THYMIDYLATE SYNTHASE: A CRITICAL TARGET IN CANCER THERAPY?

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1. ABSTRACT

For the last four decades, synthesis and testing of potentially active drugs (e.g., antimetabolites) have focused on structural modification of existing metabolites as precursors of DNA and RNA synthesis. In recent years, the focus has shifted to synthesis of target-specific agents. Thus, the current emphasis of drug development is directed at inhibiting specific target(s) expressed preferentially, if not exclusively, in tumor tissues, with the ultimate goal of improving the therapeutic efficacy and selectivity of these new agents. Preclinically, proof-of-principle studies were carried out in tumors with specific expression of the intended target. With the hope of translating preclinical findings to the design of implementation of clinical trials.

Thymidylate synthase (TS) continues to be a critical target for 5-fluorouracil (5-FU) and its prodrugs, UFT/LV (Orzel), capecitabine (Xeloda), and S-1, primarily because this enzyme is essential for the synthesis of 2'-deoxythymidine-5'-monophosphate, a precursor for DNA synthesis. While fluoropyrimidine antimetabolites have other sites of action, antifolates ZD1694 (ralitrexed, Tomudex®) and AG337 (Thymitag™) are more specific and potent TS inhibitors. Thus, it is hoped that pronounced and sustained inhibition of this enzyme could result in downstream regulation of molecular markers associated with sensitivity and resistance to these agents. It is also critical to recognize that the degree and duration of inhibition of the target enzyme may depend on the expression level of the target enzyme, thymidylate synthase.

Correlative studies in preclinical and clinical systems demonstrated a close relationship between the enzyme level (mRNA and protein) and response to therapy of colorectal cancer patients treated with fluoropyrimidine or Tomudex®. However, significant overlap was demonstrated between responders and non-responders. These data are consistent with the hypothesis that prediction of response to anticancer drugs is multifactorial, and TS is one target.

Clinically, although overall response of colorectal cancer patients to a variety of TS inhibitors is similar, toxicity profiles are different. The availability of the 5-FU prodrugs offers the possibility of greater therapeutic selectivity based on the demonstration that thymidine phosphohydrolase, the activating enzyme for 5-FU, is expressed at a higher level in tumor tissue compared with normal tissue counterparts. It is likely that successful application of TS inhibitors will not only be based on measurement of the TS level in tumors vs. normal tissues, but on the delineation of the consequences of this inhibition on molecular markers associated with cellular proliferation, apoptosis and cell cycle regulation.

2. INTRODUCTION

For more than four decades, thymidylate synthase (TS) has been recognized as a critical enzyme for de novo synthesis of thymidylates for DNA synthesis and as a target for cancer chemotherapy, in particular, pyrimidine and antifolate antimetabolites (Figure 1).

Thymidylate pools as precursors for DNA synthesis are derived through two major metabolic pathways: the de novo pathway by the conversion of deoxyuridine monophosphate to thymidine monophosphate via thymidylate synthase; and the salvage pathways by the conversion of thymidine to thymidine monophosphate via thymidine kinase. The relative contribution of these pathways in the generation of thymidylate pools has not been fully documented and remains controversial, and is likely to be tissue dependent.

Although it has been long accepted that systematic tumoral tissue thymidine pools play a critical
Although considerable data have been published documenting the correlation between the level of TS expression and response to 5-FU/LV, Xeloda and Tomudex®, these results, however, do not establish a cause-effect relationship. Downstream molecular alterations from pronounced and sustained inhibition of tumoral TS are likely to be critical determinants of response to TS inhibitors. Thus, obtaining pronounced and sustained TS inhibition per se may not be sufficient to predict the outcome of therapeutic efficacy.

Identification of predictive markers of response by TS inhibition requires the evaluation of multiple relevant factors, including levels of TS expression. In this review, the status of TS inhibitors will be briefly reviewed. TS inhibitors will be presented and discussed, including those identified as direct inhibitors (antifolates, Figure 3) and indirect inhibitors (5-FU and its prodrugs Xeloda and Tegafur/Uracyl/LV, Figure 4). The therapeutic selectivity and efficacy of these agents will be presented, as will the future prospects of these agents administered alone and in combination with other drugs will also be presented.

3. 5-FLUOROURACIL/LEUCOVORIN (5-FU/LV)

Results of preclinical in vivo and in vitro studies have demonstrated that augmentation of the therapeutic efficacy of 5-FU by LV is LV dose and schedule dependent. Further, augmentation of drug response was clearly associated with the degree and duration of TS inhibition through stabilization of the ternary complex formation between 5-fluorodeoxyuridine monophosphate (FdUMP), the active metabolite of 5-FU, the target enzyme TS, and 5, 10 methylenetetrahydrofolate. Preclinical results provided the basis for the clinical development of 5-FU/LV and verification of strong association between the TS level and response to 5-FU/LV therapy. Clinical trials also confirmed that LV can increase the response to bolus 5-FU with no significant impact on overall survival in patients with advanced colorectal cancer. In patients with advanced colorectal cancer, the overall response rate to protracted continuous intravenous administration of 5-FU was similar to that achieved with daily (Mayo Clinic) or weekly (Roswell Park) schedules of 5-FU/LV. Thus, bolus 5-FU/LV appears to mimic the response rate achieved with protracted infusion of 5-FU, but was associated with more dose limiting toxicity.

3.1. Metabolic Activation

Figure 2 outlines the key steps involved in metabolism of 5-FU and its prodrugs by three main pathways: (1) Activation to 5-FU by thymidine phosphorylase (TPase) and to deoxythymidine monophosphate (FdUMP), the active metabolite of 5-FU, the target enzyme TS, and 5, 10 methylenetetrahydrofolate. Preclinical results provided the basis for the clinical development of 5-FU/LV and verification of strong association between the TS level and response to 5-FU/LV therapy. Clinical trials also confirmed that LV can increase the response to bolus 5-FU with no significant impact on overall survival in patients with advanced colorectal cancer. In patients with advanced colorectal cancer, the overall response rate to protracted continuous intravenous administration of 5-FU was similar to that achieved with daily (Mayo Clinic) or weekly (Roswell Park) schedules of 5-FU/LV. Thus, bolus 5-FU/LV appears to mimic the response rate achieved with protracted infusion of 5-FU, but was associated with more dose limiting toxicity.

3.1. Metabolic Activation

Figure 2 outlines the key steps involved in metabolism of 5-FU and its prodrugs by three main pathways: (1) Activation to 5-FU by thymidine phosphorylase (TPase) and to deoxythymidine monophosphate (FdUMP) by thymidine kinase; (2) Activation of 5-FU to 5-fluorouracil triphosphate (FUTP) via several kinases and subsequent incorporation into cellular RNA; and (3) Deactivation of 5-FU initially via dihydropyrimidine dehydrogenase (DPD) and subsequently into various metabolites leading to CO₂. Thus, possible mechanisms of action are multifunctional involving incorporation of FUTP into cellular RNA, TS inhibition by FdUMP and degradation or inactivation of 5-FU by DPD.
Thymidylate synthase inhibitor

While it is likely that in the presence of LV, incorporation into RNA is likely associated with host toxicity, TS inhibition is associated with antitumor activity. Degradation of 5-FU by DPD is associated with resistance to 5-FU, due to rapid depletion of 5-FU available for metabolism into FdUMP and/or FUTP, and toxicity due to rapid accumulation of therapeutically inactive, but toxic, metabolites, 5-FU, such as FUH₂.

Several approaches have been utilized to modulate the metabolic pathways of 5-FU and its prodrugs with the hope of improving on the therapeutic efficacy and selectively. These include the use of uracil (U) and 5-chloro-2,4-dihydroxypridine (CDHP) and eny luracil (EU) as non-competitive inhibitors of DPD, and potassium oxonate (OX) as a non-competitive inhibitor of PRP transferase the enzyme responsible for conversion of FU into FdUMP.

Further, the reportedly high levels of (TPase) in tumor tissue provided the basis for the preclinical development of capecitabine (Xeloda) and Tegafur (FT). Although, TS is the intended target of fluoropyrimidines, these drugs affect other targets such as protein and RNA synthesis and function. The availability of antifolates as directly specific and potent TS inhibitors provided the opportunity to determine whether these drugs could provide greater therapeutic selectivity and efficacy. Clinical results, however, do not support this premise.

4. TEGAFUR/URACIL/LEUCOVORIN (UFT/LV)

Recognizing that therapy with 5-FU/LV has limited selectivity and efficacy, several 5-FU prodrugs have been developed with the ultimate hope of overcoming therapeutic limitations of 5-FU/LV. Based on several reports documenting the critical role of DPD in the catabolic pathway of 5-FU (Figure 2), the competitive inhibitor of this enzyme, U, was evaluated in combination with the 5-FU prodrug FT in the molecular ratio of 1:4 of FT to U. The addition of LV was based on the increased response rate of bolus 5-FU documented when combined with LV.

Two randomized clinical trials of intravenous 5-FU/LV vs. orally administered UFT/LV in previously untreated advanced colorectal cancer patients have demonstrated that UFT/LV was inferior in terms of response rate and survival. The UFT/LV regimen was better tolerated in terms of less mucositis, diarrhea and hematologic toxicities; however, based on these results, the United States Food and Drug Administration (FDA) did not approve this treatment modality for advanced colorectal cancer.

Although the FDA had reservations about UFT/LV efficacy, the fact remains that UFT is orally available, better tolerated than conversional 5-FU/LV (bolus) and, as a result, could impact on quality of life and hospitalization costs. In the opinion of the authors, this class of drugs deserves a different set of criteria for approval, as long as these drugs are as active as 5-FU administered i.v. but with less toxicity and can be taken on outpatient basis.

The rationale for the development of DPD inhibitors was based on the important role of DPD in the catabolism of 5-FU, resulting in a low level remaining to antabolic metabolism to active components. The disappointing clinical effectiveness of UFT/LV, however, is consistent with the original hypothesis. It is possible, however, that the 4:1 FT to U ratio may not be optimal. Pronounced inhibition of DPD by U may reduce the dose of 5-FU delivered to tumor that is sufficient to exert the desired effect, particularly in tumors with high TS expression. Since the DPD level in the normal liver is at least 100-fold higher than levels in tumors, the use of DPD inhibition that completely shuts down DPD in normal tissues may not be desirable. Thus, the desired DPD inhibitor should be to selectively produce prolonged inhibition of DPD in tumor tissues leaving some DPD in normal tissues to de-activate 5-FU.

5. TEGAFUR/5-CHLORO-2,4-DIHYDROXYPYRIDINE/POTASSIUM OXONATE (S-1)

In an effort to improve the therapeutic selectivity of 5-FU against colorectal cancer, S-1, a combination of 5-FU and two modulators, was recently developed by Taiho.
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Pharmaceuticals Co. S-1 is a combination of Tegafur (FT), 5-chloro-2,4-dihydroxyxpyridine and potassium oxonate in the molar ratio of 1.0:0.4:1.0, with the latter two components are inhibitors of DPD and phosphoribosylpyrophosphate transferase, respectively. The therapeutic selectivity and efficacy of S-1 (oral) was compared with FT (oral) and 5-FU (i.v. infusion) in rats bearing advanced colorectal cancer by using clinically relevant schedules and the maximum tolerated doses (MTDs) of S-1, FT, and 5-FU. The therapeutic index of S-1 was 4- to 5-fold higher than that of either FT or FU. S-1 achieved 100% complete tumor regression (CR) at its MTD on both 7-day and 28-day schedules. Further, the high incidence of stomatis, alopecia, and diarrhea observed with 5-FU and FT were not observed with S-1. In an attempt to understand the basis for the observed superior therapeutic selectivity with S-1, pharmacokinetic analysis of 5-FU, drug-induced apoptosis, suppression of mitosis, and TS inhibition after S-1, FU, or FT administration were studied. The peak plasma concentrations of 5-FU derived from 5-FU, FT or S-1 at comparable MTDs were similar, but the plasma level of 5-FU derived from S-1 was higher than FU S-1. Induction of high and sustained apoptosis was achieved with S-1. Although the initial level of apoptosis induced by 5-FU was comparable to S-1, it was not sustained. The sustained level of apoptosis appears to correlate with tumor growth inhibition. Studies on TS inhibition indicated that, although both S-1 and 5-FU caused a 4- to 6-fold induction of total TS protein, single oral administration of S-1 was superior to 24-h infusion of FU in suppressing free TS. The data are consistent with the observation that the therapeutic efficacy of S-1 (100% cure) over 5-FU is associated with high and sustained levels of drug-induced apoptosis, greater suppression of mitosis, and inhibition of free TS in tumor tissues.

In brief, the data demonstrated that inactivation of a 5-FU degradative enzyme, DPD, with concurrent inhibition of 5-FU incorporation into the RNA of normal tissues and with selective tumor tissue activation of FT to 5-FU can lead to a highly active and selective treatment for colon cancer. These data suggest that this approach could offer a therapeutic advantage in the treatment of colorectal cancer and other malignancies amenable to fluoropyrimidine therapy. Unlike other fluoropyrimidines, S-1 is highly effective at lower doses than the MTD and may offer a greater therapeutic selectivity when combined with other drugs that have different mechanisms of action and toxicity profiles. For example, the combination chemotherapy using drugs directed against targets other than TS, e.g., CPT-11 (a topoisomerase I inhibitor) or cisplatin (a DNA cross-link agent) with limited overlapping toxicity, could offer the hope of maximizing antitumor activity with manageable toxicity. However, the validity of this concept must be verified clinically.

Clinical trials of S-1 revealed a similar overall response rate. Diarrhea was the dose limiting toxicity. Clinically, S-1 is being evaluated further with the hope of incorporating this agent into a therapeutic regimen for the treatment of colorectal cancer.

6. 5-FLUOROURACIL/ENALURACIL (5-FU/EU)

In a continuing effort to stabilize the overall pharmacology of 5-FU, a potential limiting factor in the therapeutic efficacy of 5-FU, Enaluracil (EU), a potent and specific DPD inhibitor, was developed. Preclinically, the combination of 5-FU/EU was highly active. These results provided evidence to suggest that DPD inhibition by EU was associated with the observed response, especially when 5-FU administered as bolus or by infusion were less active. These preclinical data provided the rationale for the clinical development of oral FU/EU. DPD inhibition by EU allowed for the oral administration of 5-FU. In both preclinical and clinical trials, the dose of 5-FU delivered in combination with EU had to be reduced significantly, from what is conventionally administered, 400-500 mg/m² of 5-FU alone and/or in combination with LV to a maximum of 10 mg/m² 5-FU when combined with EU. Unfortunately, the results of phase III clinical trials of 5-FU/EU vs. 5-FU/LV were disappointing, failing to show equivalent efficacy. Clinical trials clearly demonstrated that DPD inhibition can significantly alter the pharmacology and metabolism of 5-FU.

The failure of DPD inhibition in combination with 5-FU to alter the therapeutic effectiveness of 5-FU and to provide a more effective and selective alternative therapy may be a failure in the rationale design of the clinical trials as far as drug dosage and schedule as well as in determining the optimal DPD inhibitor/FU ratio. It is possible the use of DPD in a concentration and schedule that yields almost total depletion of DPD is not a desirable endpoint to achieve. Under these conditions, the dose of 5-FU that can be delivered safely may be too low to be effective against tumor tissues. If the desired end is complete DPD inhibition in tumor tissues, the dose of DPD inhibition needed would be significantly less allowing higher doses of 5-FU to be delivered. It is unfortunate that the negative results of clinical trials with two independent DPD inhibitors (EU and U) closed the door, at least for now, on the potential use of these inhibitors with 5-FU and its prodrugs such as capecitabine (Xeloda) and UFT. Perhaps this treatment modality might be effective in tumors with high level of TS and DPD. The wheel of clinical trials may turn in this direction if sufficient rationale and data are generated in the future.

7. CAPECITABINE (XELODA)

Capecitabine, unlike other 5-FU prodrugs, was designed to take advantage of the differential expression of various metabolite enzymes in normal and tumor tissues, including carboxylesterase and cytidine deaminase preferentially expressed in normal tissues and thymidine phosphorylase preferentially expressed in tumor tissues. Further, unlike UFT/LV, FU/EU and S-1, capecitabine has no DPD inhibitor. The rationale is based on the hypothesis that high levels of thymidine phosphorylase will result in a high level of 5-FU in tumor tissues that is sufficient to override the need for DPD inhibition. Results of clinical and preclinical pharmacokinetic and pharmacodynamic studies are consistent with the hypothesis that the level of 5-FU in tumor tissue derived from oral capecitabine is
Several fold higher than that achieved by 5-FU administered i.v..

Two randomized phase III clinical trials in patients with advanced colorectal cancer established superior activity of capecitabine over the daily schedule of 5-FU at the Mayo Clinic. While diarrhea, mucositis and hematologic toxicity observed with capecitabine were less than 5-FU, hand and foot syndrome was the dose-limiting toxicity (G3/4) in approximately 15-20% of patients. A higher incidence of dose-limiting toxicity was observed with high dose of capecitabine (2500 mg/m^2 bid x 14 MTD d) compared with lower doses (200 mg/m^2 bid x 14 d) of capecitabine, in recent clinical trials the incidence of hand and foot syndrome appeared to be reduced without compromising efficacy.

Capecitabine is the only 5-FU prodrug approved by the FDA for patients with advanced colorectal cancer and in patients with advanced breast cancer previously treated with taxanes. This rationally designed oral agent, with manageable toxicity, is being widely utilized alone and in combination with irinotecan, oxaliplatin, the EGFR inhibitor (Iresssa) and the VEGF inhibitor (Avastin). The future challenge presented by the optional use of capecitabine is likely to depend on the use of alternative schedule/dose and targeted therapy, particularly in patients with overexpression of TS and in tumors with low levels of apoptosis. Recent unpublished data suggest that capecitabine can alter the expression of preapoptotic gene, Bax, and anti-apoptotic gene, bcl-2. In addition, the demonstrated ability of irinotecan, oxaliplatin, Taxol, and radiation therapy to activate thymidine phosphorylase in time/dose dependent manner provides the rationale for the use of capecitabine in sequences, rather in simultaneous combinations with these agents. These principles are now being tested and verified in preclinical and clinical settings.

8. ANTIFOLATES

Several antifolate TS inhibitors, have been under active preclinical and clinical evaluation, including CB3717, a quinazoline-base antifolate analysis, raltitrexed (ZD1694, Tomudex®), an analog of CB3717, pemetrexed (LY231514), a multitargeted antifolate TS inhibitor.

Raltitrexed was extensively evaluated in phase I-III trials in colorectal cancer in both the United States and Europe. The response and overall survival rates were at least comparable with the conventional doses/schedules of 5-FU/LV. Due to lack of superior efficacy, the US FDA did not approve this drug. This agent, however, has been widely used and is approved clinically in same parts of Europe, particularly in England. Since 5-FU has multiple targets including, TS and mRNA among others, and with the increasing recognition that TS is a critical target, it was hypothesized that agents with high specificity by TS offer greater therapeutic efficacy than the standard 5-FU/LV regimen. Unfortunately, results obtained to date do not provide optimism for continued evaluation of this agent at levels and with the dose and schedule of this agent that have been utilized. Factors contributing to the clinical failure of this agent may be related to the doses and schedules used. Alternative schedules may offer a window of opportunity for the use of this drug in combination with other clinically active agents.

8.1. Mechanism of action

Although TS is a key and critical target in the development of cancer, results of preclinical and clinical studies with drugs that are direct inhibitors of the enzyme (antifolates) and indirect inhibitors of the enzyme (UFT/LV, 5-FU/EU, Xeloda, S-1) demonstrated equal efficacy (antitumor activity in terms of response rate and survival), but with different toxicity profiles. This suggests that TS expression may be a good prognostic marker of outcome of therapy (sensitive vs. resistance), but not the only mechanism that predicts for therapeutic outcome. (1) To date, published data demonstrated an association between TS level and response. It is likely that mechanisms predictive for clinical outcome to treatment with TS inhibitors are multifactorial. Alteration of downstream cellular signaling pathways could be directly related to the degree and duration of TS inhibition. Such inhibition is likely to be influenced by the level of the enzyme and the cellular concentrations of the drug achieved and retained at the target site. Thus, while the level of TS is critical, the degree and the duration of TS inhibition are critical steps. (2) It is possible that with current clinically effective drugs and schedules, TS inhibition and effective alteration of downstream molecular pathway may indeed be predictive of the response seen in 15-20% of patients with low expression of TS treated with these agents. (3) In patients with TS overexpression, it is likely that sufficient intratumor drug concentrations could not be achieved with standard doses and the delivery of higher doses is limited by undesirable host toxicity.

In brief, with the available new drugs and with different toxicity profiles, schedules and routes of administration, and the availability of sensitive methodology such as DNA microarray and proteomics, it should be possible to develop a new understanding as to the role of TS as a mechanism for predicting the clinical response to existing chemotherapeutic agents. However, it is likely that due to heterogeneity of tumor tissues, mechanism(s) of drug response is multifactorial, and that TS is one target among others. With the observed increase in clinical outcome with the combination of TS inhibitors with other drugs, it is possible that these new agents alter the expression of TS and consequently increase response.

9. PERSPECTIVE

Preclinical studies demonstrated that mechanism(s) of action of 5-FU vary depending on the schedule of drug administration; predominantly incorporation into cellular RNA with bolus injection and predominantly TS with protracted continuous i.v. infusion and in combination with LV. Further, in tumors in which TS is not a key marker for response to bolus i.v. administration (cytotoxicity could not be reversed by exogenous thymidine), TS became a key target when bolus 5-FU is combined with LV. Clinical trials demonstrated that the overall response in advanced colorectal cancer was
Thymidylate synthase inhibitor

increased from less than 10% for bolus 5-FU to 15-20% with the addition of LV, a response rate similar to that generally obtained with protracted continuous i.v. infusion of 5-FU. Further, daily (Mayo Clinic, Machover, etc) and weekly (RPCI) schedules of 5-FU/LV yielded similar overall response and survival rates in patients with advanced colorectal cancer. There were significant differences in the toxicity profiles (neutropenia, mucositis, and diarrhea as the dose limiting toxicity with daily x 5-FU/LV, and primarily diarrhea as the dose limiting toxicity with the weekly RPCI schedule). Present clinical trials are using the weekly schedule in combination with other drugs.

Because of the broad and significant toxicity documented with bolus 5-FU/LV, but not with continuous i.v. infusion of 5-FU (except for grade 3/4 head and foot syndrome) with comparable therapeutic outcome, several orally bioavailable 5-FU prodrugs were identified and evaluated extensively in preclinical and clinical settings. In general, overall response and survival rates with these agents (Xeloda in particular) were at least similar, if not better, with less toxicity.

Clinical experiences with antifolate TS inhibitors were somewhat disappointing. These agents are not being used widely in the United States. Clinical results suggest that although the antifolates are potent and specific TS inhibitors, the outcome is similar to 5-FU and its prodrugs, agents with multiple sites of action, including TS.

Thus, while TS is a critical prognostic marker in colorectal cancer, the level of TS expression may determine the degree and duration of its inhibition and consequently the cascade downstream molecular effects that determine response. While affecting TS as a proximal target is essential first step, it is not necessarily sufficient to predict response to TS inhibitors.

At the present time a number clinically active drugs are available. The challenge is to determine how to use these drugs in combination with the standard agents. Sensitive and quantitative methodologies for assessing multiple targets, including TS and downstream molecular markers associated with apoptosis, cell cycle control and DNA damage and repair are critical for the identification of mechanism(s) predictive for therapeutic outcome.

Although the focus has been on identification on tumor markers predictive for response, the need to understand the biology and therapy modification of normal tissues is highly critical. Due to dose-limiting toxicities, it is possible that effective drug concentrations to inhibit effectively the intended target and are not sufficient with the available therapeutic modality. Thus, it is important that efforts to identify and develop agents that selectively protect against normal tissue toxicity allowing the administration of higher drug doses should be pursued. For example, the use of selenium containing compounds is under extensive preclinical and clinical evaluation at Roswell Park. It has been documented that selenium can selectively protect against normal tissue toxicity induced by a variety of chemotherapeutic agents and enhance the therapeutic efficacy in several preclinical xenograft models that are de novo resistant to conventional drug doses. These data provided the basis for the development of phase I/II clinical trials at RPCI to validate this approach clinically.

10. ACKNOWLEDGEMENT

Supported in part by NCI CA65761, and an Institute Cancer Center Support Grant 15056-26 for the National Cancer Institute, Bethesda, MD.

11. REFERENCES


Diasio R: Clinical implications of dihydropyrimidine dehydrogenase on 5-FU pharmacology. *Oncology* 15(suppl 2), 21-26 (2001)

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Key Words: 5-Fluorouracil prodrugs, Antifolates, Mechanism, Perspectives, Review

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