VOL 26, NO 2, SUPPL 6 APRIL 1999

Supplement to

Seminars in Oncology



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# MTA, A Novel Multitargeted Antifolate From Preclinical to Phase I and Beyond

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# MTA: Summary and Conclusions

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## BACKGROUND

SINCE the introduction of methotrexate into clinical practice in the late 1940s, there have been continual attempts to introduce improved or different antifolates. Although antifolates have been successfully introduced as anti-infective agents (for example, pyrimethamine for malaria and trimethoprim for bacterial infections), the discovery of additional folate-based therapeutic agents against cancer has been more elusive. A number of dihydrofolate reductase (DHFR) inhibitors have been tested, including metoprine[1] (2,4-diamino-5-(3',4'-dichlorophenyl)-6-methylpyrimidine), trimetrexate,[2] and edatrexate.[3] While trimetrexate shows some activity as an anticancer agent and as an antifungal agent (*Pneumocystis carinii* pneumonia) and edatrexate has demonstrated activity in lung cancer, none of these compounds has so far achieved a mainstream role in cancer treatment. The development of antifolate drugs targeted against alternative enzymes of folate metabolism has met with rather more success. It has been argued that a selective folate-based inhibitor of thymidylate synthase (TS) would provide a therapeutic advantage compared with compounds inhibiting DHFR.[4] The first clinically tested folate-based TS inhibitor (CB 3717) showed antitumor activity, but clinical development was discontinued due to sporadic and unpredictable toxicity.[5] However, this experience led to the development of raltitrexed, a drug that has activity in colon cancer[6] and is licensed for this indication in a number of countries. Several folate-based inhibitors of glycinamide ribonucleotide formyl transferase (GARFT) have entered early clinical studies, but so far none has progressed beyond the phase I/early phase II stage.[7,8] The lesson to be learned from almost 50 years of experience with antifolate drugs must be that it is difficult to discover new agents that produce sufficiently compelling clinical results to give them a role in everyday practice. This review will examine the results presented in this supplement on MTA to project whether this drug will be a broadly useful anticancer agent and whether it will differ substantially from those already available.

It has been argued that antifolates capable of inhibiting two loci in folate metabolism could offer advantages compared with those inhibiting only one,[9] since the development of drug resistance might be rendered less likely. If resistance were to develop by an increase in the level of one target enzyme, the folate pathways would still be inhibited by the action of the drug on the alternative target. Counter to such an argument is the observation that different folate-dependent enzymes are present in differing activities. For example, a 50% inhibition of the flux through a rate-limiting enzyme such as TS will result in a corresponding inhibition of the rate of DNA synthesis, while a greater than 90% inhibition of DHFR will be necessary to achieve a similar reduction in the rate of DNA synthesis, since this latter enzyme is normally present in excess.[10] Thus, in order for a drug to be capable of reducing the rate of DNA synthesis due to inhibiting either TS or DHFR, it would be necessary for its $K_i$ for DHFR to be considerably lower than that for TS. However, such an argument does not take into account the idea that the level of expression of TS and other enzymes of folate metabolism may be both variable between cell types and inducible in response to exposure to an inhibitor. In this event, loci other than the primary target of an antifolate could become important in response to exposure to the drug.

## MTA AS A NEW DRUG

### *Is MTA Functionally More Than a Pure Thymidylate Ssynthase Inhibitor?*

As has been described in this supplement, MTA was discovered during the evaluation of a series of compounds originally intended to be inhibitors of GARFT. Initial testing suggested that it was in

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*Sponsored by Eli Lilly and Company.*

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*0093-7754/99/2602-0617$10.00/0*

fact functionally a TS inhibitor, but further evaluation showed that it also inhibited DHFR, as well as GARFT, and aminoimidazole carboxamide ribonucleotide formyltransferase. The $K_i$s for TS, DHFR, GARFT, and aminoimidazole carboxamide ribonucleotide for the $Glu_5$ derivative are reported by Chen et al in this supplement as 1.3, 7.1, 65, and 260 nmol/L, respectively. These figures lead one to believe that the dominant locus of MTA would be TS, a supposition that is supported by the observation that in most of the studies presented, end-product reversal of a mildly toxic concentration MTA can be achieved in vitro by the addition of thymidine alone (Chen et al and Smith et al, this supplement). However, MTA at a concentration 10-fold higher than the $IC_{50}$ (7 μmol/L; Smith et al, this supplement) also required a purine source for reversal, suggesting that an alternative locus, most likely GARFT, was coming into play. Pharmacokinetic data show that clinically achieved plasma levels of MTA following the administration of 600 mg/m$^2$ exceed 200 μmol/L at the peak and remain over 7 μmol/L for many hours (Robert D. Johnson, PhD, Eli Lilly and Company, personal communication).[11] Furthermore, biochemical evidence shows a direct effect of MTA on purine synthesis (Mendelsohn et al, this supplement) and a difference in the accumulation of deoxyadenosine triphosphate (Chen et al, this supplement) compared with the more specific TS inhibitor, raltitrexed. All these observations lend weight to the idea that when administered in clinically relevant doses to humans, the alternative targets of MTA will play a significant part in its actions. Also of great interest are the data on resistant cell lines (Schultz et al, this supplement) in which cell lines are described that are significantly more resistant to raltitrexed than to MTA, in which a purine source is required to protect from MTA toxicity in the resistant line. These data again suggest that a second biochemical target may be important in circumventing drug resistance.

In vivo antitumor data derived in mice, while not directly establishing a mechanism of action for MTA, are encouraging. It is well-known that mice have higher circulating levels of thymidine than humans[12]; this fact leads them to be poor models for the antitumor efficacy of TS inhibitors, unless the tumors concerned are low in thymidine kinase and therefore unable to use the circulating thymidine. Nevertheless, MTA showed significant activity in a range of thymidine kinase-competent human tumor xenografts grown in mice.

## DOES MTA HAVE CLINICAL CHARACTERISTICS LIKELY TO MAKE IT A PRACTICAL DRUG?

To be broadly applicable to the treatment of human cancer, a drug needs to have a reasonably convenient mode of administration, reasonably consistent and predictable toxicities, and amenable to drug combinations.

The phase I experience with MTA (Rinaldi, this supplement) reports three schedules of administration. The weekly ×4 schedule was excluded from phase II evaluation on account of the possibility of cumulative toxicity, but the other two schedules (single dose every 3 weeks or five daily doses repeated every 3 weeks) were both judged feasible. The single 3-weekly schedule was chosen for the phase II work on account of its convenience. All the trials showed similar toxicities, with leucopenia and thrombocytopenia being dose-limiting. Non-dose-limiting toxicities comprised transient transaminase elevations, rashes, mucosal toxicity, general malaise, diarrhea, and skin rashes. Symptomatic and "patient unfriendly" toxicities, such as acute nausea and vomiting or alopecia, were noticeably infrequent. Sporadic cases of severe myelosuppression with severe gastrointestinal toxicity and sepsis were seen in all the phase I studies. However, such toxicities have not been a serious problem in those phase II studies in which the patients were in general of a good performance and nutritional status.[13] The recently presented study of the use of plasma homocysteine as a marker for folate deficiency[14] shows a correlation between elevated pretreatment homocysteine levels and the subsequent occurrence of grade 3 or 4 toxicity. Thus, it seems that it will be possible to administer MTA every 3 weeks without significant symptomatic toxicities and with a good level of safety.

So far in oncology practice the use of cytotoxic drugs as single agents has been unusual. The optimal therapeutic regimens derived for the common tumors have nearly always been combinations of active drugs for the disease concerned. In order for a drug to be generally useful it should be amenable to use in combinations with other major agents. At the preclinical level (Teicher et al, this sup-

plement), MTA was tested in combination with cisplatin, methotrexate, 5-fluorouracil, paclitaxel, docetaxel, doxorubicin, irinotecan, and fractionated radiation therapy in vivo using the EMT-6 mammary carcinoma, the human HCT 116 colon carcinoma, and the human H460 non-small cell lung carcinoma grown as xenografts in nude mice. It was possible to use full doses of each of these agents in the combination, and additive or synergistic antitumor results were seen. Two phase I clinical trials of MTA in combination are presented in this supplement. The combination with cisplatin allowed the administration of a full dose (600 mg/m$^2$) of MTA and a dose of 75 mg/m$^2$ of cisplatin to be given on a repeated basis. The cisplatin dose is the same as that generally used in a large number of combinations and cannot be considered suboptimal. The combination with gemcitabine (Adjei and Erlichman, this supplement) is ongoing, but shows that a full dose of gemcitabine (1 g/m$^2$ days 1 and 8) can be combined with at least a dose of 400 mg/m$^2$ MTA, again suggesting that full doses of the combination will be possible.

### *Does MTA Have Promising Activity?*

The phase II studies summarized here (O'Dwyer et al, this supplement) report responses in six tumor types. In metastatic breast cancer responses were seen in 31% of 36 patients. The previous treatment of responding patients included both taxanes and anthracyclines and there was no evidence for a lower response rate in those with more extensive pretreatment. In previously untreated non-small cell lung cancer, two studies have shown response rates of 23% and 17%. In previously untreated colon cancer, response rates of 20% and 17% have been reported in two independent studies. The early results of a bladder cancer study suggest activity, with 7 of 25 patients being reported as showing responses. Lesser levels of activity also have been seen in hypernephroma and cervical cancer. Of particular interest is the observation made in the combination phase I study of MTA and cisplatin in which four of seven patients with mesothelioma have been reported as responding. If confirmed in a larger study this is a truly exceptional result in a very refractory tumor. Overall the breadth and consistency of the phase II activity reported with MTA is remarkable and unusual in a new drug of any class at this stage of its development.

## CONCLUSIONS

MTA is clearly mechanistically distinct from existing antifolates. The biochemical data make a strong case for the role of more than one locus in its cytotoxic action and this feature may inhibit or preclude the development of certain mechanisms of drug resistance. The phase I and II experiences suggest that although MTA displays typical "antifolate" toxicities, these are manageable and predictable so that broad scale clinical use will be feasible. Of interest is the relative lack of toxicities that induce unpleasant symptoms of concern to patients, such as nausea, vomiting, and alopecia. Also noteworthy are the early clinical results showing evidence of significant activity in a broad range of common tumors, some of which were resistant to the major agents currently available. These observations taken together suggest that MTA will become a major addition to the armamentarium of drugs currently available to oncology practice.

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