Chapter 7. The 6MP story 220720aa3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 7

The 6-mercaptopurine (6MP) story.

When I arrived at NIH in 1957 and was assigned to NCI's childhood leukemia ward -- for historical record, it was on the 2nd floor (2East) on the South side of the Clinical Center -- I helped to care for very sick children with acute leukemia. Using methotrexate or 6-mercaptopurine (6MP), we sometimes could reverse blast crisis, a late event in the disease that otherwise would be rapidly fatal. Although neither drug extended survival very much, both had a role in developing drug combinations that eventually led to lasting cures of the disease. The methotrexate story was related in Chapter 5. This chapter focusses on 6MP and its relative 6-thioguanine, how they were discovered, their mechanisms of action, and clinical application.

The 6MP story began in 1949, perhaps a few years earlier than the 5-fluororuracil story related in Chapter 6. It began with the work of Gertrude Elion and George Hitchings (Figure 7.1), who shared a Nobel Prize in 1988. In 1951, they published their investigation of 100 compounds related to purines (adenine and guanine) (Figure 7.2). They studied the effects of the compounds in inhibiting or stimulating the growth *Lactobacillus casei* bacteria. An example of one of their early experiments is shown in Figure 7.3. Their major and most enduring finding was that their most potent inhibitory compound was a purine with a sulfur atom added at the 6-position (6-mercaptopurine, 6MP) (Figure 7.2); the inhibition was reversed by purines such as adenine (Elion and Hitchings, 1950; Elion et al., 1951). (Similar effects were produced by the earlier known, but less potent, 2,6-diaminopurine (Figure 7.3).)

They went on to test the effect of 6MP on cancers in mice and were impressed that the drug inhibited the S180 sarcoma tumor and even cured a few of the animals (Clarke et al., 1953). Some of their other purine compounds had inhibited the growth of more sensitive tumors but had little effect on S180.

In those early days (prior FDA approval had not yet been established), the progress from a new finding in the laboratory to a clinical test was remarkably rapid. Thus within two years after 6MP's inhibition of bacteria was noted in 1951 and the same year as the first report of inhibition of tumors in animals in 1953, a test in humans was also published -- by investigators led by Joseph H. Burchenal in coordination with Hitchings and Elion (Burchenal et al., 1953). They began clinical tests after finding that the drug inhibited the growth of many different transplanted tumors in animals and after carrying out toxicity tests in mice, rats, cats, and dogs to determine a safe starting dose in patients. It was a concerted effort by at least 10 well-established clinicians and researchers from the Sloan-Kettering Institute and Cornell University Medical College in New York. Those early clinical tests were considered justified by the desperate need of the patients, including children who were dying of acute leukemia.

The clinical trials began in April 1952 and included 107 patients with various advanced cancers who had been recruited to the study by March 1953 (Burchenal et al., 1953). The study included 45 children and 18 adults with acute leukemia. The staff carefully monitored the patients' blood counts, bone marrow cellularity, as well as kidney and liver functions. The children tolerated the treatment relatively well with only rare serious toxicity. Of the 45 children with acute leukemia, 15 had good remissions lasting a few weeks to a few months. As a successful clinical trial carried out so soon after the discovery of a new drug, it was a remarkable achievement and launched 6MP as a promising new anticancer drug candidate. Further studies soon confirmed that the drug increased the survival time of some of the acute leukemia children (Burchenal et al., 1954). Those early results were the foundation for the studies in which I participated in 1957 on the childhood leukemia ward at the National Cancer Institute.

The 6-thioguanine story.

In 1958, Donald Clarke and Chester Stock together with Elion and Hitchings went on to test 102 close structural relatives of 6MP for their ability to inhibit the growth of the S180 tumor in mice. All of the compounds were less effective than 6MP, except for 6-thioguanine which was about as effective as 6MP (Clarke et al., 1958). The only difference in their chemical structures was that that 6MP lacked the amino (NH2) of the guanine part of the molecule (Figure 7.3). Moreover, the two drugs were cross-resistant: a tumor that was resistant to one was also resistant to the other. Jack D. Davidson, then at Columbia University, had shown that both drugs inhibited the synthesis of DNA in tumors (Davidson and Freeman, 1955). From those lines of evidence, Clarke and coworkers suspected (correctly) that enzymes in the tissues converted one of the drugs to the other.

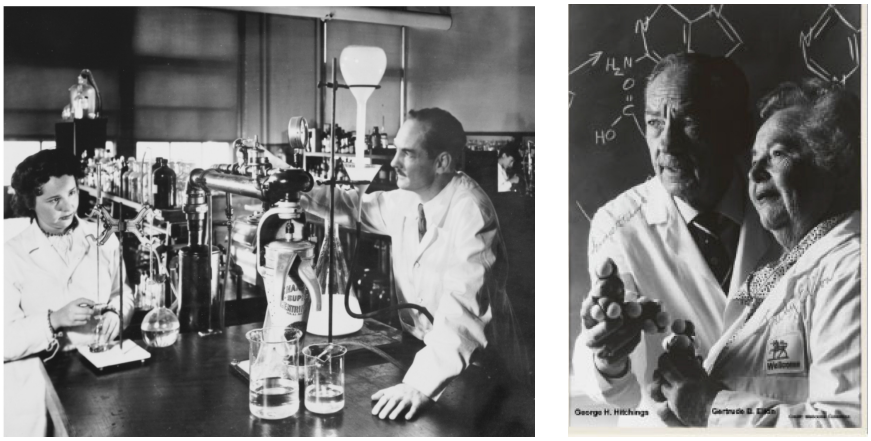

Figure 7.1. Gertrude B. Elion (1918-1999) and George H. Hitchings (1905-1998) before and after their Nobel Prize in Physiology or Medicine in 1988.

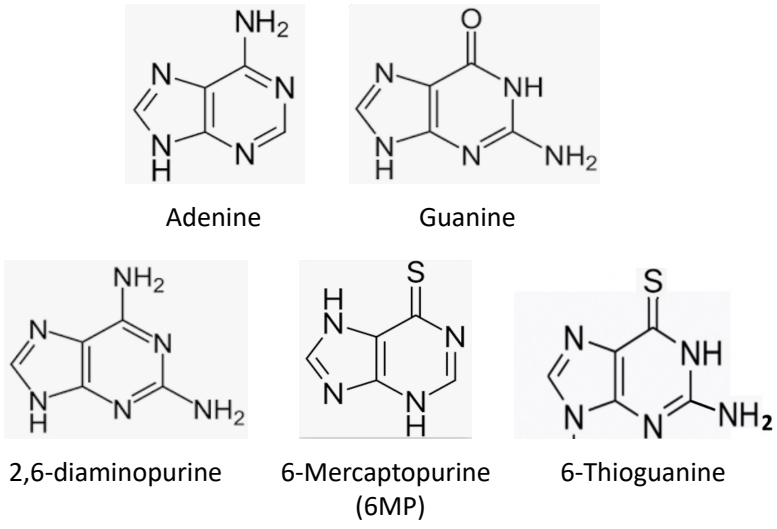

Figure 7.2. Among the first variants of adenine or guanine found by Gertrude Elion and George Hitchings to inhibit nucleic acid function was 2,6-diaminopurine. The most potent were 6-mercaptopurine (6MP) and 6-thioguanine. In cells, 6MP is converted to 6-thioguanine, which was responsible for most of the therapeutic actions of 6MP.

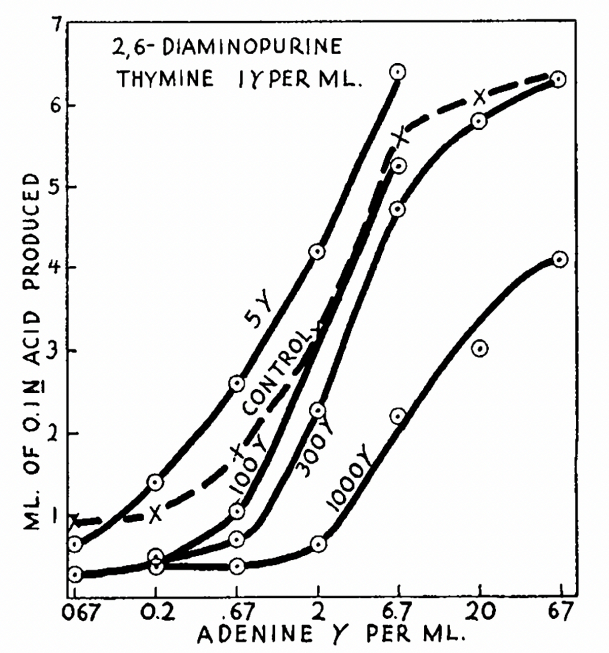

Figure 7.3. An early experiment by Elion and Hitching in 1950 showing the inhibition by 2,6-diaminopurine of the growth of *Lactobacillus casei* and its reversal by adenine (Elion and Hitchings, 1950). The bacteria were unable to grow in the absence of adenine. When adenine concentration was low, 2,6-diaminopurine inhibited growth (lower 3 curves), while higher adenine concentrations restored the ability to grow. The vertical axis was a measure of growth. The horizontal axis showed the concentration of adenine. The inference was that 2,6-diaminopurine competed with adenine as a requirement for growth.

The crucial role of 6-mercaptopurine (6MP) for continuation (maintenance) therapy needed for cure of acute lymphocytic leukemia (ALL).

The cure of acute lymphatic leukemia in children had two parts. First, the malignant cells had to be decimated using drugs, such as vincristine and dexamethasone, to induce an initial remission of the clinical disease. To get a lasting cure, however, this had to be followed by continuation therapy for 2 or 3 years or longer with 6MP plus methotrexate; no better drugs for this purpose had been found (Schmiegelow et al., 2014). That was a bit of a surprise, because 6MP was not very good for the first (induction) phase of the therapy, and the addition of the drugs that were best for the induction phase reduced the effectiveness or increase the toxicity of the continuation therapy.

In an editorial reflecting on this surprising circumstance, Barton A. Kamen in 2009 invoked a definition of "serendipity" by Horace Walpole in 1754 as "sagacity of being able to link together apparently innocuous facts to come to a valuable conclusion" (Kamen, 2009). Kamen's commentary addressed a report of a clinical trial by Kjeld Schmiegelow and coworkers that appeared in the same issue of the journal. His comments converged on

three points: (a) the success of clinical trials for children who had acute lymphocytic leukemia, (b) some characteristics of the leukemic stem cells, and (c) that tumor stem cells may be primarily on the de novo pathway for purine (guanine and adenine) synthesis, the de novo and salvage pathways being critically regulated and tied to cell differentiation and proliferation. Therefore, tumor stem cells, which must be suppressed during continuation therapy, would be particularly sensitive to inhibition of the de novo pathway by 6MP and methotrexate (Figure 7.4). Inhibition of the de novo pathway by methotrexate would enhance the utilization of 6MP via the purine salvage pathway and increase its cytotoxic action. 6MP and 6-thioguanine enter the purine salvage pathway by way of an enzyme that adds to them a phosphoribosyl group (Karran and Attard, 2008). Kamen argued that it was by Walpole's definition of serendipity that consideration of those three points led to the counter-intuitive use of 6MP plus methotrexate for the continuation therapy that was needed to cure acute lymphocytic leukemia.

In 2011, William E. Evans and coworkers at St. Jude Children's Research Hospital in Memphis, Tennessee reported that some children with acute lymphocytic leukemia (ALL) lacked MSH2, one of the proteins required for DNA mismatch repair (the subject of Chapter 25) (Diouf et al., 2011). The lack of MSH2 was not due to inadequate expression of its gene but rather was caused by overactivity of an enzyme that destroyed the MSH2 protein in the leukemia cells. The ALL children whose leukemic cells lacked MSH2 and therefore were deficient in DNA mismatch repair had a reduced cure rate: their 10-year survival was 78.7% compared to 97.5% ($P = 0.009$) for those who had higher MSH2 levels. A high mismatch repair activity therefore helped long-term survival.

That may seem counter-intuitive. The ability of the leukemia cells to repair damage to their DNA one might think should help the cells to survive, and that is usually the case. For mismatch repair, however, the opposite was the case (as we have seen for temozolomide in Chapter 2 and fluorouracil in Chapter 5). When 6-thioguanine was incorporated in DNA, it paired with C like a normal G would do. But there was an enzyme that added a methyl group to the sulfur atom of 6-thioguanine in DNA (step *18* in Figure 7.4). The resulting 6-methylthioguanine was recognized as a mismatch by the repair system. But the repair was futile: it usually left a new mismatch after each repair attempt, as will be explained in connection with step *19* in Figure 7.4.

How 6-mercaptopurine (6MP) and 6-thioguanine kill cancer cells.

Figure 7.4 shows the chemical steps and biological actions of 6-mercaptopurine (6MP) and 6–thioguanine, as well as methotrexate. In what follows, I explain the essential steps and processes as currently understood, using the number assigned to each step in the diagram. These steps and actions determine whether the drug-treated cell survives or dies.

To begin with, there are inactivation steps. The more drug that is inactivated in a given patient, obviously the less effect the drug could have. After 6MP is absorbed from the intestines, it is subject to inactivation in the liver, which converts 6MP to an inactive product, 6-thiouric acid [*1*]. Another enzymatic inactivation process adds a methyl group to the sulfur atom of 6MP or 6-thioguanine [*2*, *2a*]. Variants of that enzyme (thiopurine-

methyltransferase, TPMT, explained later in this Chapter) affect its activity and therefore the sensitivity of children with acute lymphatic leukemias to toxicity from 6MP treatments (Chan et al., 2019).

Both 6MP and 6-thioguanine can become incorporated as a thioguanine component in place of guanine in nucleic acids. The first step along the pathway for incorporation into DNA is the addition of a deoxyribose-phosphate group to 6MP [*3, 7*] or to 6-thioguanine [*6*]. 6MP and 6-thioguanine converge to the same DNA synthesis precursor, 6-thioguanine-deoxyribose-phosphate, to which two more phosphates are added to form the triphosphate [*8*]. The latter becomes incorporated as a thioguanine in place of guanine in DNA [*10*]. Once thioguanine is in the DNA structure, it inhibits DNA synthesis [*14*] and causes additional trouble, as we shall see.

Thioguanine incorporation into DNA however was recently found to be limited by an enzyme called NUDT15 that removes two phosphates from thioguanine-deoxyribose-triphosphate and sends it back to the monophosphate level [*9*] (Chan et al., 2019; Singh et al., 2017). The enzyme normally protects DNA from incorporation of 8-oxoguanine, a troublesome product of reactive oxygen species – it selectively removes phosphates from 8-oxoguanosine triphosphates. The *NUDT15* gene was found to have single-nucleotide polymorphisms (SNIPs). The particular *NUDT15* SNIP in a patient's genome affected how sensitive the patient was to bone marrow suppression by 6MP. If a patient's *NUDT15* SNIP was such that activity of the enzyme was unusually low or absent, the 6MP dose would be reduced for safety – because low activity of reaction [*9*] would allow more 6-thioguanine (as well as more 8-oxoguanine) to enter the DNA.

After thioguanine was incorporated into DNA [*10*], an S-methyltransferase enzyme added methyl groups to the sulfur atom of 6MP or 6-thioguanine [*2, 2a*] and methyl groups to the DNA-incorporated thioguanines [*18*]. As already mentioned above, the resulting S-methyl-thioguanines are recognized by the DNA mismatch repair system (the topic of Chapter 25). The repair system removed a section of either the strand containing the S-methyl-thioguanine or a section containing the base mispaired with it. In either case however the repair process would be apt to produce a new mismatch. This cycling of futile repair attempts eventually causes the cell to give up and die [*19, 20*].

While all of that is going on, normal guanine is also being incorporated into DNA [*11, 12*]. However, normal guanine and 6-thioguanine compete for incorporation into DNA [*15*]. Thus, when guanosine-triphosphate is high, the DNA-incorporation of thioguanine would be relatively low. The amount of guanine nucleotides would depend on the rate that guanine is made available by de novo synthesis [*16*] and by recovery from degraded nucleic acids ("salvage pathway") [*17*]. The de novo pathway is inhibited by methyl-mercaptopurine-ribose-phosphate [*5*], which is produces by the S-methyltransferase that adds a methyl group to the sulfur [*4*]. De novo purine synthesis is also inhibited by methotrexate [*22*]. Both inhibitions reduce the production guanine nucleotides, which reduces competition with thioguanine and consequently allows increased incorporation of thioguanine into DNA [*15*]. Methotrexate enhances the action of 6MP both by inhibiting de novo purine synthesis and by inhibiting the destruction of 6MP by blocking its oxidation to

6-thiouric acid [*1*] (Larsen et al., 2021; Schmiegelow et al., 2014).

Although treatment of children who have acute lymphocytic leukemia, including long-term maintenance (or "continuation") therapy with 6MP plus methotrexate, cured most of them, many eventually relapsed (Figure 7.5). Relapse was thought perhaps related to low activity of a necessary enzyme in the metabolic scheme shown in Figure 7.4. This possibility was investigated by testing whether relapsing children sometimes were heterozygous in a critical gene, in other words having one normal gene and an inactivating mutation in the other one. The net effect would be reduced activity of that gene.

The investigation pointed particularly to the gene for thiopurine-methyltransferase (TPMT), which is responsible for reactions *2, 2a, 4,* and *18* in Figure 7.4 (Schmiegelow et al., 2014). It would seem that deficiency in reactions *2* and *2a* would increase 6MP and 6-thioguanine actions, whereas deficiency in reactions *4* and *18* would have the opposite effect (assuming that all those inhibitions involve exactly the same gene). Heterozygous mutation that inactivated only one copy of the gene was found in about 10% of patients, although 1 in 300 had inactivating mutations in both copies of the gene that put them at risk of life-threatening toxicity if treated with customary 6MP doses. Patients with a heterozygous *TPMT* mutation had higher 6-thioguanine levels in their cells, more toxicity but higher cure rates -- but they were thought to have higher risk of developing new cancers in coming years. In 2018, it was recommended that the genotypes of both *TPMT* and NUDT15 be evaluated in deciding on the dose of 6MP to be used.

The *TPMT* gene, which is located on chromosome 6, had about 40 variant forms resulting from single-nucleotide polymorphisms (SNPs), of which four accounted for 80-95% of the TPMT variant cases found in patients; each of these four variants had an amino acid change from the normal gene and encoded a protein with relatively low enzymatic activity (Franca et al., 2021). The normal function of TMPT remained unknown; the absence of the gene seemed not to cause disease. However, the TMPT protein was reported to methylate the selenium atom that replaces sulfur in selenocysteine, a rare encoded form of the normal amino acid cysteine (selenium is below sulfur in the periodic table and is highly nucleophilic).

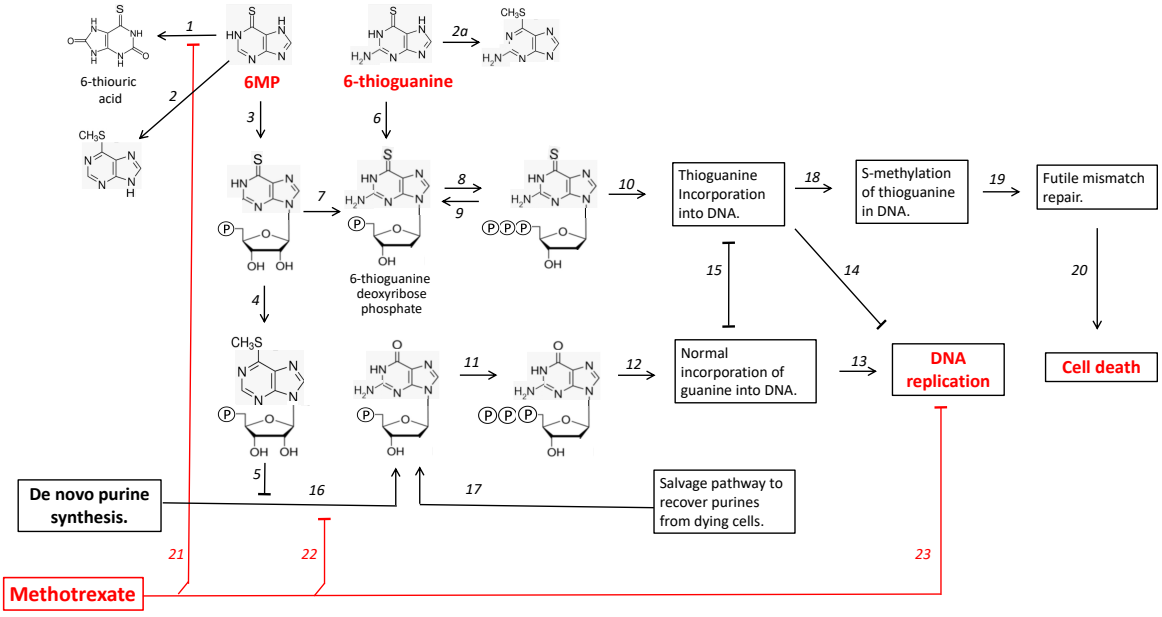

Figure 7.4. The chemical changes and biological effect of 6-mercaptopurine (5MP), 6-thioguanine, and methotrexate in the cancer cell. See the preceding text for explanation of the steps and processes shown in the diagram.

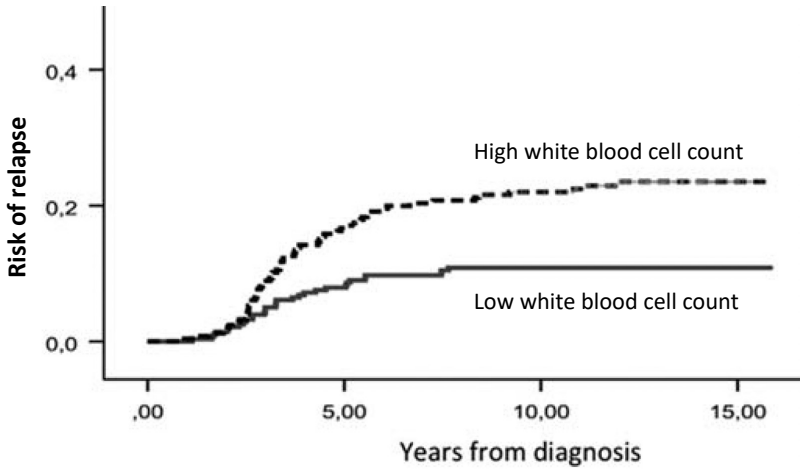

Figure 7.5. Relapse rates of children with acute lymphocytic leukemia after long-term maintenance therapy with 6-mercaptopurine (6MP) and methotrexate. If the effect of the therapy was strong enough to suppress the white blood cell count to a relatively low level (lower curve), then relapse rate was relative low (10.9%), compared with patients whose treatment had a lower effectiveness as indicated by a higher white blood cell count, who had a higher relapse rate (upper curve, 23.5%; P<0.001) (Schmiegelow et al., 2014).

Final word

We saw (again, as in the case of 5-fluorouracil) how drugs with simple chemical structures, discovered on the basis of simple principles, entered metabolic pathways and caused multiple complex perturbations of those pathways, leading to sometimes surprising therapeutic and toxic actions. Also remarkable was how quickly those early discoveries, crucial to the eventual cures of acute leukemias and lymphomas, moved from discovery to treatment of patients.

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