Purine Analogs

6-Mercaptopurine (6-MP) was among the first purine analogs that demonstrated antineoplastic activity, and it remains useful in the treatment of acute leukemia. This derivative of hypoxanthine is a relatively insoluble, amphoteric compound that is stable, except in alkaline solutions. Metabolic activation primarily occurs by reaction with PRPP via hypoxanthine-guanine pyrophosphorylase (HGPRT) to form 6-MP riboside 5′-phosphate, more properly called thioinosine monophosphate (TIMP).

TIMP is believed to exert its major effect on purine nucleotide metabolism by inhibition of the first step in purine biosynthesis, the formation of 1-NH₂-ribose-5-PO₄, via a pseudofeedback inhibition in which TIMP mimics the regulatory action of adenine or guanine nucleoside monophosphates. An early precursor of purine biosynthesis, 5-aminoimidazol-4-carboxamide, which can be converted to the corresponding ribonucleotide, protects cells in culture against the inhibition of growth by 6-MP. This finding is consistent with the view that the primary action is limitation of an early step in de novo synthesis. TIMP also blocks the subsequent metabolism of inosinic acid, the initial purine nucleotide, to 2'-deoxyadenosine 5′-phosphate.
adenylic acid by inhibiting adenosylsuccinate synthase. Similarly, synthesis of guanine nucleotides is reduced by inhibition of the oxidation of inosinic acid to xanthyllic acid. TIMP is not incorporated into nucleic acids as such, but minor amounts are converted to thioguanylic acid, which is incorporated into both RNA and DNA. It has not been established, however, that this incorporation is significant to the toxic or antineoplastic actions of 6-MP. TIMP was recently shown to be a potent inhibitor of DNA exonuclease, which could excise ara-CMP from terminal DNA. This may partly explain the synergistic interaction of 6-MP and ara-C. A summary of 6-MP metabolism is presented in.

Metabolic activation and targets of thiopurines. 6-MP = 6-mercaptopurine; PRPP = pyrophosphorylribose-5-PO₄.

6-Mercaptopurine is generally administered orally (90 mg/m²) for several weeks. Absorption is variable, incomplete, and associated with a half-life of 20 to 45 min in plasma, where it is minimally bound to serum proteins. The rapid turnover largely results from oxidation by xanthine oxidase, which converts it to inactive thiouric acid, the primary urinary excretion product. In patients who are receiving allopurinol to control uricemia, the dose of 6-MP must be reduced by approximately 75% because drug catabolism is sharply reduced with the attendant risks of toxicity. No selective advantage in tumor therapy is achieved by this combination. Another metabolite, the 5-methyl derivative of 6-MP, is found in cells as methyl mercaptopurine ribonucleotide, where it inhibits purine metabolism; it is excreted in urine as methyl mercaptopurine riboside.

The dose-limiting toxicity of 6-MP is myelosuppression. It is slow in onset, 2 to 4 weeks, and rapidly reversed after the dose is either reduced or discontinued. All formed elements (thrombocytes, granulocytes, and erythrocytes) can be affected. Although gastrointestinal mucositis or stomatitis is minimal, approximately 25% of treated patients experience nausea, vomiting, and anorexia, and a small number display hepatotoxicity.

Therapeutic action depends on the formation of the nucleotide 6-MP ribonucleoside monophosphate. In experimental tumor systems, resistance commonly is associated with a decreased rate of activation to the nucleotide form, resulting from deletion or modification of HGPRT activity. Limited studies in humans, however, suggest that resistance is caused by increased activity of a 5'-phosphatase that limits the concentration and duration of intracellular 6-MP ribonucleotide.

6-Mercaptopurine is effective in combination with prednisone for inducing remission in children with acute lymphoblastic leukemia. Currently, it is a regular component of consolidation and maintenance therapy for this disease. It also is of some value in adult acute lymphocytic leukemias. It no longer is commonly used in myeloid leukemias of adults, but it does have modest activity in combination therapy.

Although many 6-MP derivatives have been synthesized and evaluated in model systems, only one, azathioprine, is available at present. This methyl-nitro-imadazole derivative of the thiol group on 6-MP is cleaved in vivo, presumably by thiol, to liberate 6-MP. It generally is not used in cancer therapy, but it remains an important element of immunosuppressant therapy for allograft transplantation and selected autoimmune states.

Thioguanine (see Figure 50-8) is the 6-thiol derivative of guanine corresponding to 6-MP and also depends on activation via HGPRT. Unlike 6-MP, however, di- and triphosphates of
thioguanine ribonucleotide are formed and incorporated into RNA. After conversion to thioguanine deoxynucleotide triphosphate, it can substitute for deoxyguanosine triphosphate (dGTP) in DNA polymerase reactions. This incorporation is thought to be the primary mechanism of cytotoxicity. Thioguanylate monophosphate is the predominant acid-soluble nucleotide, but it does not appear to exert the major effects on de novo purine synthesis that have been observed with 6-MP nor to deplete pools of normal purine nucleotides.

Like 6-MP, thioguanine, after deamination to thioxanthine by guanase, is readily catabolized to thiouric acid by xanthine oxidase. S-methylation also is observed, yielding S-methyl-thioguanine and thioxanthine. Dethiolation contributes to metabolism as well, as evidenced by the urinary excretion of $^{35}$S-SO$_4$ after administration of $^{35}$S-thioguanine. The primary use of thioguanine is in acute myeloid leukemia, where it may be combined with arabinosyl cytosine. Recent studies question its value in this disease, however. A summary of thioguanine metabolism is presented in Figure 50-9.

Allopurinol (4-hydroxypyrazolo-3,4-d-pyrimidine) is an important adjuvant to antineoplastic therapy (Figure 50-10). This agent and its primary metabolite, oxypurinol, are potent inhibitors of xanthine oxidase. As such, they limit the formation of uric acid from the degradation of purine nucleotides and nucleic acids. It is interesting to note that oxipurinol is formed by the target enzyme xanthine oxidase and is a potent inhibitor of this enzyme. In addition to this mechanism, allopurinol has been shown to inhibit purine nucleotide biosynthesis by feedback inhibition of the first reaction in the pathway and to deplete pyrophosphoryl ribose-5-PO$_4$, presumably by formation of the corresponding allopurinol and oxypurinol ribonucleotides. These nucleotides are inhibitors of orotidylate decarboxylation as well, and they result in the excretion of urinary orotate and orotidine. These actions may relate to the ability of allopurinol to selectively reduce the toxicity of 5-FU to some normal tissues, as described previously.

![Image](image-url)

(Pentostatin or deoxycoformycin,)

Deoxycoformycin (pentostatin) is a natural product first isolated in 1974 from the culture of Streptomyces antibioticus (see Figure 50-9). Its structure mimics the transitional-state form of adenosine in an adenosine deaminase-catalyzed reaction, and it is one of the most potent inhibitors of adenosine deaminase ($K_i$, $5 \times 10^{-10}$-$10^{-12}$ M depending on the source of the enzyme).
Because adenosine deaminase is not essential for cell growth in culture, this compound did not show antitumor activity in preclinical screenings.

The initial clinical development of deoxycoformycin centered on its activity as an adenosine deaminase inhibitor for the potentiation of adenosine arabinoside, which also was deaminated by adenosine deaminase to yield less toxic compounds. During early Phase I studies, the profound lymphotoxic effect of deoxycoformycin was noted. Other studies described a congenital syndrome of severe combined immunodeficiency associated with low or undetectable levels of adenosine deaminase in lymphocytes, and these results suggested the importance of adenosine deaminase in lymphocyte function, leading to intensive development of deoxycoformycin as a single agent for the treatment of lymphoproliferative diseases.

The most responsive tumor identified is hairy cell leukemia, in which durable remissions are achieved in over 90% of patients with a relatively brief course of treatment. Other responsive lymphoid diseases include chronic lymphocytic leukemia and prolymphocytic leukemia, mycosis fungoides, and acute T-cell leukemia/lymphoma. Considerable variation exists in the susceptibility of patients to deoxycoformycin toxicity. This includes immunosuppression, CNS disturbances, impaired renal function, conjunctivitis, and muscle and joint pain. Impaired renal function and poor performance status place patients at high risk for toxicity, even with low dosages of this drug.

In the search for more effective compounds than adenine arabinoside (ara-A, vidarabine), which has limited clinical usefulness because of its rapid deamination by adenosine deaminase, 2-fluoroadenosine arabinoside (9-β-d-arabinofuranosyl-2-fluoradenine) was synthesized. It has been found to be relatively resistant to adenosine deaminase and has impressive antitumor activities in vivo as well as in cell culture. Its limited solubility and consequent difficulties in formulation led to the synthesis of a prodrug, the 5'-monophosphate of 2-F-ara-A (Fludara IV).
Fludara IV entered clinical trials in 1982, and it is one of the most active agents in the treatment of chronic lymphocytic leukemia. A high level of activity also has been observed in a variety of indolent lymphoproliferative neoplasms, including low-grade non-Hodgkin lymphoma, cutaneous T-cell lymphoma, macroglobulinemia, and hairy cell leukemia. The dose-limiting toxicities during Phase I trials were myelosuppression and leukopenia. Delayed onset of severe neurotoxicity also was noted with doses of 96 mg/m²/d for 5 to 7 days. Other toxicities noted during Phase I trials included somnolence, mild to moderate nausea and vomiting, and rare but reversible interstitial pneumonitis. Fludara IV is converted by phosphatases to 2-F-ara-A within several minutes of injection; it is not further catabolized in plasma.}

( تصوير بالا ) Cladribine

The rationale for the development of 2-chlorodeoxyadenosine (Cl-dAdo, cladribine) was that the death of lymphocytes in patients with adenosine deaminase deficiency was associated with the accumulation of deoxynucleotides. This deoxyadenosine analog was selected for its resistance to adenosine deaminase. Its specific action on lymphoid cells is attributed to the high level of deoxycytidine kinase and low 5′-nucleotidase activity in these cells. This compound is highly cytotoxic to a variety of cell lines in culture, and it has potent antileukemic activity in mice. Cladribine was shown to have potent and lasting effects in the treatment of low-grade B-cell neoplasms, such as chronic lymphocytic leukemia, non-Hodgkin lymphoma, and hairy cell leukemia. In addition, Cl-dAdo has demonstrated clinical activity against acute myeloid leukemia in children, including those with leukemic blast cells in the CNS and in T-cell lymphoproliferative disorders. The spectrum of clinical activity is similar to that of Fludara IV; however, a few patients who do not respond to Fludara IV were sensitive to Cl-dAdo. The major toxicity encountered is bone marrow suppression that is associated with severe infections. The degree of suppression relates to the rate of administration, cumulative dose, and tumor burden at the start of therapy.
New studies demonstrated the activity of pentostatin, fludarabine monophosphate, and 2-chlorodeoxyadenosine (2-CDA) in relapsed patients. The structure of each of these molecules, as well as that of other purine analogues, is illustrated in Figure 4.

Figure 4. Structure of purine analogues used in the treatment of CLL.

Two early studies demonstrated that fludarabine had activity in salvage treatment. The largest study was from MD Anderson Cancer Center, which reported a CR rate of 15% and an OR rate of 44%. This led to the definition of a response group called nodular partial remission, in which patients demonstrated complete remission except on bone marrow biopsy, where nodules persisted.

A number of studies have demonstrated a response rate of approximately 50% for fludarabine as salvage therapy. However, no schedule has shown to be superior to the traditional regimen of 25 mg/m² per day for 5 days. Cladribine has demonstrated a similar level of activity compared with fludarabine, but with a higher incidence of thrombocytopenia. By contrast, in this setting, low response rates are noted with pentostatin. Of note, all 3 drugs are now being combined with alkylating agents in clinical trials. Fludarabine has been predominantly investigated in the United States, whereas cladribine is being widely investigated in Europe.

In previously untreated patients, fludarabine has a high OR rate of 80%, with 30% of patients achieving CR and a remission duration of 2.5 to 3 years, leading researchers to note that the quality of response is associated with remission duration. Cladribine has a similarly high OR rate in this population, but is associated with a slightly lower CR rate and shorter remission durations. Overall, purine analogues are more established than any other class of drugs as the most active single agents in the management of both previously treated and untreated patients with CLL.

What is chronic lymphocytic leukemia?
Chronic lymphocytic leukemia (CLL) is a type of cancer that starts from white blood cells (called lymphocytes) in the bone marrow. It then invades the blood. Leukemia cells tend to build up over time, and many people don’t have any symptoms for at least a few years. In time, it can also invade other parts of the body, including the lymph nodes, liver, and spleen. Compared with other types of leukemia, CLL usually grows slowly.

منابع تحقيق:

/http://www.ncbi.nlm.nih.gov/books/NBK12627
(Holland-Frei Cancer Medicine. 6th edition)
www.medscape.com