Μπλεομυκίνη, Bleomycin**
- **Ακτινομυκίνη D, Actinomycin D**

Antibiotics

Adriamycin and Daunorubicin :

Properties:

- **Adriamycin and Daunorubicin are tetracycline rings with the sugar daunosamine. They are DNA intercalating agents that block the synthesis of DNA and RNA.**
- **These agents are primarily toxic during the S phase of cell cycle.**
- **These agents imparts a red tinge to the urine.**
- **Adramycin is used to treat acute leukemias, lymphoma, and a number of solid tumors.**

Αντιβιοτικά

Mitomycin C:

Μηχανισμός

- **Αλκυλίωση DNA. Αναστολή σύνθεσης, επαγωγή διπλών σπασιμάτων**

Ενδείξεις

- **Χρήση σε συνδυασμό με άλλες ουσίες (π.χ., vinvristine) έναντι καρκίνου του μαστού.**

Δυσμενείς επιπτώσεις

- **Μυελοκαταστολή.**

Antibiotics

Actinomycin D:

- **Actinomycin D intercalates DNA and thereby prevents DNA transcription and messenger RNA synthesis.**
- **The drug is given intravenously, and its clinical use is limited to the treatment of trophoblastic (gestational) tumors and the treatment of pediatric tumors, such as Wilms' tumor and Ewing's sarcoma.**

Antibiotics

Bleomycin:

Mechanism:

- **The drug has its greatest effect on neoplastic cell in the G2 phase of the cell replication cycle. Although bleomycin intercalates DNA, the major cytotoxicity is believed to result from iron-catalyzed free radical formation and DNA strand breakage.**

Indications:

- **It is useful in Hodgkin's and non-Hodgkin's lymphomas, testicular cancer, and several other solid tumors.**

Adverse Effects:

- **Bleomycin produces very little myelosuppression. The most serious toxicities of Bleomycin are pulmonary and mucocutaneous reactions.**

Anti-Cancer Plant Allaloids

- **Tubulin-Binding Agents**

Vinca Alkaloids: **The cellular mechanism of action of vinca alkaloids is the prevention of microtubule assembly, causing cells to arrest in the late G2 phase by preventing formation of mitotic filaments for nuclear and cell division.**

Anti-Cancer Plant Allaloids

- **Tubulin-Binding Agents**
- **Vinca alkaloids:**

Vinblastine,vincristin, vindesine and vinorelbine are all alkaloids derived from the periwinkle plant (Vinca rosea).

Indications:

- **Vinblastine is used in combination with Bleomycin and Cisplatin for metastatic testicular tumors.**
- **Vincristine is used in combination with prednisone to induce remission in childhood leukemia.**
- **Vinorelbine is used to treat non-small-cell lung cancer and breast cancer.**

Anti-Cancer Plant Allaloids

- **Tubulin-Binding Agents**
- **Paclitaxel:**

Taxanes enhance all aspects of tubulin polymerization, an action that is the opposite to that of vinca alkaloids, but they are also cytotoxic, emphasizing the dynamic importance of tubulin polymerization as a target for cytotoxic drugs.

Paclitaxel, Taxotere

Anti-Cancer Plant Allaloids

- **Interfere the Function of Ribosome:**
- **Cephalotaxus Alkaloids :**

  Harringtonine

  Homoharringtonine

Platinum Compound

Cisplatin:

Mechanism of Action:

- **Cisplatin binds to guanine in DNA and RNA, and the interaction is stabilized by hydrogen bonding. The molecular mechanism of action is unwinding and shortening of the DNA helix.**

Platinum Compound

Cisplatin:

Indications:

- **Cisplatin has efficacy against a wide range of neoplasms. It is given intravenously as a first-line drug for testicular, ovarian, and bladder cancer, and it is also useful in the treatment of melanoma and a number of other soild tumors.**

Adverse Effect:

- **Cisplatin produces relatively little myelosuppression but can cause severe nausea, vomiting, and nephrotoxicity.**

Platinum Compound

Carboplatin:

Indication:

- **Carboplatin has a similar spectrum of activity, but it is approved only as a second-line drug for ovarian cancer.**

Hormones

- **Several types of hormone-dependent cancer (especially breast, prostate, and endometrial cancer) respond to treatment with their corresponding hormone antagonists.**
- **Estrogen antagonists are primarily used in the treatment of breast cancer, whereas androgen antagonists are used in the treatment of prostate cancer. Corticosteroids are particularly useful in treating lymphocytic leukemias and lymphomas.**

Hormones

Estrogens:

- **Estrogens inhibit the effects of endogenous androgens and androgen-dependent metastatic prostatic carcinoma. Diethylstilbestrol is usually the agent of choice.**
- **Cardiac and cerebrovascular complications and carcinoma of the male breast are potential adverse effects.**

Hormones

Progenstins:

- **Progestins are useful in the management of endometrial carcinoma and back-up therapy for metastatic hormone-dependent breast cancer.**

Hormones

Antiestrogen: *Tamoxifen*

- **Tamoxifen is the drug of choice in postmenopausal women with or recovering from metastatic breast cancer. It is most effective in patients who have estrogen receptor-positive tumors.**
- **Tamoxifen is also used as adjunvctive therapy to oophorectomy to leuprolide or goserelin in premenopausal women with estrogen receptor-positive tumors.**

Hormones

Androgens:

- Androgen activity in breast cancer is similar to that of estrogens, perhaps for the same mechanistic reasons.
- Virilizing effects and hepatic toxicity make them unacceptable to most patients.
- Fluoxymesterone is the most widely used agent.
- Danazol has use in hematology in aplastic anemia and congenital anemias.

Hormones

Glucocorticoids:

- They are integral components of curative therapy for acute lymphoblastic leukemia, non-Hodgkin's lymphoma, and Hodgkin's disease.
- Glucocorticoids have essential roles in the prevention of allergic reaction, emesis control, relief of intracranial hypertension or spinal cord compression in neurologic complications, and pain relief.

Κατάταξη αντικαρκινικών φαρμάκων

- **Σύμφωνα με βιοχημικούς μηχανισμούς αντικαρκινικής δράσης:**

1. **Αναστολή βιοσύνθεσης νουκλεϊκών οξέων**
2. **Αμεση επίδραση στη δομή και λειτουργία του DNA**
3. **Παρεμβολή μεταγραφής και παρεμπόδιση σύνθεσης RNA**
4. **Παρεμβολή σύνθεσης και λειτουργίας πρωτεϊνών**
5. **Επίδραση ομοιόστασης ορμονών**
6. **Αλλα**

Block Nucleic Acid (DNA, RNA) Biosynthesis

Antimetabolites:

- ***Folic Acid Antagonist:*** **inhibit dihydrofolate reductase (methotrexate)**
- ***Pyrimidine Antagonist:***

  inhibit thymidylate synthetase (fluorouracil) ;

  inhibit DNA polymerase (cytarabine)
- ***Purine Antagonist:***

  inhibit interconversion of purine nucleotide (mercaptopurine)
- ***Ribonucleoside Diphosphate Reductase Antagonist:*** **(hydroxyurea)**

Interfere Transcription and Block RNA Synthesis

- **Bind with DNA to block RNA production.**

  doxorubicin

Influence the Structure and Function of DNA

- ***Alkylating Agent:*** **mechlorethamine, cyclophosphamide and thiotepa**
- ***Platinum:*** **cis-platinium**
- ***Antibiotic:*** **bleomycin and mitomycin C**
- ***Topoismerase inhibitor:*** **camptothecine and podophyllotoxin**

Influence Hormone Homeostasis

These drugs bind to hormone receptors to block the actions of the sex hormones which results in inhibition of tumor growth.

- Estrogens and estrogen antagonistic drug
- Androgens and androgen antagonistic drug
- Progestogen drug
- Glucocorticoid drug
- gonadotropin-releasing hormone inhibitor: leuprolide, goserelin
- aromatase inhibitor: aminoglutethimide, anastrazole

Κατάταξη των αντικαρκινικών φαρμάκων

- **Σύμφωνα με την φάση του κυτταρικού κύκλου:**

1. **Μη ειδικά σχετικά με τον κύκλο cell cycle nonspecific agents (CCNSA)**
2. **Ειδικά για τον κύκλο cell cycle specific agents (CCSA)**

The Basic Concept of Cell Generation Cycle

- **The cycle of cell replication includes:**

  M（Mitosis）phase

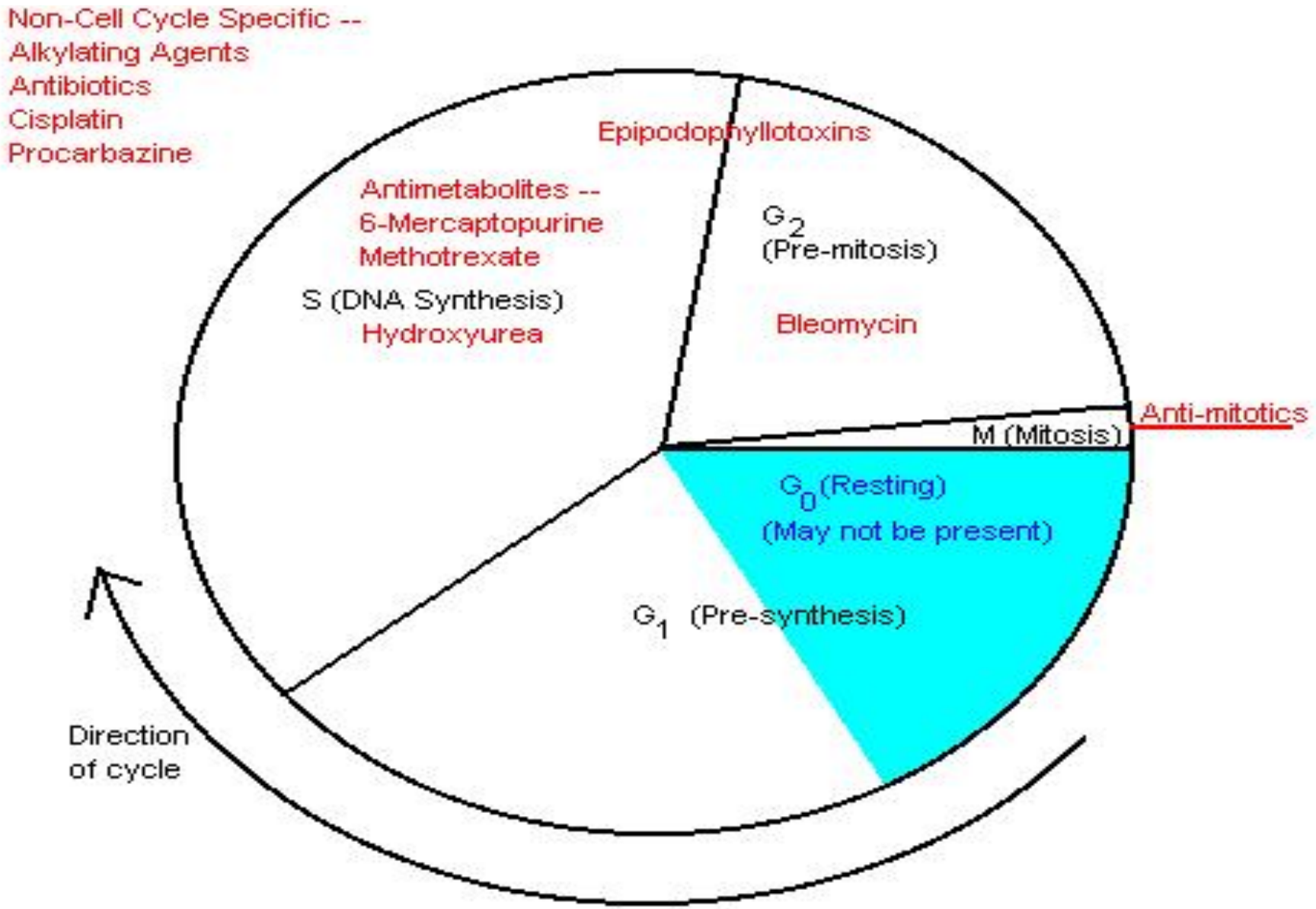
  G1（Gap1, period before S）phase

  S（DNA synthesis）phase

  G2（Gap2, period after S）phase

- **Growth Fraction (GF）**

Site-of-action of Cell Cycle specific Antineoplastics



Cell Cycle and anticancer drugs

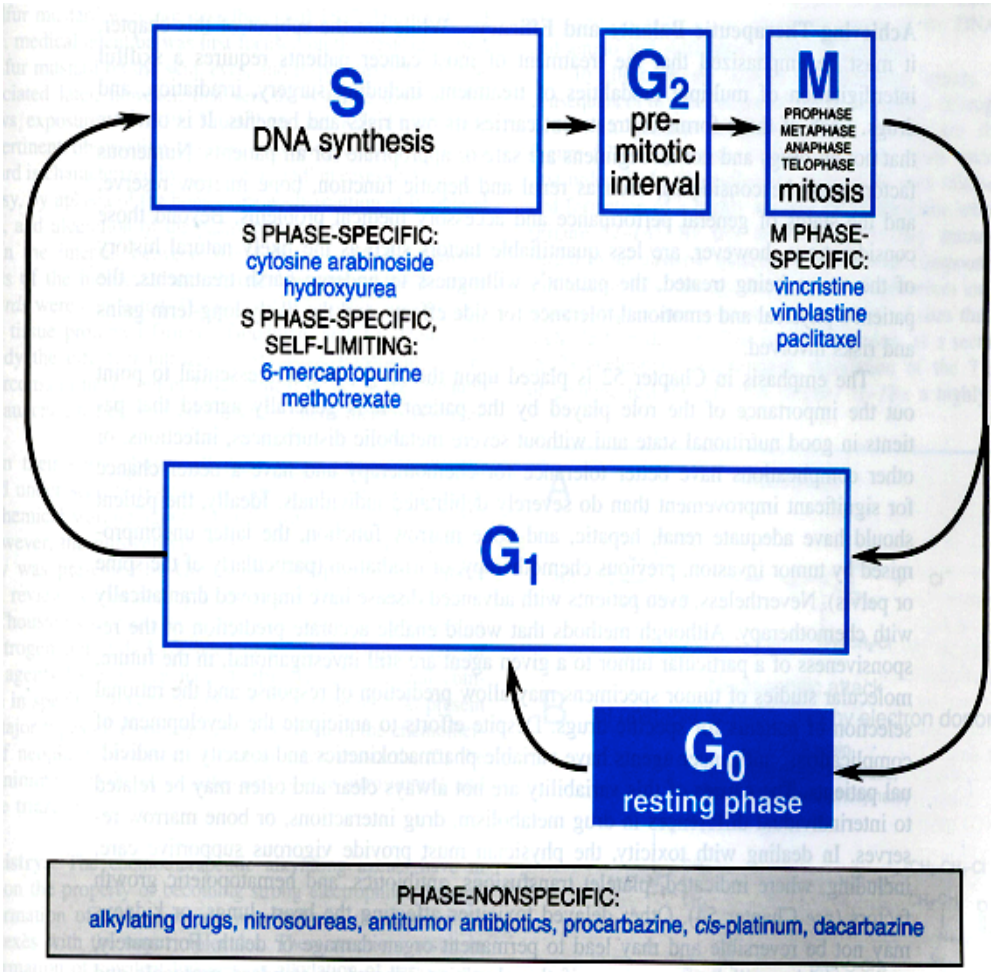


Figure IX–2. The cell cycle and the relationship of antitumor drug action to the cycle.

G_1 is the period between mitosis and the beginning of DNA synthesis. Resting cells (cells that are not preparing for cell division) are said to be in a subphase of G_1, G_0. S is the period of DNA synthesis; G_2 the premitotic interval; and M the period of mitosis. Examples of cell-cycle–dependent anticancer drugs are listed in blue below the phase in which they act. Drugs that are cytotoxic for cells at any point in the cycle are called cycle-phase-nonspecific drugs. (*Modified from* Pratt *et al., 1994 with permission.*)

Hardman JG, Limbird LE (eds, Gilman AG (cons. ed).
The Pharmacological Basis of Therapeutics. 10th ed., 2001, McGraw-Hill

The Cell Cycle. An understanding of cell-cycle kinetics is essential for the proper use of the current generation of antineoplastic agents. Many of the most potent cytotoxic agents act by damaging DNA. Their toxicity is greater during the S, or DNA synthetic, phase of the cell cycle, while others, such as the vinca alkaloids and taxanes, block the formation of the mitotic spindle in M phase. These agents have activity only against cells that are in the process of division. Accordingly, human neoplasms that are currently most susceptible to chemotherapeutic measures are those with a high percentage of cells undergoing division. Similarly, normal tissues that proliferate rapidly (bone marrow, hair follicles, and intestinal epithelium) are subject to damage by most antineoplastic drugs, and such toxicity often limits the usefulness of drugs. On the other hand, slowly growing tumors with a small growth fraction (for example, carcinomas of the colon or lung) often are unresponsive to cytotoxic drugs. Although differences in the duration of the cell cycle occur between cells of various types, all cells display a similar pattern during the division process. This cell cycle may be characterized as follows (*see* Figure IX–2): (1) There is a presynthetic phase (G_1); (2) the synthesis of DNA occurs (S); (3) an interval follows the termination of DNA synthesis, the postsynthetic phase (G_2); and (4) mitosis (M) ensues—the G_2 cell, containing a double complement of DNA, divides into two daughter G_1 cells. Each of these cells may immediately reenter the cell cycle or pass into a nonproliferative stage, referred to as G_0. The G_0 cells of certain specialized tissues may differentiate into functional cells that no longer are capable of division. On the other hand, many cells, especially those in slow-growing tumors, may remain in the G_0 state for prolonged periods, only to reenter the division cycle at a later time. Damaged cells that reach the G_1/S boundary undergo apoptosis, or programmed cell death, if the p53 gene is intact and if it exerts its normal checkpoint function. If the p53 gene is mutated and the checkpoint function fails, damaged cells will not be diverted to the apoptotic pathway. These cells will proceed through S phase and some will emerge as a drug-resistant population. Thus, an understanding of cell-cycle kinetics and the controls of normal and malignant cell growth is crucial to the design of current therapy regimens and the search for new drugs.

Η P53 αντιλαμβάνεται βλάβες στο DNA και ενεργοποιεί την απόπτωση σε απόκριση με αλκυλίωσή του. Μεταλλάξεις στο p53 οδηγεί σε αντίσταση σε αλκυλιωτικούς παράγοντες

Growth Fraction (GF)

$$GF = \frac{\text{Proliferating cell group}}{\text{Total tumor cell group}}$$

CCNSA: **drugs that are active throughout the cell cycle.**

CCSA: **drugs that act during a specific phase of the cell cycle.**

Cell cycle specific agents and Cell cycle Non-specific agents

- **Cell Cycle Nonspecific Agents (CCNSA)**

  drugs that are active throughout the cell cycle

  1. **Alkylating Agents**
  2. **Platinum Compounds**
  3. **Antibiotics**

Cell cycle specific agents and Cell cycle Non-specific agents

- **Cell Cycle Specific Agents (CCSA)**

  drugs that act during a specific phase of the cell cycle

S Phase Specific Drug:

Antimetabolites, Topoisomerase Inhabitors

M Phase Specific Drug:

Vinca Alkaloids, Taxanes

G2 Phase Specific Drug:

Bleomycin

Problems With Cancer Chemotherapy

- **Drug Resistance**
- **Drug Toxicity**

Drug Resistance

- De novo Resistance
- Acquired Resistance
- Multidrug Resistance (MDR)

Drug Resistance

De novo resistance:

- **De novo resistance can be de novo genetic (i.e. the cells are initially inherently resistant), or can arise because drugs are unable to reach the target cells because of permeability barriers such as the blood-brain barrier.**

Drug Resistance

Acquired Resistance:

- **Acquired drug resistance may result from genomic mutations, such as the induction or deletion of enzymes involved in drug inactivation or drug activation, respectively.**

Drug Resistance

Multidrug Resistance (MDR):

- **P-glycoprotein transports many naturally occurring drugs out of neoplastic cells, and its induction may lead to multidrug resistance.**
- **As scientific understanding of the mechanisms of drug resistance increases, new treatments may be developed to counteract resistance.**

Drug Toxicity

- **The most common toxicities of antineoplastic drugs result from inhibition of cell replication in the bone marrow, gastrointestinal epithelium, and hair follicles.**

- **Many antineoplastic drugs also stimulate the chemoreceptor trigger zone in the medulla and thereby elicit nausea and vomiting.**

Immunomodulating Drugs

Immunosuppressive Agents:

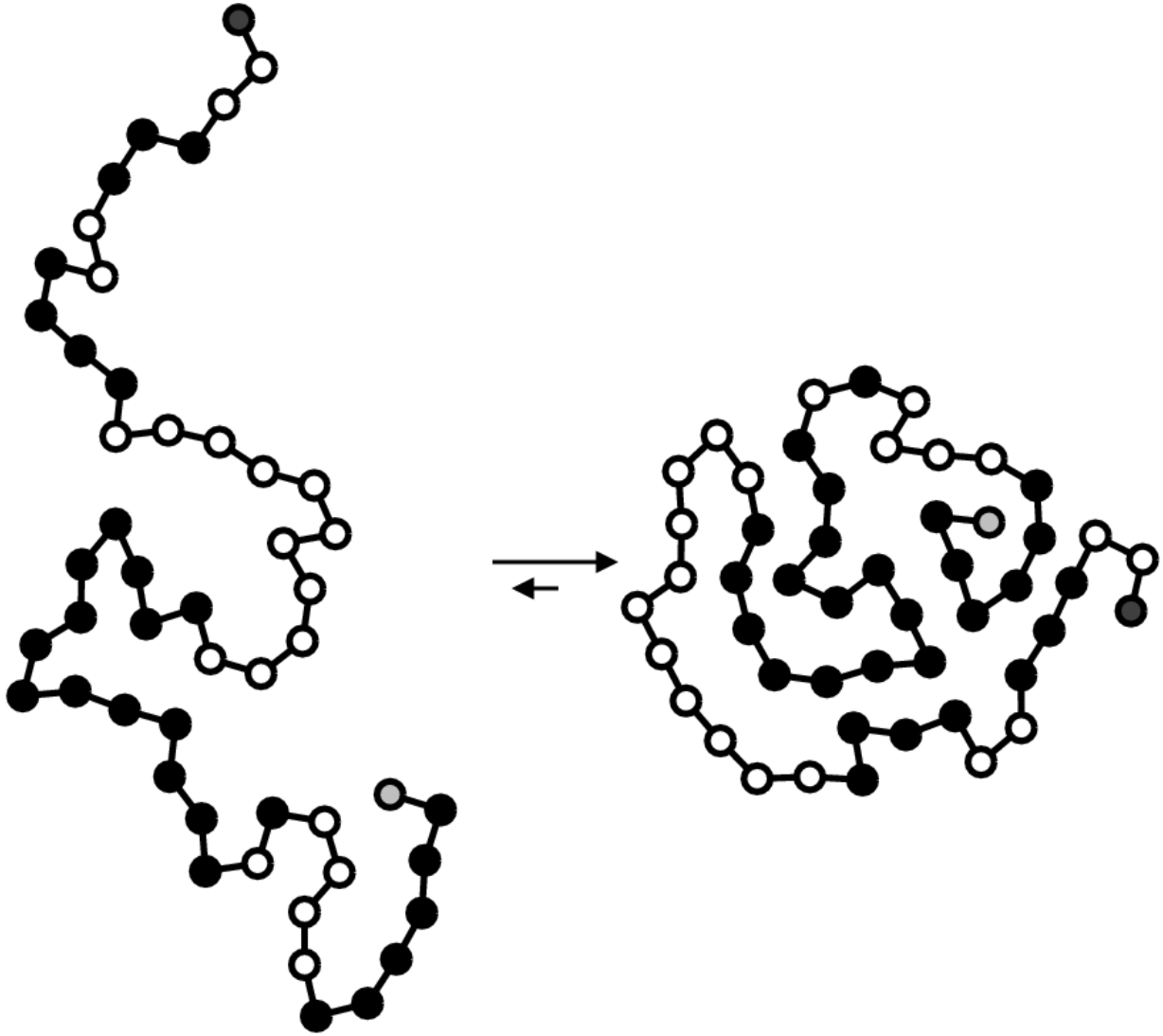
- **Act to suppress immune mechanisms and are used to treat autoimmune diseases or to prevent graft rejection following tissue transplantation.**
- ***Ciclosporin, Tacrolimus, adrenocortical hormones, antimetabolites, alkylating agent, antilymphocyte globulin, Mycophenolate Mofetil***

Immunomodulating Drugs

Immunopotentiator :

- **Enhance antitumor immunity and are used to treat neoplastic disease.**
- ***Recombinant Interferons and Cytokines.***

Αναδίπλωση πρωτεϊνών (protein folding)



Το δόγμα του Anfinsen

Η φυσιολογική αναδίπλωση (ή διαμόρφωση)
(native structure)
μιας πρωτεΐνης καθορίζεται μόνο από
την αλληλουχία αμινοξέων στο περιβάλλον
όπου γίνεται η αναδίπλωση
(Θερμοκρασία, συγκ/ση και σύσταση διαλύτη, κλπ)

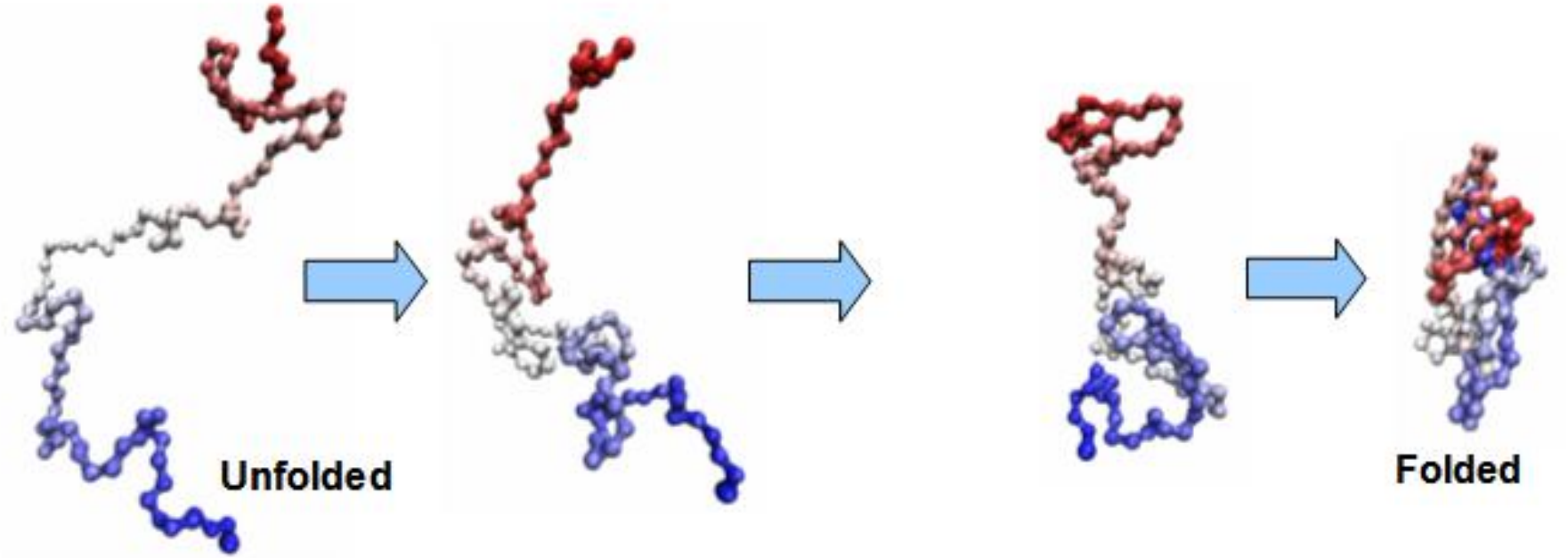
Anfinsen C.B.
1916-1995

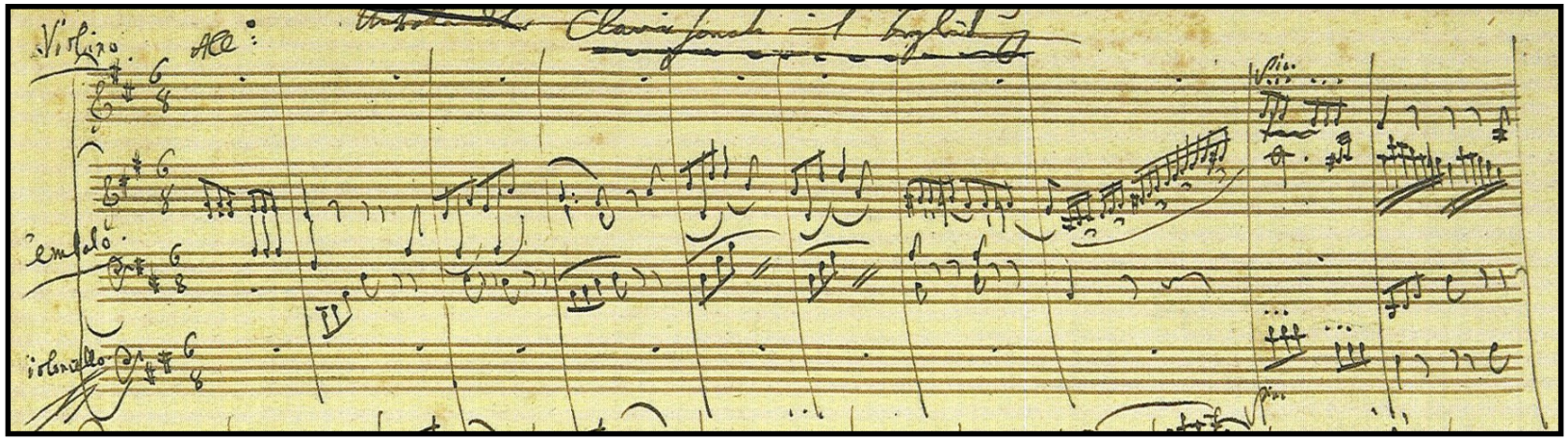
1972

Η φυσική αναδίπλωση χαρακτηρίζεται από:

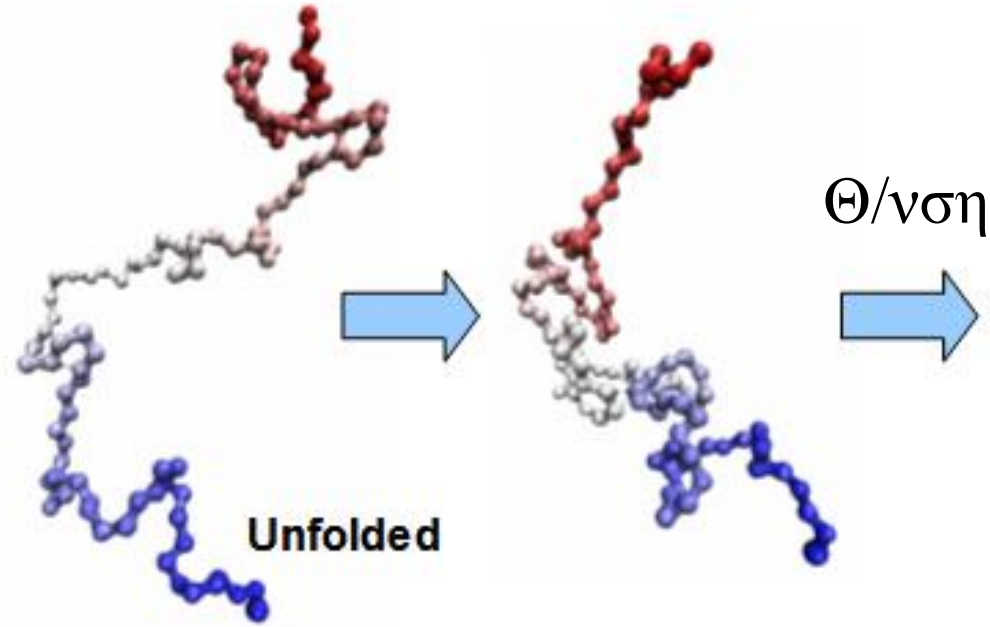
- ***Μοναδικότητα (uniqueness)***
- ***Σταθερότητα (stability)***
- ***Κινητική προσβασιμότητα (kinetical accessibility)***

Αναδίπλωση πρωτεϊνών (protein folding)

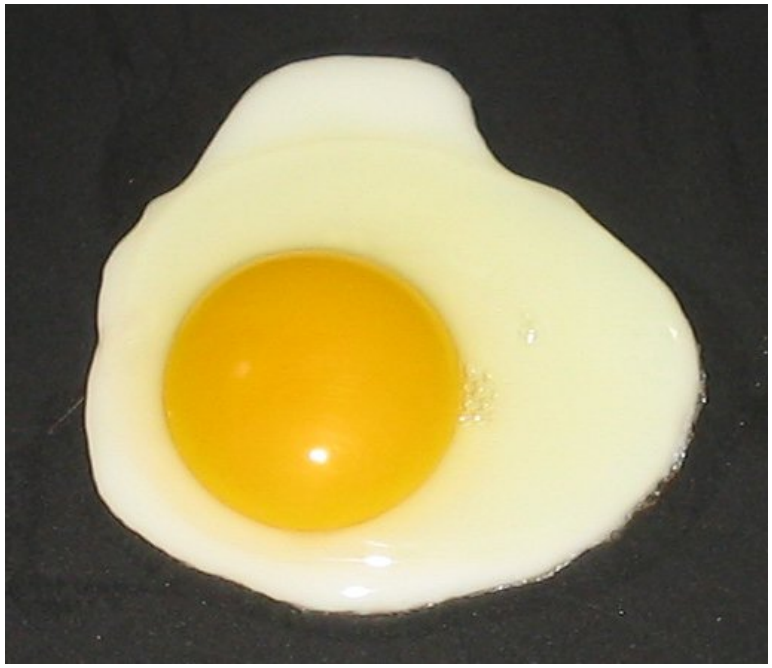


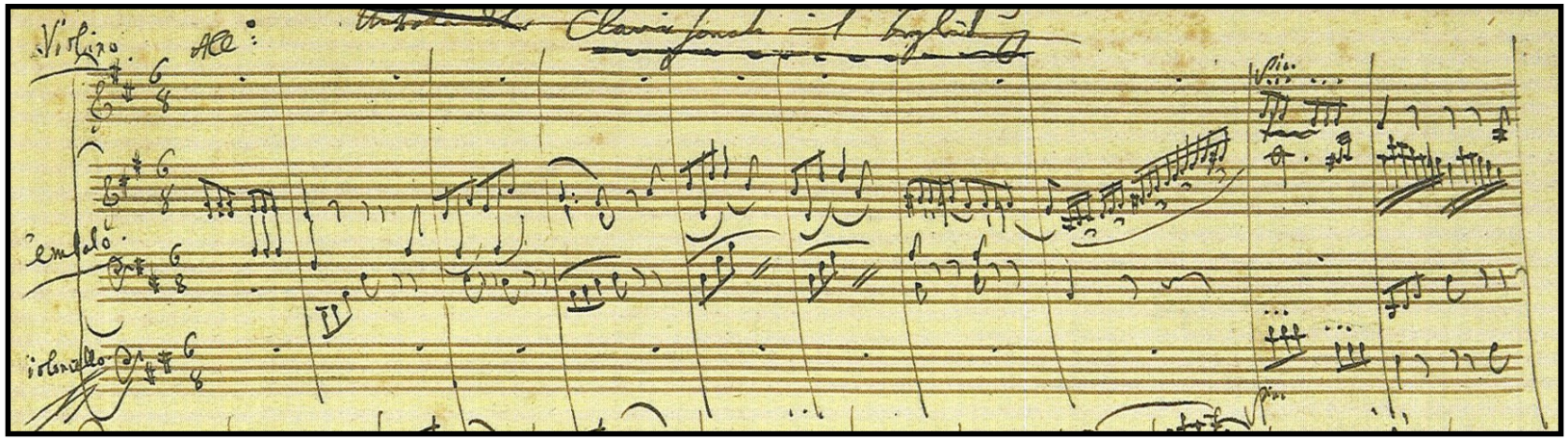


Αναδίπλωση πρωτεϊνών (protein folding)



Θ/νση





Παρατηρήσεις

- Αρχές δεκαετίας ‘60 F. Ritossa: ανακάλυψε τη θερμική απόκριση (HS) response ενώ μελετούσε τους σιελογόνους αδένες της *Drosophila melanogaster*.

- Θέρμανση των κυττάρων προκαλούσε επαγωγή θυλάκων (puffs) σχηματίζοντας πολυτενικά χρωμοσώματα.
  Περαιτέρω ανάλυση έδειξε οτι οι θύλακες ήταν περιοχές αυξημένης μεταγραφής συγκεκριμένων ομάδων πρωτεϊνών.

- Η απόκριση ονομάστηκε “απόκριση θερμικού σοκ” γιατί η θέρμανση ήταν ο πιό κοινός επαγωγέας.

- Επάγονται όμως και σε καταστάσεις ψύχους, έλλειψης οξυγόνου.

Heat-shock (HSP) proteins

Υπάρχουν σε όλα τα κύτταρα

Επάγονται σε καταστάσεις στρές

- Θέρμανση
- Ψύχος
- Ελλειψη οξυγόνου

Σε φυσιολογικές συνθήκες λειτουργούν ως chaperones

- Διατήρηση δομής πρωτεϊνών
- Μετακίνηση πρωτεϊνών μεταξύ κυτταρικών διαμερισμάτων
- Μετακίνηση πεπτιδίων στην κυτταρική μεμβράνη

Δράση HSPs

Ανάκαμψη από στρες:

Επαναδιάταξη (refolding)

Αποικοδόμηση

πρωτεϊνών που υπέστησαν βλάβη

Διατήρηση ομοιόστασης

Κυτταρική επιβίωση

The main families of Heat-shock proteins

HSP family	Members	Intracellular location
Small HSPs	HSP10, GROES, HSP16, -crystallin, HSP20, HSP25, HSP26, HSP27	Cytosol
HSP40	HSP40, DNAJ, SIS1	Cytosol
HSP47	HSP47	Endoplasmic reticulum
Calreticulin	Calreticulin, calnexin	Endoplasmic reticulum
HSP60	HSP60, HSP65, GROEL	Cytosol and mitochondria
HSP70‡	HSP72, HSC70 (HSP73), HSP110/SSE, DNAK	Cytosol
	SSC1, SSQ1, ECM10	Mitochondria
	GRP78 (BiP), GRP170	Endoplasmic reticulum
HSP90	HSC84, HSP86, HTPG	Cytosol
	gp96 (GRP94, HSP108, endoplasmin)	Endoplasmic reticulum
HSP100	HSP104, HSP110§	Cytosol
	CLP proteins	Cytosol
	HSP78	Mitochondria

Association of specific antigen peptides with HSPs

Antigen source	HSP with which associated	MHC class I molecule that presents epitope
Tumour antigens		
PRL1a mouse leukaemia	Hsp70, Hsp90, gp96	H–2L^{d}
Human melanoma MART-1	HSP70	HLA-A2
Human melanoma tyrosinase	HSP70	HLA-A2
Human melanoma gp100	HSP70	HLA-A2
Viral antigens		
Vesicular stomatitis	gp96	H–2K^{b}
Herpes simplex -2	gp96	H–2^{d}
Influenza	gp96, Hsp70	H–2K^{b}
Simian virus 40	gp96	H–2D^{b}, H–2K^{b}
Hepatitis B	gp96	
Intracellular bacterial antigens		
Mycobacterium tuberculosis	gp96	H–2^{d}
Listeria monoctyogenes	gp96	H–2^{d}
Model antigens		
-Galactosidase	gp96	H–2L^{d}
Ovalbumin	gp96, Hsp70	H–2K^{b}
Normal cellular antigens		
Minor histocompatibility antigens	gp96	H–2K^{d}, H–2K^{b}

HSPs και ανοσοαπόκριση

Οι HSPs ενεργοποιούν το ανοσοποιητικό σύστημα μέσω

- Ενδοκυττάριων
- Εξωκυττάριων δραστηριοτήτων

HSPs και καρκίνος

- Καρκινικά κύτταρα μπορούν να εξασθενήσουν ή *καθυστερήσουν* (*attenuated)* ,
- Τότε μπορούν να ενεθούν (εμβολιασμός) σε ποντίκια.
- Αν στη συνέχεια τα ίδια κύτταρα -όχι όμως εξασθενημένα- ενεθούν στα ποντίκια, αποβάλλονται και δεν αναπτύσσεται όγκος.
- Αν τα ποντίκια δεν ενεθούν με αυτόν τον τρόπο, δεν προστατεύονται.

Tumor used for challenge

Tumor used for vaccination

	A	B	C	D	E
A	+	-	-	-	-
B	-	+	-	-	-
C	-	-	+	-	-
D	-	-	-	+	-
E	-	-	-	-	+

+ Tumor rejected
- Tumor grows

HSPs και εξειδίκευση

- Ο παράγοντας που προστάτευε τα ποντίκια ήταν οι HSPs.

- **Η δράση τους δεν γινόταν από μόνη της, αλλά όταν συνδεόταν σε πεπτίδια**

- Ιδρυση της εταιρείας Antigenics.

- Η εταιρεία έλαβε την πρώτη πατέντα το 1998 για εμβολιασμό με HSPs για χρήση έναντι καρκίνων.

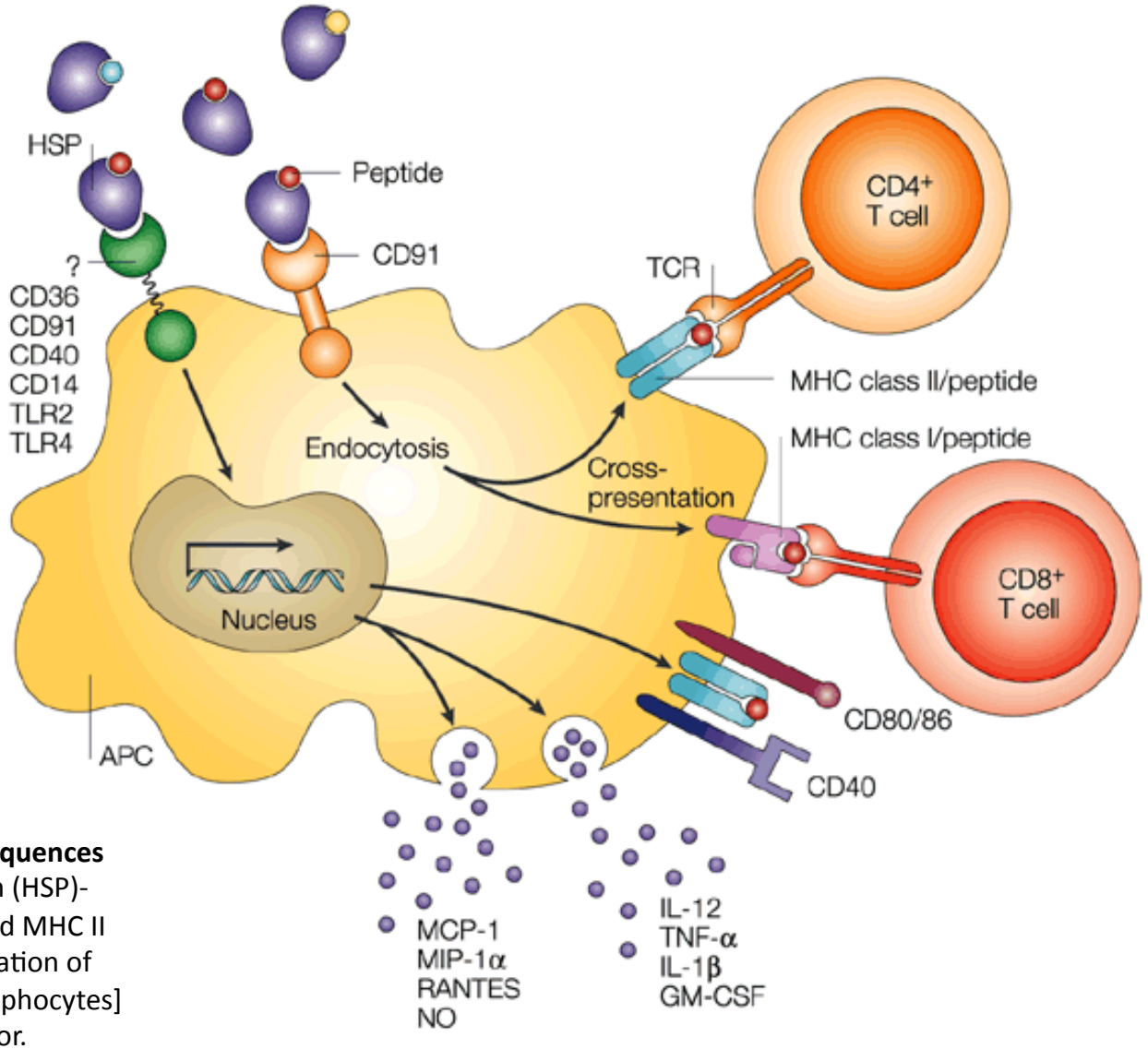
Dr. Pramod Srivastava

Τι έδωσε στις HSPs την τόσο μεγάλη εξειδίκευση;

HSPs και ανοσοαπόκριση

- Τα άρρωστα κύτταρα πεθαίνουν ελευθρώνοντας σύμπλοκα HSP-πεπτιδίων
- Τα σύμπλοκα αναγνωρίζονται από περιφερόμενα κύτταρα του ανοσοποιητικού (*antigen-presenting cells*, APCs):
  μακροφάγα (macrophages) και
  δενδρίτες (dendritic cells).
- Τα σύμπλοκα HSP-πεπτιδίων προσδένουν τον CD91 υποδοχέα των APC κυττάρων και έτσι παίρνουν το σύμπλοκο.
- Κατόπιν τα APCs πηγαίνουν στους λεμφαδένες
- Εκεί, τα APCs εμφανίζουν τα πεπτίδια στην επιφάνειά τους
- Τα πεπτίδια είναι αντιγονικά (προκαλούν ανοσοαπόκριση)
- Τα Τ-κύτταρα “διαβάζουν” τα πεπτίδια και τα αναζητούν σε άλλα κύτταρα
- Τα πεπτίδια αυτά είναι μοναδικά για κάθε ασθενή

The HSP-APC interaction integrates adaptive and innate immune events

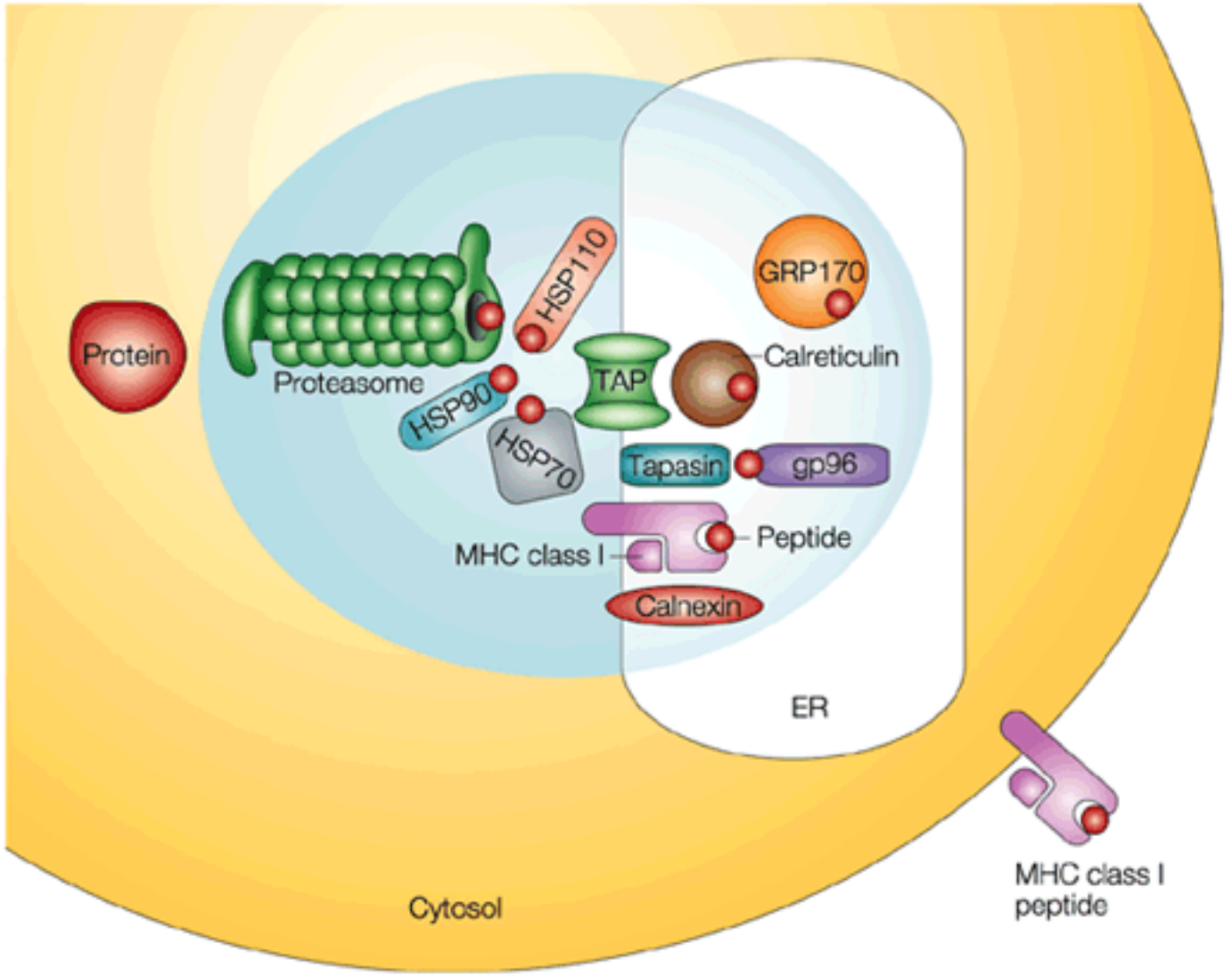


The adaptive (antigen-specific) consequences
[re-presentation of heat-shock protein (HSP)-chaperoned peptides by the MHC I and MHC II and the consequent respective stimulation of peptide-specific CD8+ and CD4+ T lymphocytes] are mediated by the CD91 HSP receptor.

The non-antigen-specific consequences
(e.g. cytokine and chemokine release, dendritic-cell maturation) are mediated by other receptors.

Nature Reviews | Immunology

HSPs in antigen presentation: The presentosome



Nature Reviews | Immunology

Εμβολιασμός με καρκίνο έναντι του καρκίνου

- Επιβεβαιώθηκε σε διαφορετικές σειρές ποντικιών και σε άλλα είδη
- Αύτη είναι η βασική αρχή της ανοσίας έναντι του καρκίνου.
- Ισχύει για σειρά καρκίνων όπως:
  - Δέρματος
  - Εντέρου
  - Μαστού
  - Ηπατος
  - Νεφρών
  - Εγκεφάλου
  - Ογκους από καρκινογόνα
  - Αυθόρμητους καρκίνους

Tumor used for challenge

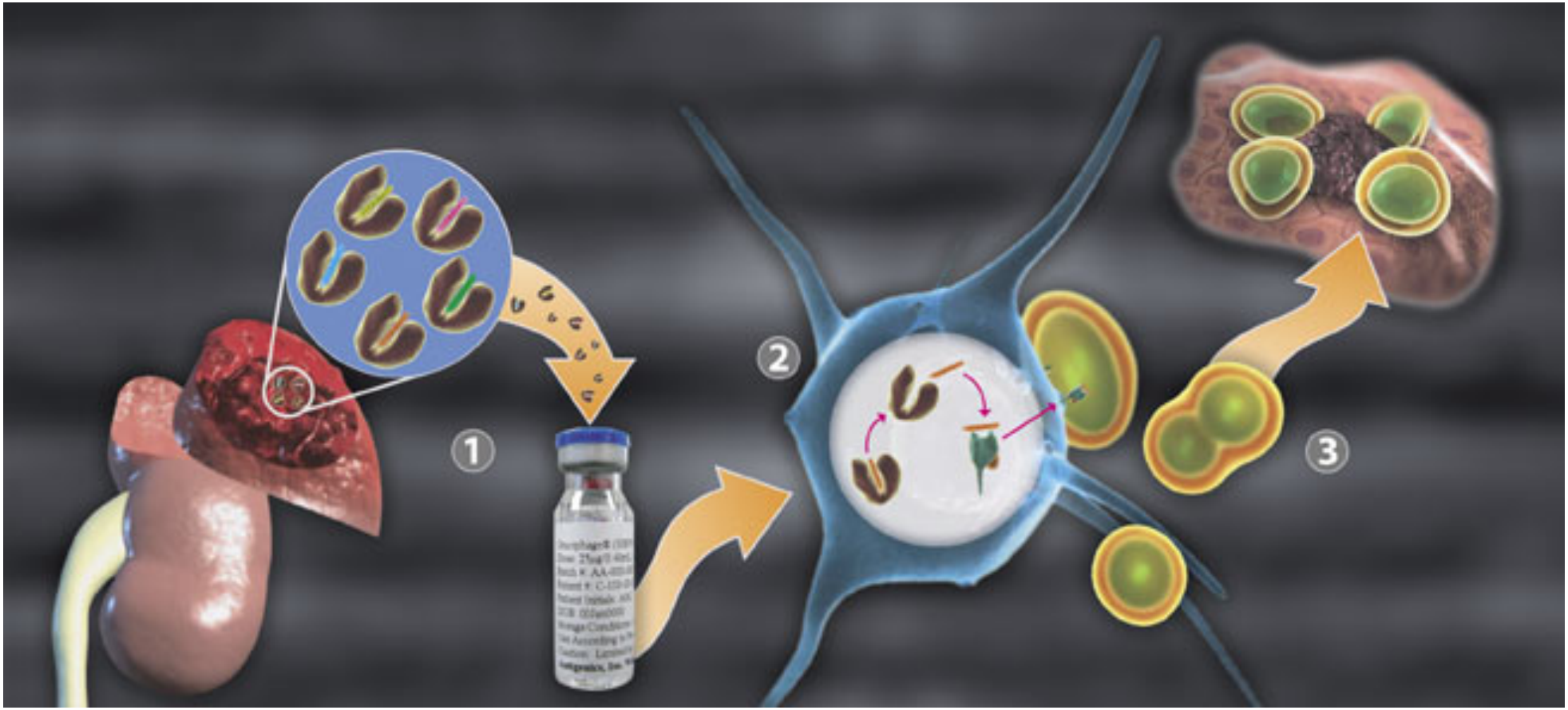
Tumor used for vaccination	**A**	B	C	D	E
A	+	-	-	-	-
B	-	+	-	-	-
C	-	-	+	-	-
D	-	-	-	+	-
E	-	-	-	-	+

+ Tumor rejected

- Tumor grows

- Οι HSPs έδειξε πως η δράση τους είναι πολύ εξειδικευμένη
  Κύτταρα από όγκο Χ μπορούν να εμβολιάσουν έναντι καρκίνου Χ και όχι έναντι του καρκίνου Υ.

Hpss ‘mechanism of action’ diagram



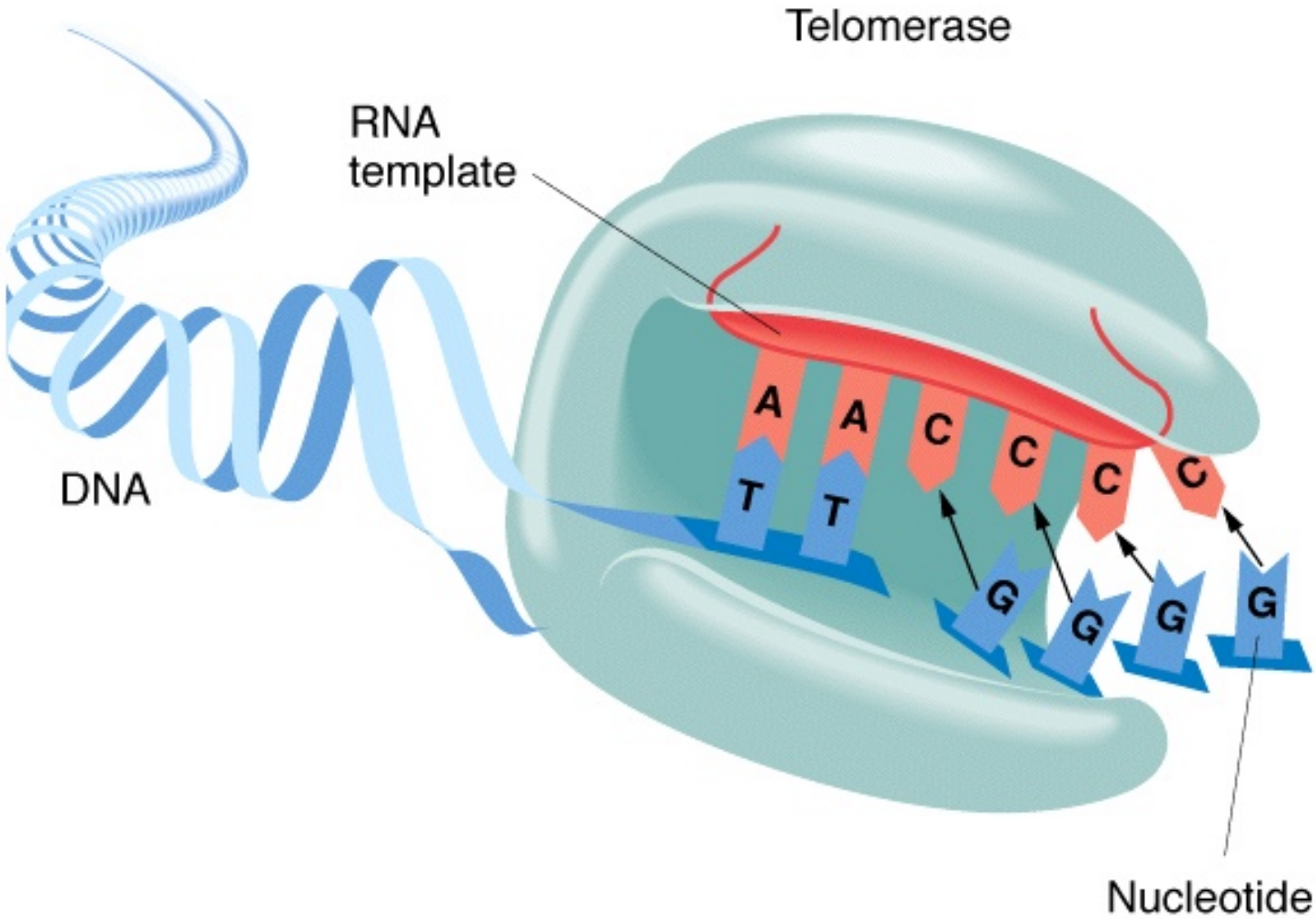
www.antigenics.com/ news/graphics/

- The pattern of heat shock proteins in mammalian brain, either synthesized in a developmentally regulated manner or in response to stress, is non-random. This has been related to specific functions of different parts of the brain, including role of Hsps, particularly the Hsp70, in short- and long-term memory and making different parts of the brain more or less susceptible to stress-induced injuries.

- * A variety of pollutants induce detectable levels of stress proteins and therefore, relatively simple methods are being developed to use the stress-proteins as important bio-markers for environmental pollution. Such studies will be of significance in public health.

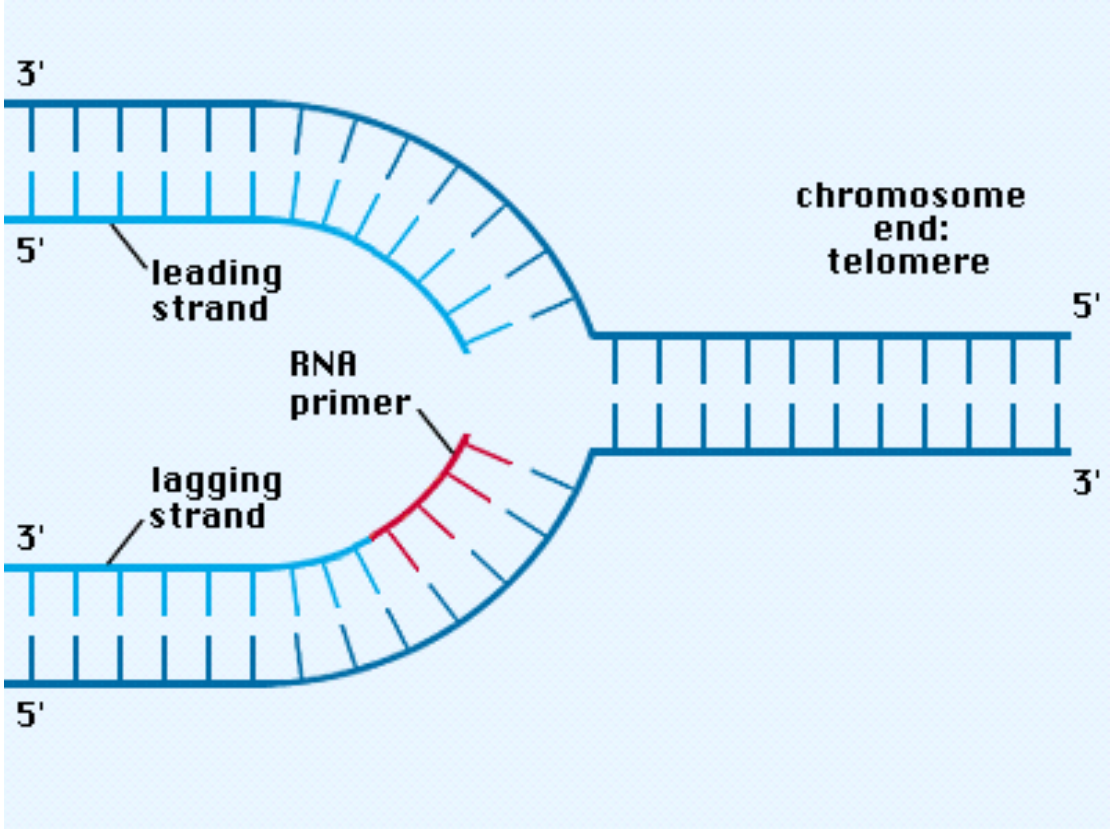
- * Recent studies have shown significant roles for different Hsps in the immune response. A number of Hsps such as Hsp70, Hsp90 and Gp96/ Grp94 have been shown to chaperone a broad array of peptides, derived from different cellular proteins,Hsp-peptide complexes, whether isolated from cells or reconstituted in vitro, have the ability to vaccinate against the complexed peptides.

Τελομερή και τελομεράση



Telomerase is a ribonucleoprotein enzyme complex (a cellular reverse transcriptase) that maintains chromosome ends and has been referred to as a cellular immortalizing enzyme. Telomerase is a ribonucleoprotein reverse transcriptase enzyme (composed of both RNA and proteins) that uses its internal RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize telomeric DNA (TTAGGG)n, directly onto the ends of chromosomes. Telomerase is present in most fetal tissues, normal adult male germ cells, inflammatory cells, in proliferative cells of renewal tissues, and in most tumor cells. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six base pair repeat. This extension of the 3' DNA template end in turn permits additional replication of the 5' end of the lagging strand, thus compensating for the end-replication problem.

Τελομερή - Τελομεράση



Η ρύθμιση του μήκους των τελομερών

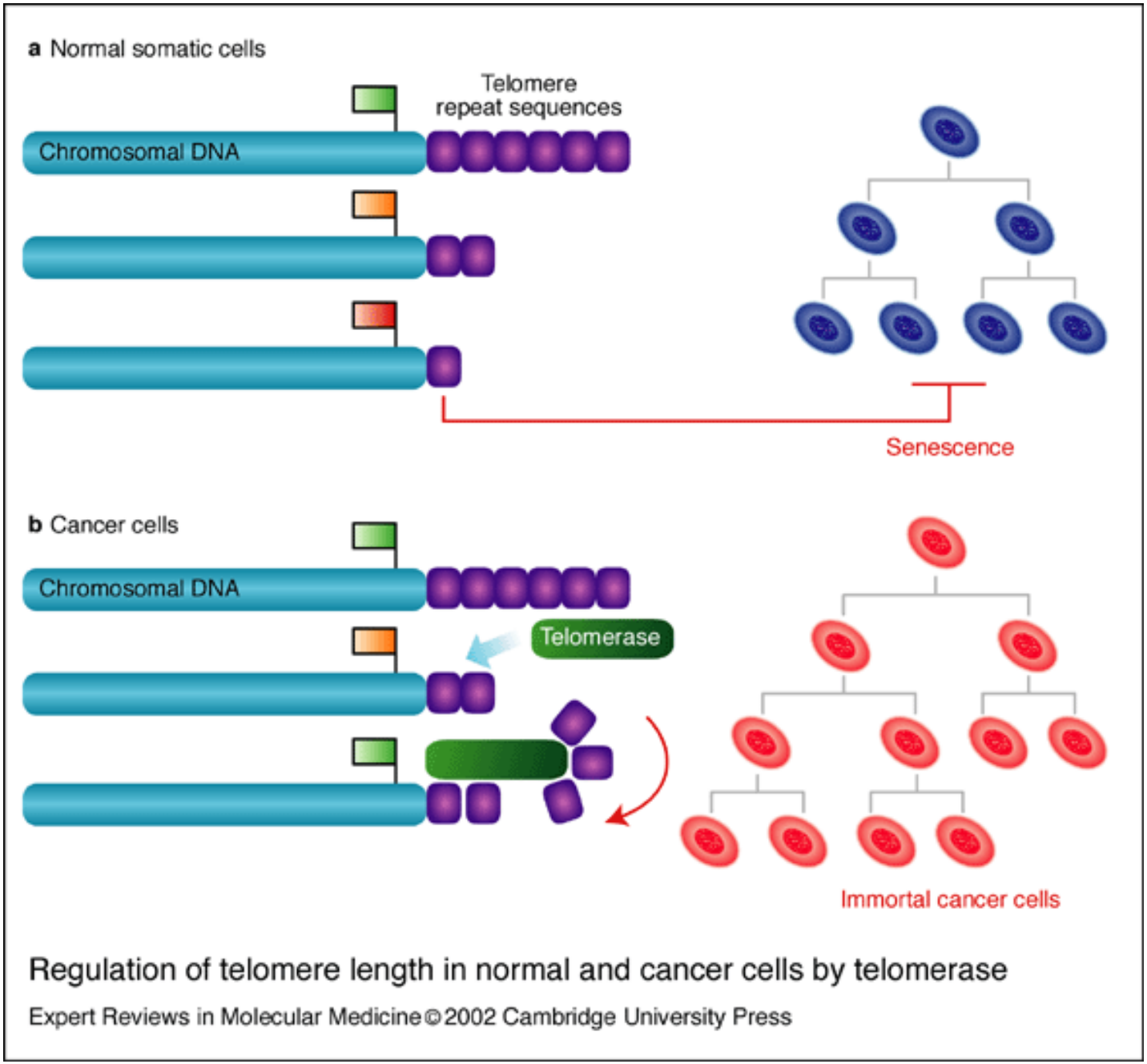
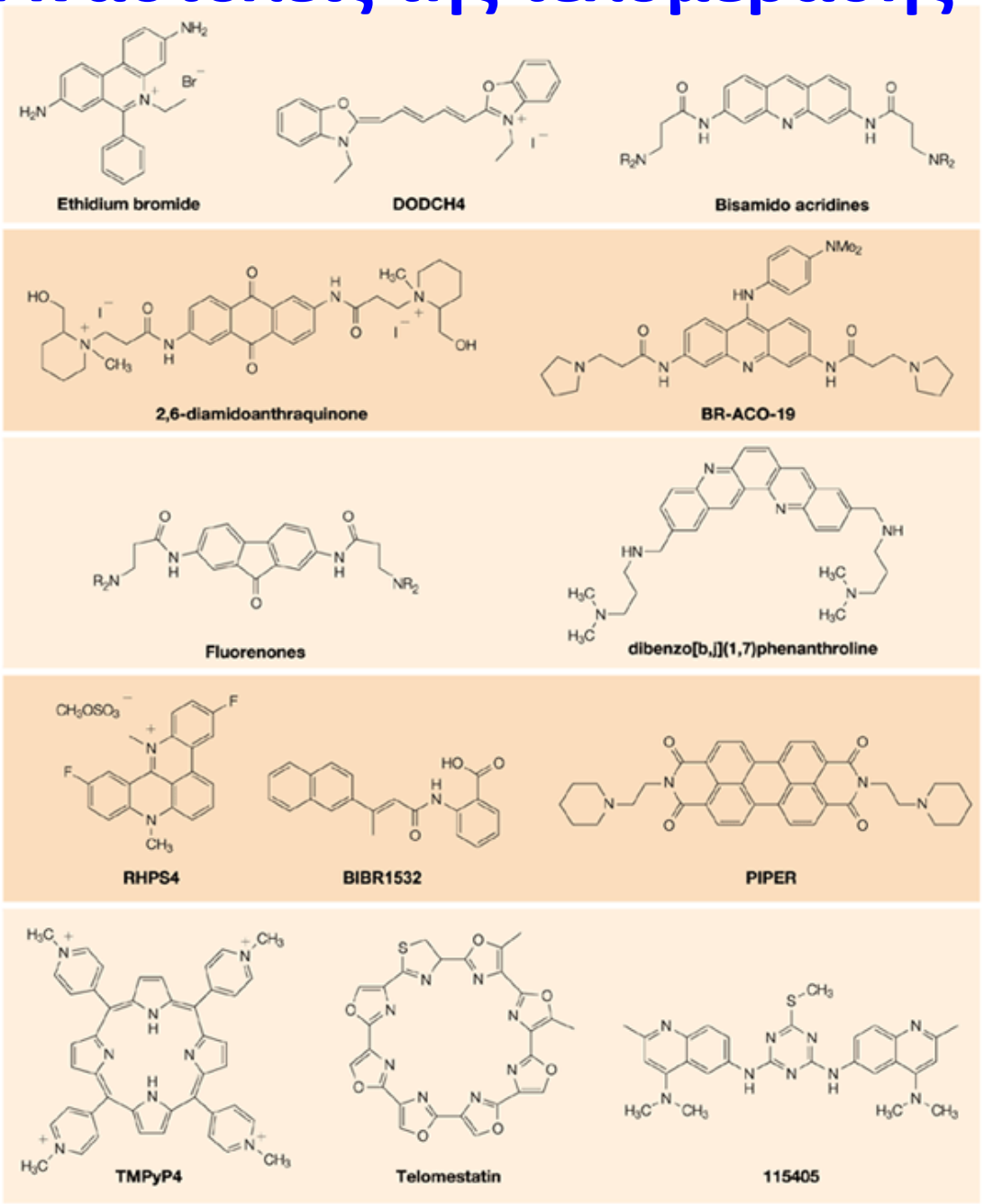


Figure 1. Regulation of telomere length in normal and cancer cells by telomerase.

(a) In normal somatic cells telomerase is absent. Every time a normal cell divides, the telomeric repeat sequence (depicted as a purple bar) is lost from the end of the chromosome. Eventually, after many cell divisions, the gradual erosion of the telomere is sensed by the cell (an orange flag on the chromosome in the diagram depicts this event) and, when the telomeres reach a critically short length (red flag), a cell-signalling pathway initiates the senescence programme, resulting in a cessation of cellular proliferation.

(b) (b) In cancer cells, the expression of telomerase allows the senescence program to be bypassed (Refs 4, 7). Once activated, telomerase maintains telomeres at a length compatible with cell proliferation through the addition of telomere repeat sequences. Thus, the cancer cell becomes immortal (fig001nkg).

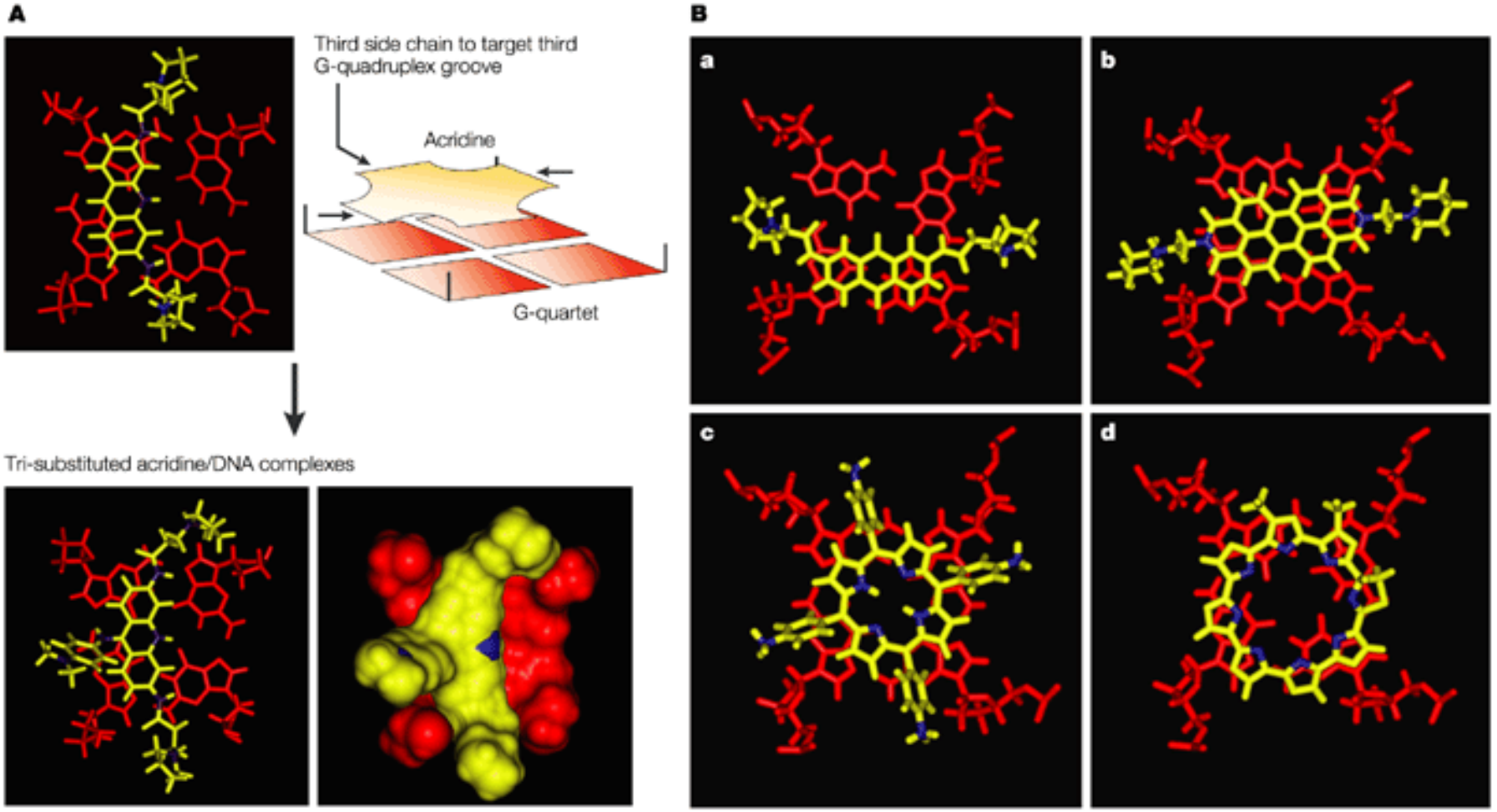
Αναστολείς της τελομεράσης



The emphasis here is on G-quadruplex-interacting ligands, which generally have planar aromatic chromophores

Neidle and Parkinson Nature Reviews Drug Discovery 1, 383-393 (2002)

Structure-based design of G-quadruplex ligands.



Neidle and Parkinson Nature Reviews Drug Discovery 1, 383-393 (2002)

A | The basis for the rational design of second-generation acridine inhibitors, showing how a third substituent on an acridine skeleton (yellow) can interact in a third groove of a quadruplex. Such a molecule has inherent selectivity over DNA-duplex-binding molecules, as the two grooves of B-form duplex DNA cannot readily accommodate tri-substitution. The views are down onto the plane of a G-quartet. B | Views from molecular modelling of several inhibitors that interact with the G-quartet surface of a quadruplex. a | A di-substituted anthraquinone, b | the PIPER molecule, c | a tetrapyridyl-porphyrin and d | the telomestatin molecule. Differences in ligand–G-quartet overlap are clearly apparent. We are grateful to Martin Read for these figures.

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Elizabeth H. Blackburn

1/3 of the prize
USA
b. 1948
(in Hobart,
Tasmania, Australia)

Carol W. Greider

1/3 of the prize
USA
b. 1961

nobelprize.org

Jack W. Szostak

1/3 of the prize
USA
b. 1952
(in London, United
Kingdom)

Gleevec®

Protein Kinase Inhibitor Therapy
for Chronic Myeloid Leukemia, CML

faculty.smu.edu/jbuynak/Gleevec.ppt

Imatinib (STI571),

Gleevec® (USA)

or

Glivec

(Europe, AUS, Latin America)

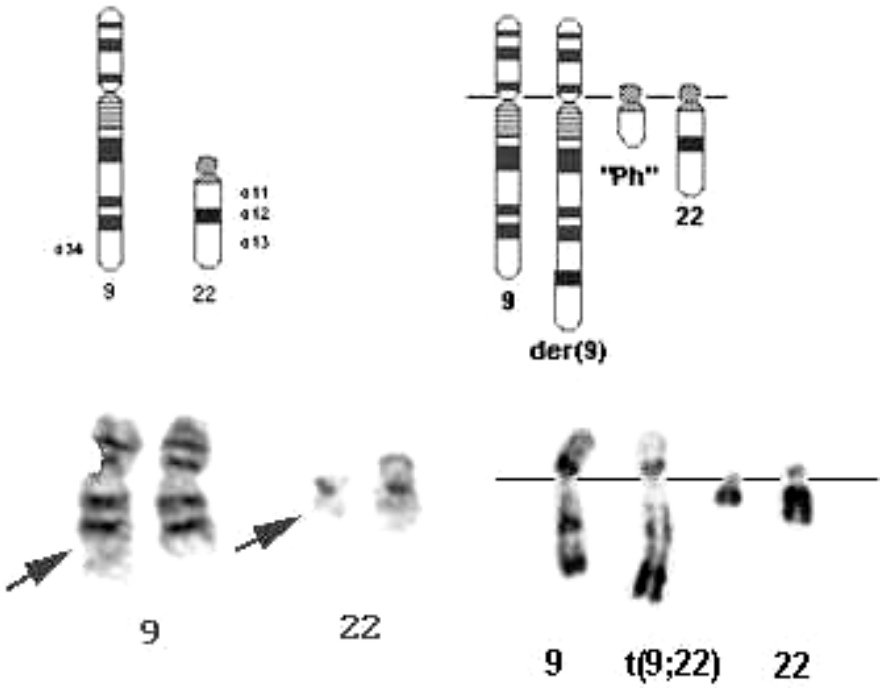
by

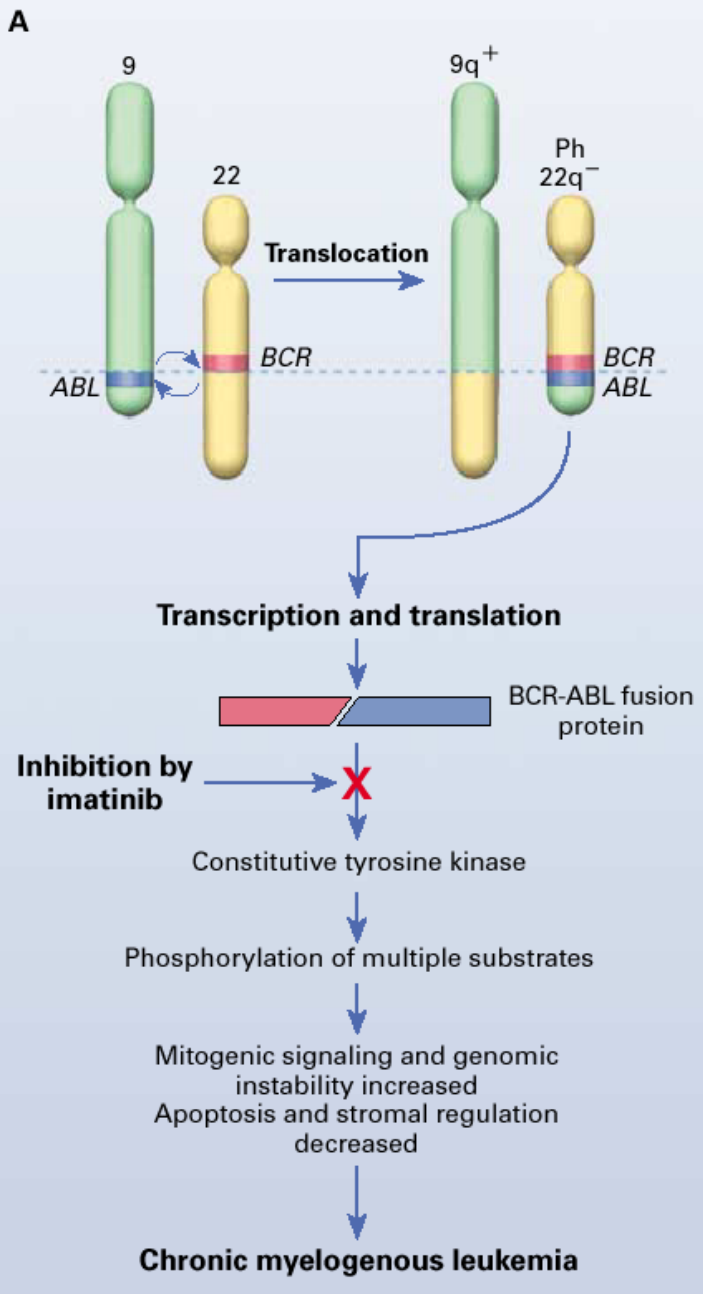
Novartis

Protein Kinase Inhibitor Therapy
for Chronic Myeloid Leukemia, CML

Chronic Myeloid Leukemia (CML)

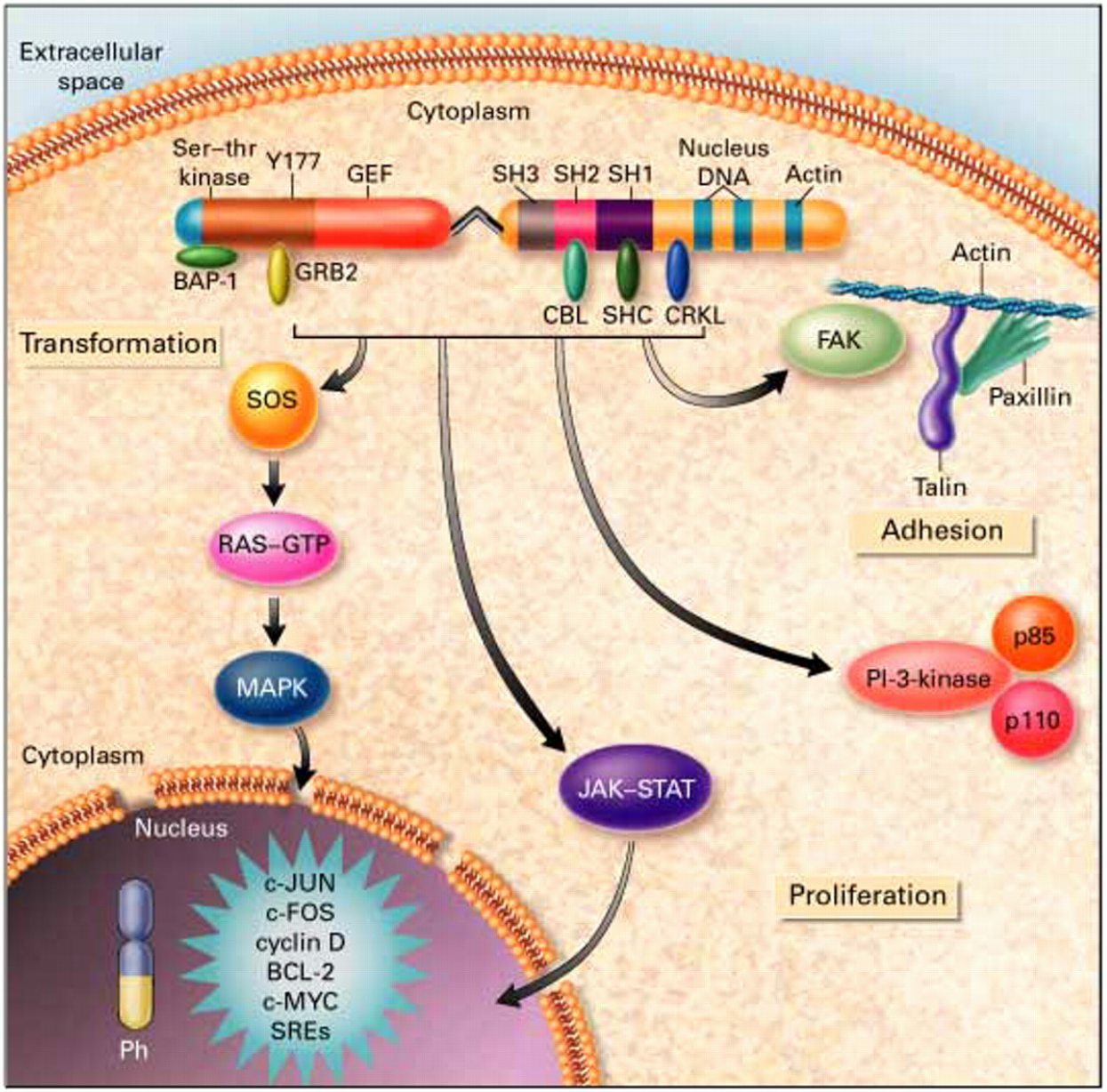
- Cancer in the bone marrow
- Abnormal overproduction of progenitor white blood cells
- Philadelphia chromosome abnormality





Philadelphia Chromosome

- A shortened chromosome 22 resulting from the translocation between chromosome 9 and chromosome 22
- Produces BCR-ABL oncogene


Extracellular space
Cytoplasm
Ser–thr kinase
Y177
GEF
SH3 SH2 SH1
Nucleus
DNA
Actin
BAP-1
GRB2
CBL SHC CRKL
Actin
FAK
Paxillin
Talin
Adhesion
Transformation
SOS
RAS–GTP
MAPK
p85
PI-3-kinase
p110
Cytoplasm
JAK–STAT
Nucleus
c-JUN
c-FOS
cyclin D
BCL-2
c-MYC
SREs
Ph
Proliferation

Three Phases of CML

Phase One: Chronic Phase

- Minor symptoms such as fatigue, infection, bleeding, and weight loss
- Progresses slowly

Phase Two: Accelerated Phase

- White blood cells increase and disease is harder to treat

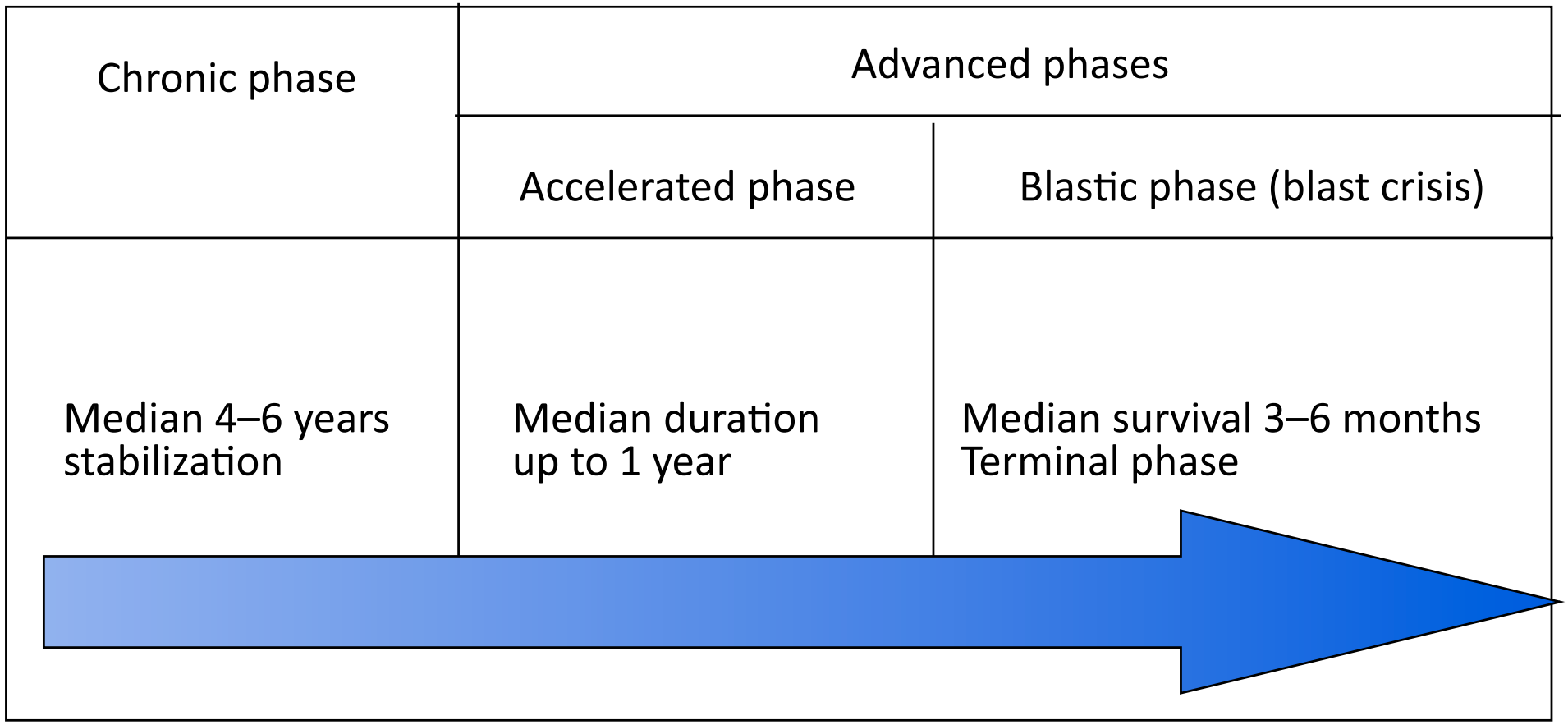
Phase Three: Myeloid Blast Crisis

Epidemiology of CML

- Median age range : 45-55 years
- Male-to-female ratio—1.3 : 1
- 50% diagnosed by routine lab tests

  85% diagnosed during the chronic phase

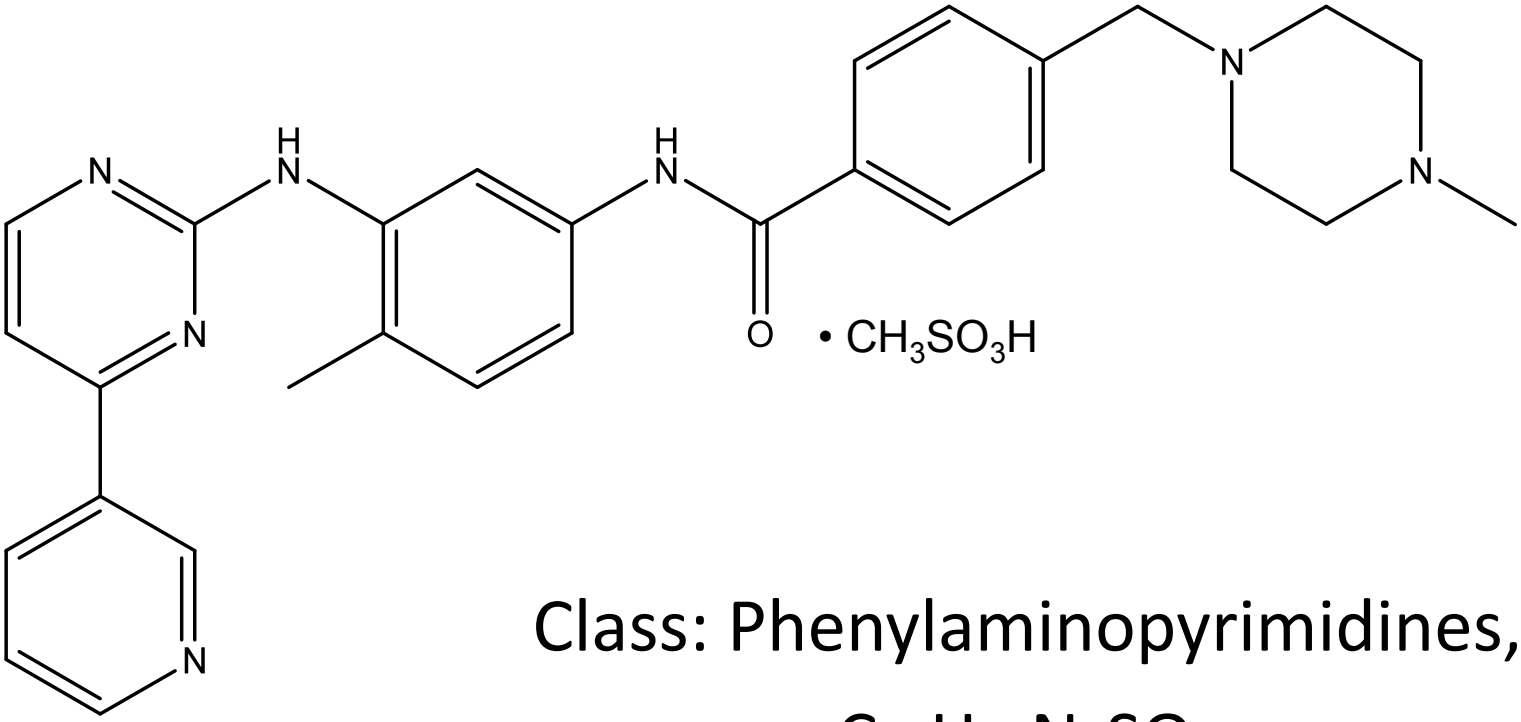
Clinical Course: Phases of CML



Alternative Treatments for CML

- Chemotherapy: Hydroxyurea, Busulfan
- Bone marrow transplant
- Interferon-alpha
- Gleevec

Structure of Gleevec®

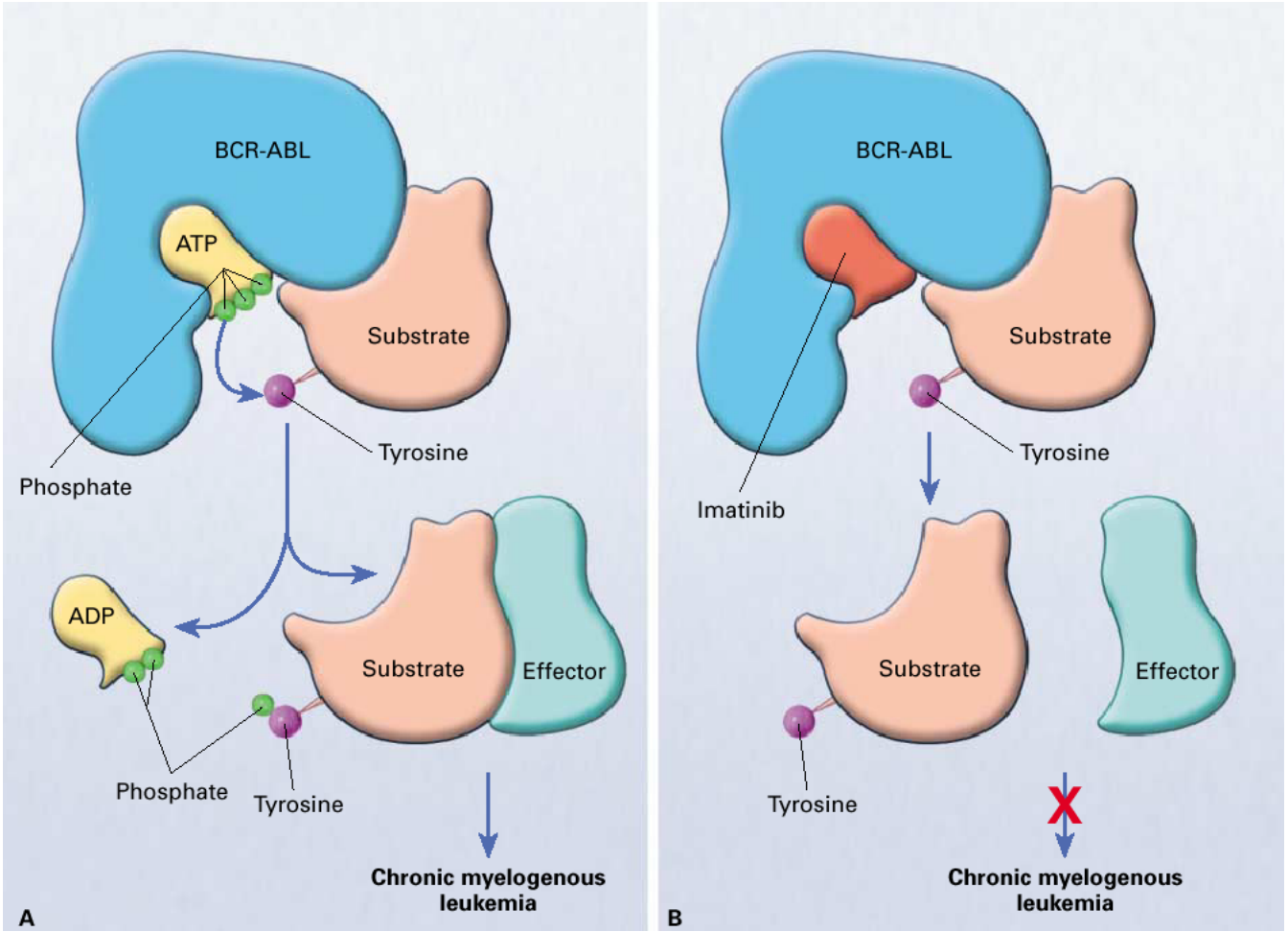


Class: Phenylaminopyrimidines,

$C_{30}H_{35}N_7SO_4$

MW=589.7

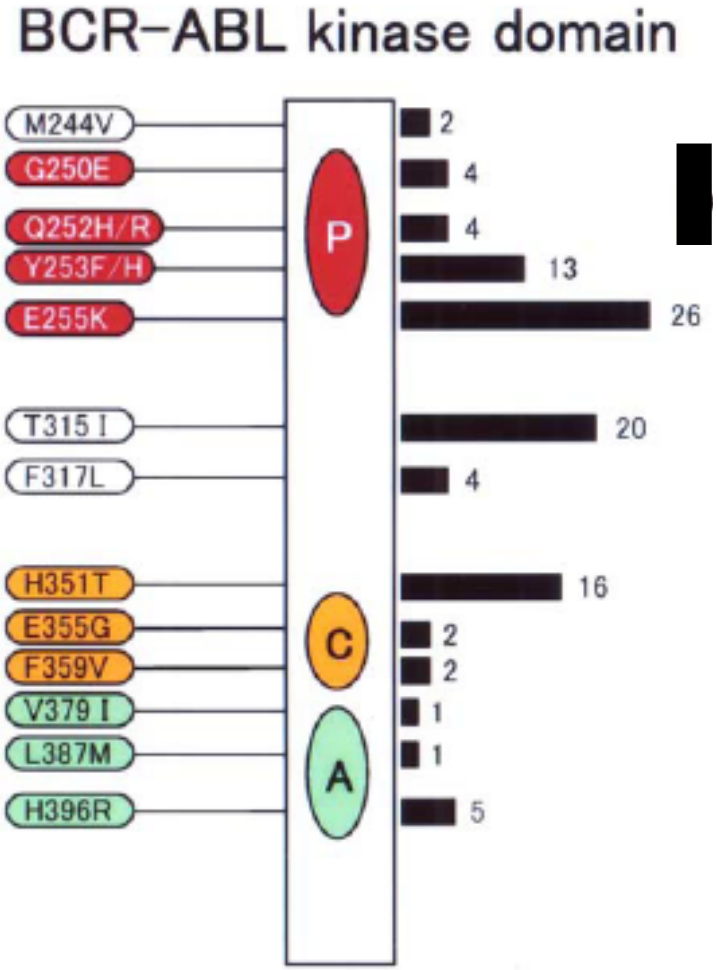
Mechanism of Gleevec

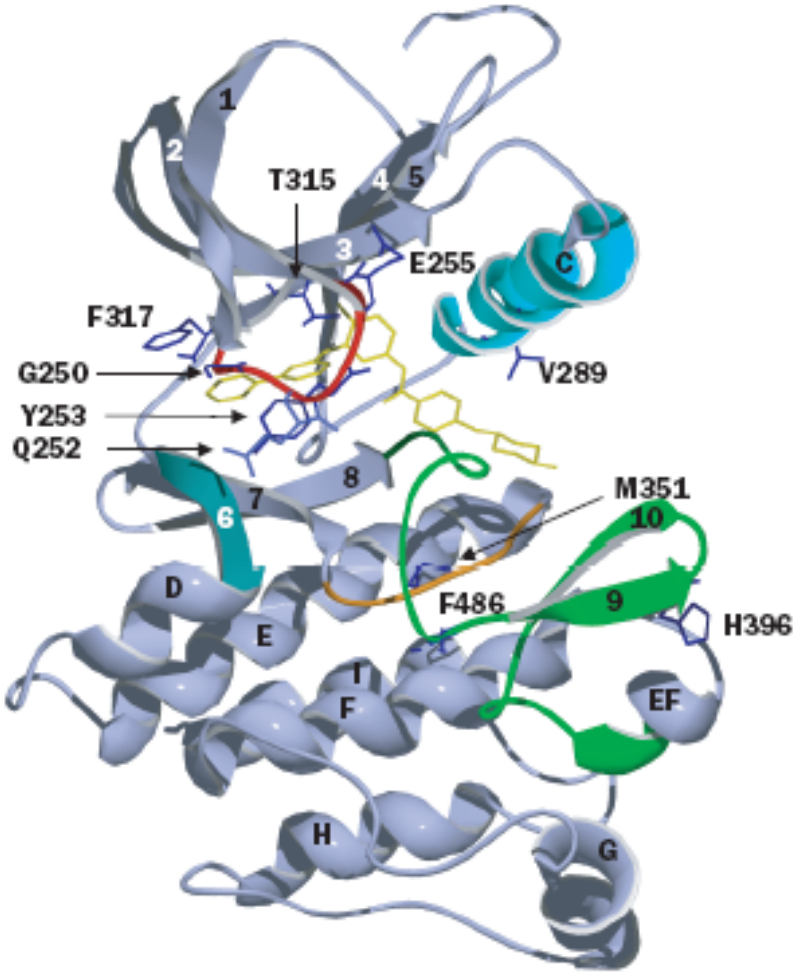


BCR-ABL Mutations

- Detected in up 90% of patients who develop secondary resistance to Gleevec
- Rare in patients with primary resistance
- Caveat: PCR techniques used may only identify most dominant clonses

BCR-ABL Kinase Domain Mutations





Kinase Domain Mutations

- Evidence for clonal selection of preexisting BCR-ABL selection
- Sequences upstream of kinase domain do not show additional mutations
- Multiple independent BCR-ABL mutations found in several patients who relaps within 3 months of therapy
- Oligonucleotide-PCR to detect specific mutations

Strategies to Overcome Gleevec Resistance

- Novel BCR-ABL kinase inhibitors
- Target other aspects of BCR-ABL protein
- Inhibit downstream signaling

New Compounds

- Hsp-90 Inhibitors
  - Geldamycin (17-AAG)
- Farnesyl Transferase Inhibitors
  - Downregulate Ras-MAPK pathway
- SRC/ABL kinase inhibitors
  - PD166326: Can bind both inactive and active abl conformations
  - BMS-354825: Active against 14/15 imatinib resistant BCL-ABL isoforms including all mutations located in phosphate binding P-loop

Side Effects

- Mild: Nausea, vomiting, diarrhea, heart burn, headache, and muscle cramps

- Severe: Ascites, Pulmonary Edema, Neutropenia, Thrombocytopenia, etc

Advantages of Gleevec

- Specifically targets an enzyme in cancer cells, not normal healthy cells
- Minimal side effects compared to other treatments
- Given orally instead of injections

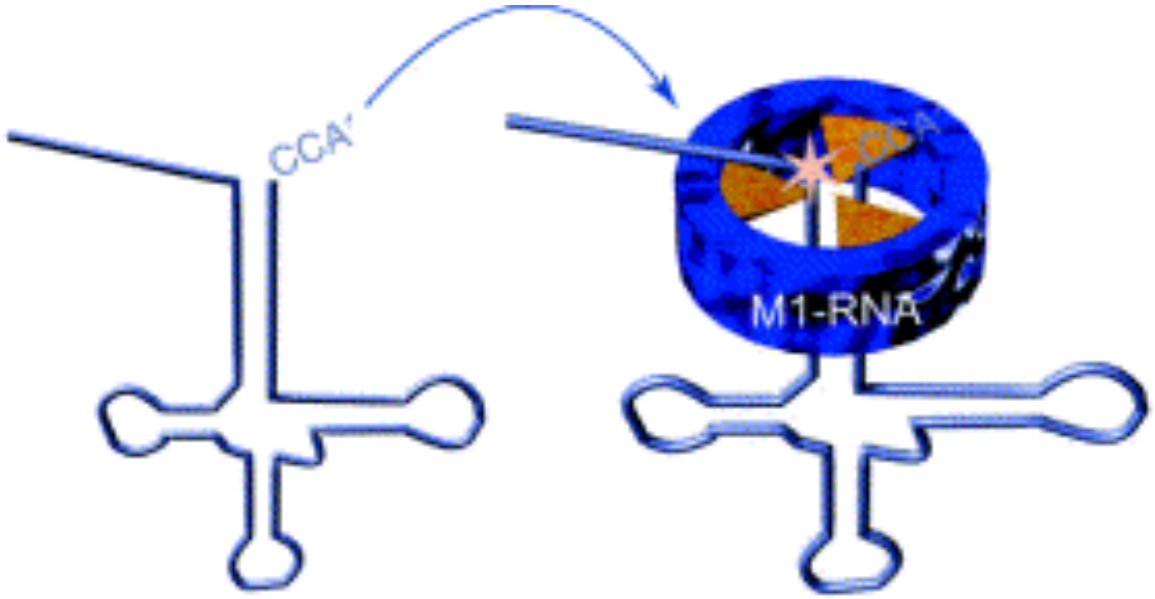
Conclusions

- Based on diversity of mutations and mechanisms causing imatinib resistance unlikely that a single agent will be effective
- Identification of resistance mechanisms may be able to guide therapy in future

Conclusions

- CML is a result of a single genetic mutation
  - the Philadelphia Chromosome
- Gleevec® is a Tyrosine Kinase Inhibitor that targets the protein product of the mutation
- Clinical Trials show Gleevec® to be effective in Chronic Phase CML>Accelerated>Blast Crisis
- Gleevec® is generally well-tolerated

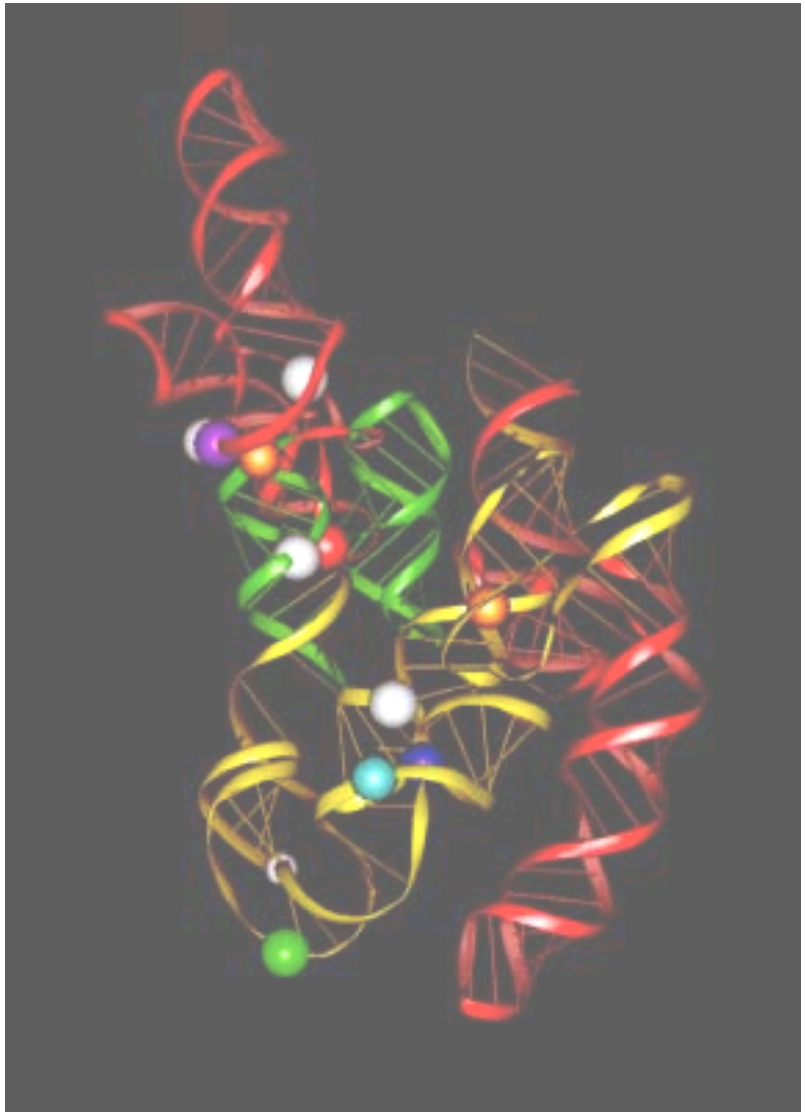
Η RNaseP και το M1 RNA

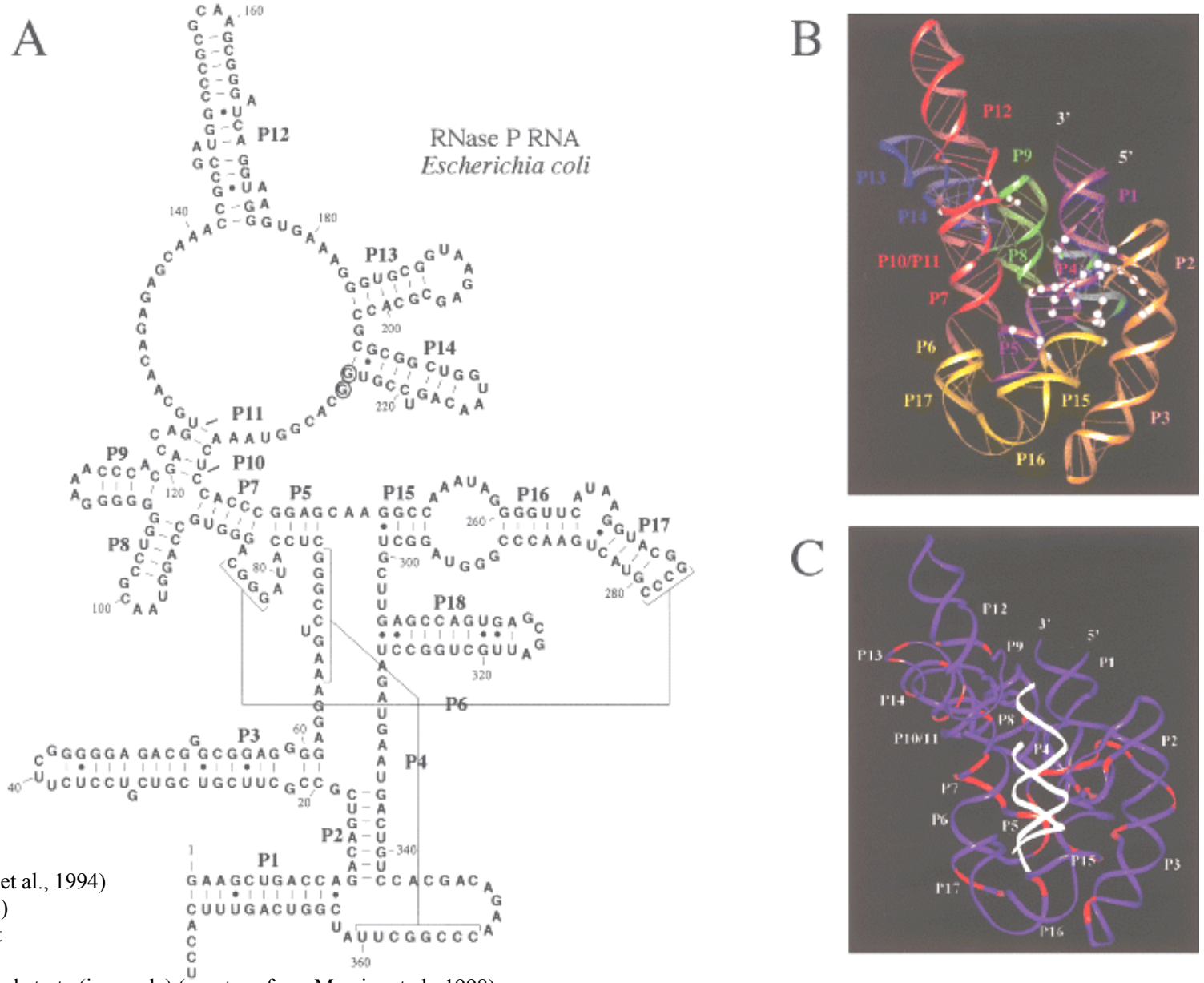


Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

Fig. 1. Biological function of M1 RNA from Escherichia coliRNase P. Pre-tRNA (left) is the natural target of RNase P, which recognizes the double-stranded RNA structure of the pre-tRNA T-stem and the 3′ CCA unpaired stretch, and cleaves the 5′ leader to release the mature tRNA.

M1 RNA και ptRNA





Liu and Altman S.
Nucleic Acids Research,
1996, 24: 2690–2696

Fig. 2.
Proposed secondary structure (**A**) (Haas et al., 1994) and three-dimensional structure (B and C) (Massire et al., 1998) of the RNA subunit (M1 RNA) of RNase P from E. coli.
B. A complex of M1 RNA and a ptRNA substrate (in purple) (courtesy from Massire et al., 1998).
C. A complex of M1 RNA (in purple and red) and a substrate consisting of a GS and a mRNA targeting sequence (in white). The red regions represent those sequences that were found in close proximity to a mRNA substrate by UV crosslinking and nuclease footprint analysis (Trang et al., 1999; Hsu et al., 2000; Kilani et al., 2000). C is generated with a SGI-O2 Workstation using the two current three-dimensional models of M1 RNA from Dr Eric Westhof's and Dr Norman Pace's laboratories (Chen et al., 1998; Massire et al., 1998). The mutated positions (G224G225) (circled) of selected variant R29 and R6 (Kilani et al., 2000) at the secondary structure of M1 RNA are also shown in (A).

Η RNaseP το M1 RNA και η τεχνολογία (E)GS

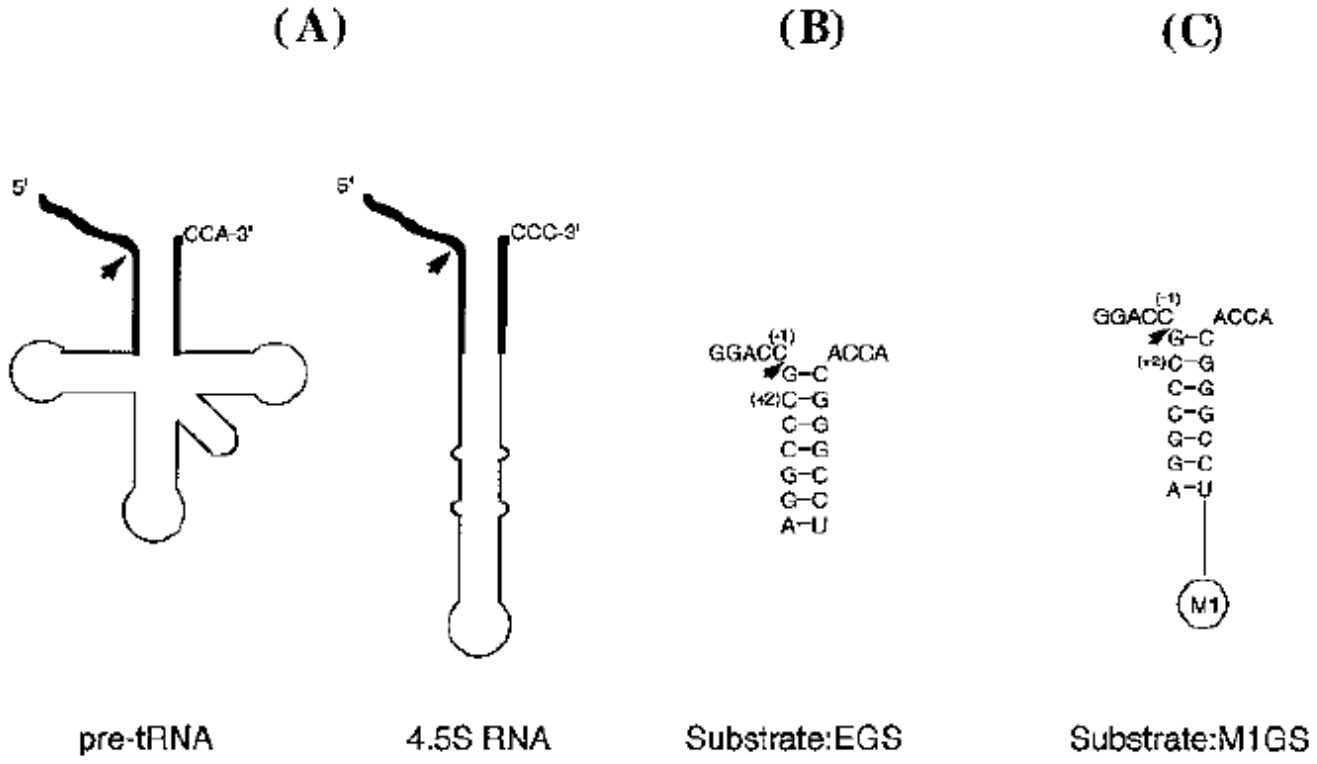
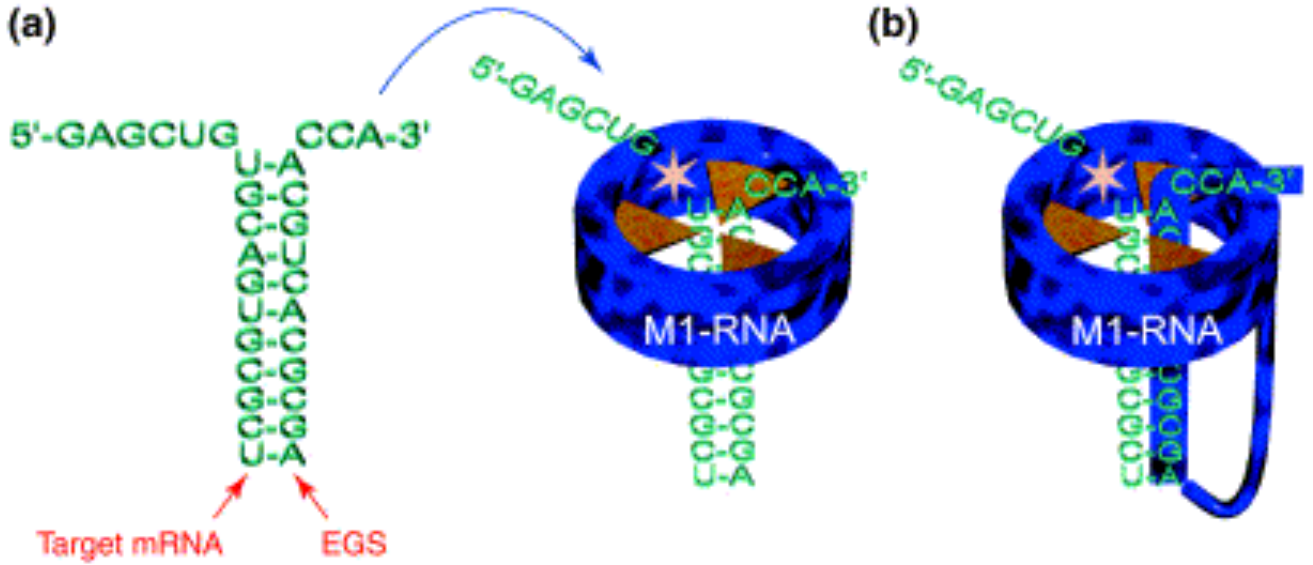


Figure 1. (**A**) Schematic representation of natural substrates (precursor tRNA and 4.5S RNA) for ribonuclease P and M1 RNA from*E.coli*. The structural components common to both precursor tRNA and 4.5S RNA are highlighted; they are equivalent to 7 bp of the acceptor stem of a tRNA. The site of cleavage by RNase P or M1 RNA is marked with a filled arrow. (**B**) Schematic diagram of a complex formed between a substrate (phe7) and an EGS (PHE). (**C**) Schematic diagram of an M1GS RNA construct (M1PHE) to which a target RNA (S) (phe7) has hybridized. In (B) and (C), the stem structure formed between the target RNA and either the EGS RNA or M1GS RNA is shown as 7 bp to mimic the structure of the tRNA acceptor stem; it can be varied from 3 to 19 bp as described in the text and in previous studies (6,11). The positions 3′ and 5′ adjacent to the scissile bond are designated as the +1 and –1 sites respectively. Accordingly, the position 3′ adjacent to the +1 site is called the +2 site. The sequence shown here are from the acceptor stem region of $tRNA^{Phe}$ (7).

Liu and Altman S. *Nucleic Acids Research*, 1996, 24: 2690–2696

Η RNaseP το M1 RNA και η τεχνολογία (E)GS



Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

Fig. 2. M1 RNA as a gene therapy tool.
(a) Use of a external guide sequence (EGS) to anneal with the
mRNA and to mimic the structure of M1 RNA natural target, by producing a 3′ CCA
unpaired tail, a doublestranded RNA region and
a 5′ leader-like region in the strand that has to be cut after interaction with the ribozyme.
The cleavage site in the target mRNA is between U7 and G8.
(b) Structure of a M1 RNA with an internal guide sequence (IGS)
covalently linked to its 3′ end, joined to its target substrate and providing all the necessary
structural requirements to cleave it at the desired point.

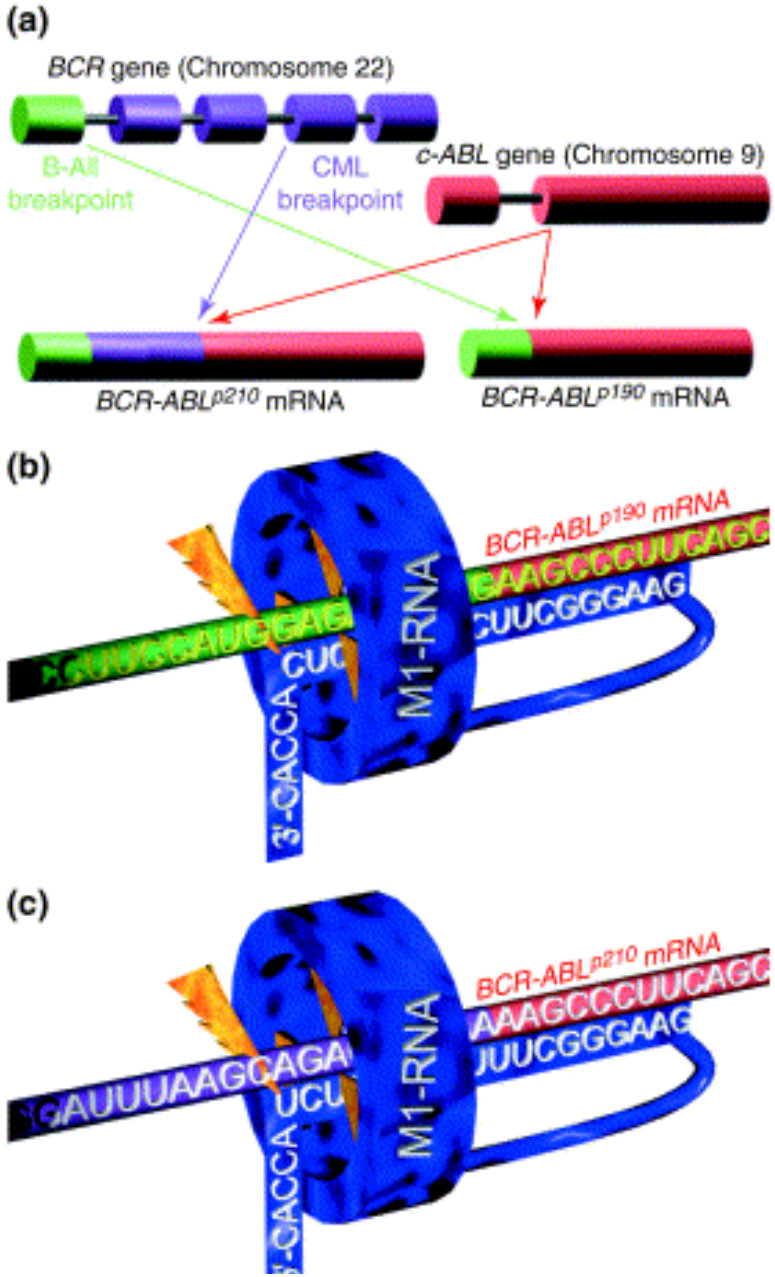
Η τεχνολογία IGS και καταστολή ογκογόνων χιμαιρικών mRNAs

Fig. 3BC. Destruction of chimeric oncogenes by M1 RNA with an internal guide sequence (M1-RNA-IGS).

(a) The products of the t(9;22)(q28;q11) translocation. Different oncogenes can arise depending on the precise breakpoints in the BCRgene. Two are shown:
BR-ABLp190, associated with B-acute lymphoblastic leukaemias (B-ALL) and
BCR-ABLp210, which produces chronic myelogenous leukaemia (CML).
They differ in the part coming from BCRgene but share the same region of ABLgene.

(b) The structure of the ***anti- BCR-ABLp190 M1-RNA-IGS***. This is bound to the BCR-ABLp190 oncogene mRNA at its fusion region by base-pairing with the IGS. The point in which the target is cut by the ribozyme is shown.

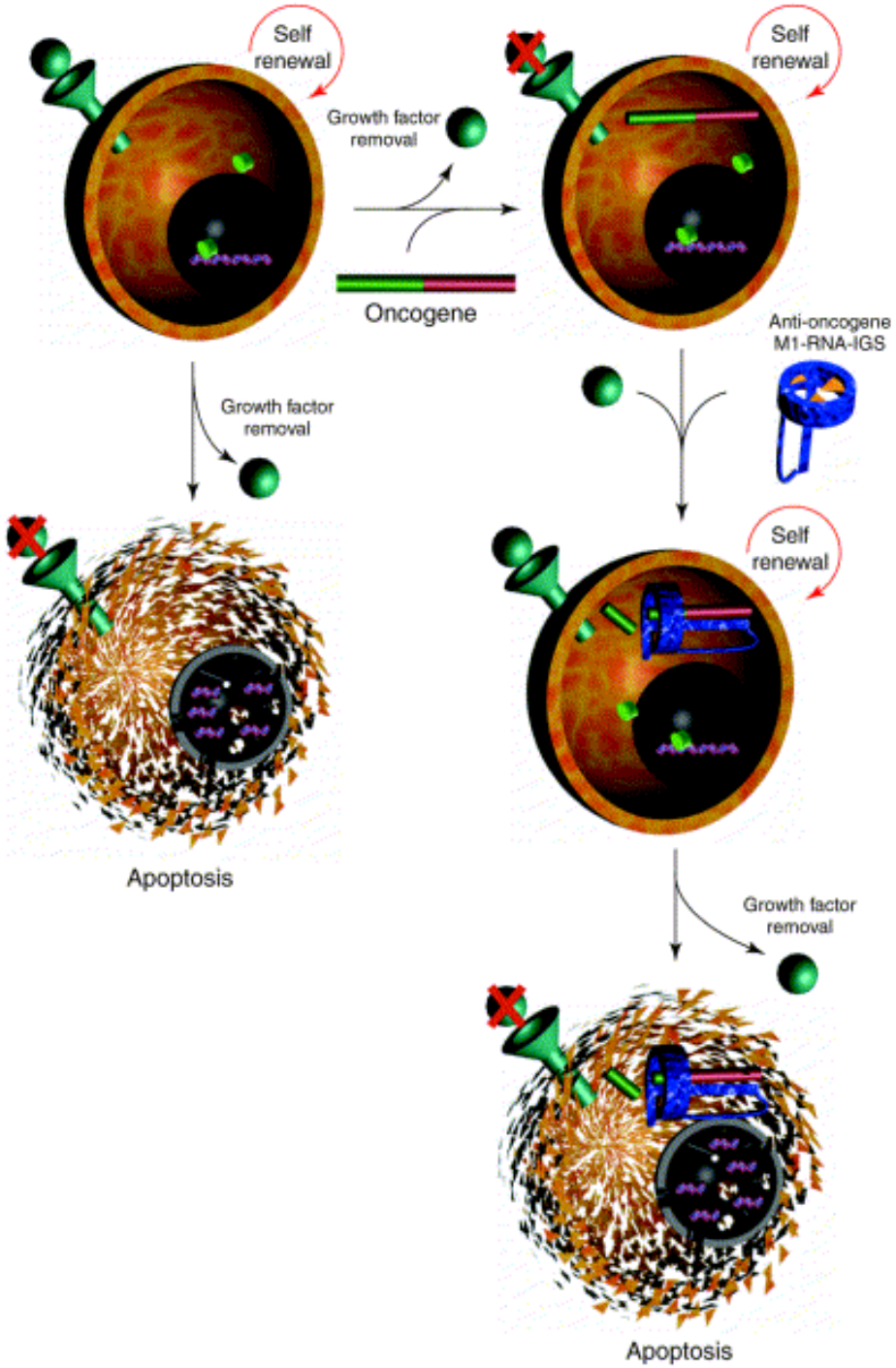
(c) The structure of the ***anti- BCR-ABLp210 M1-RNA-IGS***. This is bound to the BCR-ABLp210 oncogene mRNA at its fusion region by base-pairing with the IGS.
The point in which the target is cut by the ribozyme is shown.



Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001

Fig. 4. Cellular model system for an in vivo M1 RNA assay.

The cells require the presence of a cytokine in the medium to survive in culture;
in the absence of this factor, they die.
The presence of the oncogene makes the cells cytokine independent, because this oncogene provides the necessary survival signals.
When the oncogene is destroyed by a specific M1 RNA, the cells can be kept alive by adding the growth factor, but they again die upon cytokine withdrawal because the destroyed oncogene can no longer provide the survival signals.
If M1-RNA-treated cells die even in the presence of the cytokine, this means that the ribozyme is interacting with factors other than the oncogene and so it is not specific.



Cobaleda and Sachez-Garcia, *TRENDS Biotechnol*, 2001