Drs. Takimoto and Allegra present a comprehensive overview of the development of antifolates over the past decade and a half. The antifolates are antimetabolite antineoplastic agents that are structurally and chemically similar to naturally occurring folates required for the synthesis of purines and pyrimidines. These drugs interfere with DNA synthesis by inhibiting key enzymes. They are transported across the plasma membrane and converted intracellularly to cytotoxic species, which must compete with endogenous substrates for target enzyme binding.

The cytotoxicity of antifolates is both dose- and time-dependent, with the duration of exposure apparently becoming more important than dose once the threshold concentration for cytotoxicity has been exceeded. A concern for the rapid clinical development of novel antifolates should be optimization of administration schedule: How should the schedule be selected (based on preclinical data) to optimize the therapeutic index in the clinic? For the lipophilic antifolates (trimetrexate [Neutrexin], piritrexim) this question has not been answered, despite a profusion of schedules in phase I trials. The protracted intracellular half-lives of lometrexol and Tomudex may render this issue less important in their development.

As a consequence of their mechanism of action, the clinical toxicity of antifolates usually results from damage to rapidly dividing normal tissues; thus, stomatitis, diarrhea, and bone marrow suppression are commonly seen. However, toxicity may also occur in organs with nonproliferating cells. Of particular interest is the liver. As the authors note, chronic low-dose methotrexate may produce liver damage. Serum transaminase elevation is also observed with some of the newer antifolates, especially CB3717, Tomudex, and AG331. Presumably, these effects are independent of DNA synthesis inhibition (although it is possible that cytotoxicity could result from inhibition of repair synthesis). Further work to understand this toxicity may allow its elimination by appropriate molecular design.

A Striking Example
In fact, the antifolates represent the most striking example of the influence of careful pharmacologic study on the optimization of targeted molecules. The biochemical pharmacology of methotrexate, the most widely used antifolate, has been elucidated, and important insights into the mechanisms of drug action and cellular resistance have emerged. The authors provide a detailed description of this biochemical pharmacology and emphasize the importance of the tetrahydrofolates as essential cofactors for the production of several fundamental precursors of DNA synthesis, including, but not limited to, thymidine.

These studies have led to the design of several new antifolate compounds, such as trimetrexate, edatrexate, piritrexim, Tomudex, and lometrexol. The authors detail background information on the proposed mechanisms of action, toxicities, and results of clinical phase I trials of each of these drugs. While each new agent appears to represent an improvement over methotrexate, each has a potential problem area. For example, the lipid-soluble quinazoline antifolate trimetrexate can enter the cells by passive diffusion and is therefore active in methotrexate-resistant tumor cells that lack the reduced folate membrane transport system. However, unlike methotrexate, trimetrexate does not undergo polyglutamation and is rapidly effluxed. It is also a potential substrate for the P-glycoprotein multidrug resistant (MDR) efflux pump; thus, prolonged exposure to trimetrexate is required to maintain cytotoxic intracellular concentrations. In clinical testing, trimetrexate has not
emerged as superior to the parent compound in the treatment of solid tumors.

**Most Promising New Antifolate**

The most promising of the agents described appears to be Tomudex, also called D1694. This compound grew out of the work of Jackman, Harrap, and colleagues at the Institute for Cancer Research in the United Kingdom, who sought an antifolate that had specific inhibitory activity against thymidylate synthase (TS). Screening led to the selection of a propargyl derivative, CB3717, for clinical development. Although potent and specific for TS, this agent proved nephrotoxic in initial clinical studies.

Tomudex represented a more soluble analog that was well tolerated in phase I studies. Myelosuppression, fatigue, mucositis, and mild hepatic enzyme elevation were the major toxicities. Takimoto and Allegra describe the initial phase II data with this compound in solid tumors. Phase III studies in colorectal cancer are now complete, and the results are awaited with interest. It seems likely that Tomudex will be the first specific TS inhibitor for use in this disease. Its schedule of a single dose every 3 weeks is also likely to compare favorably with fluorouracil with respect to cost and quality of life.

While the review by Takimoto and Allegra provides insights into the development of these new antifolates, it also raises questions about how these drugs should be studied or used in the future. In this regard, current clinical research with Tomudex is also relevant. The work of Johnston, Allegra, and colleagues has shown the predictive value of measures of TS expression (messenger RNA or protein) in determining the likelihood of response to fluorouracil. In phase II trials conducted by the Eastern Cooperative Oncology Group, the activity of Tomudex in colorectal cancer will be related to TS expression as a possible means of selecting patients for treatment.

**Potential and Limitations of Antifolate Design**

This overview is to be commended for pointing out the potential of antifolate design, as well as its possible limitations. Particularly interesting is the development of novel TS inhibitors, such as AG331 and AG337, using x-ray crystallography to guide drug design. Based on the knowledge of the three-dimensional structure of the active site of TS, crystal structures can be used to analyze the binding of ligands to that site. The molecules so designed have proved to be potent inhibitors of TS with significant potential antitumor activity. It may be also advantageous to design molecules targeted to the altered TS found in certain resistant tumors.

Other specific mechanisms of resistance have been targeted, including transport (trimetrexate, piritrexim), gene amplification (Tomudex, lometrexol), and polyglutamylation defects (trimetrexate, Tomudex analogs in development). Firm conclusions regarding the success of these strategies will await the results of this broad drug development program.

As noted by the authors, research involving the antifolates is crucial not only to oncologists but also to other medical specialists. *Pneumocystis carinii* pneumonia is a frequent cause of death in individuals with AIDS. The absence of a folate transport system in parasites provides an opportunity to affect the protozoan with trimetrexate, with differential rescue of the host tissue with leucovorin (5-formyltetrahydrofolate). The development of more potent dihydrofolate reductase inhibitors as antiprotozoan agents, utilized concurrently with leucovorin rescue, could further impact on this disease.

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