Methotrexate and Pralatrexate

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KEYWORDS

• Methotrexate • Pralatrexate • Dihydrofolate reductase • Folate • Folic acid • Purine synthesis • S phase • Apoptosis

KEY POINTS

- Methotrexate (MTX) and pralatrexate (PDX) are competitive inhibitors of folate metabolism that block dihydrofolate reductase, thereby preventing thymidylate and purine synthesis and resulting in cell cycle arrest in the S phase.
- MTX and other folate inhibitors also reduce cellular levels of S-adenosylmethionine, the principal methyl donor for methyltransferases, thereby inhibiting DNA methylation.
- In CTCL, this derepresses tumor suppressor genes such as the death receptor, Fas (CD95), thereby enhancing apoptosis.
- These properties make folate antagonists useful for the treatment of lymphomas, either as single agents or in combination with other therapies that enhance or complement their effects.

INTRODUCTION

Methotrexate (MTX) is a well-known antimetabolite that blocks the action of dihydrofolate reductase, thereby inhibiting the metabolism of folic acid. It has been used widely since the 1950s to treat a variety of neoplastic and inflammatory diseases. Recently, a more potent analog, pralatrexate (PDX), has been developed and approved by the Food and Drug Administration (FDA) for the treatment of peripheral T-cell lymphomas (PTCLs). This article discusses some emerging concepts relevant to the optimal use of folate antagonists and reviews these drugs in regard to the therapy for cutaneous T-cell lymphomas (CTCLs), including clinical indications, mechanism of action, pharmacokinetics, dosing regimens, response rates, and adverse effects. According to convention, MTX will be used as the abbreviation for methotrexate. In keeping with prior publications, pralatrexate will be abbreviated as PDX, a designation derived from its alternative name: 10-propargyl-10-deazaaminopterin.[1]

EMERGING CONCEPTS RELEVANT TO THE OPTIMAL USE OF FOLATE ANTAGONISTS

The Role of Folate Antagonists in the Epigenetic Regulation of Gene Expression

The products of at least 5 tumor suppressor genes generally known to be silenced by promoter methylation have been reported to be deficient in mycosis fungoides (MF) and Sézary syndrome (SS), FAS/CD95, FAS-ligand, p16, p21, and protein phosphatase 4 regulatory subunit-1 (PP4R1).[2–11] These and other genes are also known to be silenced by promoter methylation in many other cancers (eg, TRAIL-R1, TRAIL-R2, p16, p21, hMLH1, MGMT, and RASSF1A).[12,13] These findings suggest that demethylating agents

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could benefit MF/SS patients by derepressing silenced tumor suppressor genes. Although FDA-approved for use in other diseases, traditional demethylating agents such as 5-azacytidine and decitabine have a toxicity profile that discourages their use for the treatment of chronic cutaneous lymphomas such as MF/SS. One of the most exciting aspects of folate antagonists is the recent realization that, in addition to their well-established role as S phase cell cycle inhibitors, they can also act as DNA methylation inhibitors.[2,14] Most of the relevant experiments have been performed using MTX; however, all related folate antagonists should share the same basic properties (see later discussion).

The Importance of Combination Therapy for Cancer

Inhibition of DNA methylation constitutes a novel mechanism of action and rationale for the use of MTX and related compounds in the management of cutaneous lymphomas. It also provides a new justification for their use in combination with other treatments that produce effects complementary to those of folate antagonists. The advantages of combination therapy relative to monotherapy for cancer treatment have been calculated recently by Bozic and colleagues.[15] In brief, they used mathematical modeling to show that by the time a tumor reaches a few millimeters in diameter it is likely to harbor hundreds to thousands of mutant cells that are resistant to any particular monotherapy. This typically results in short-term clinical benefit followed by treatment failure because resistant mutant tumor clones proliferate in response to the selection pressures of monotherapy. In contrast, dual therapy results in long-term disease control in most cases, if there are no mutations in a single cell that cause cross-resistance to both agents. The chances of cross-resistance are diminished if the 2 agents target different pathways. For patients with large disease burden in which the number of resistant mutants is greater, triple therapy is needed. The mathematical models also showed that simultaneous therapy with 2 agents is much more effective than when they are used as sequential therapies.

The implications of these mathematical models are relevant to folate antagonists because these drugs can be used in combination with other treatments that have different mechanisms of action and affect multiple cellular pathways. **Table 1** summarizes examples of MTX in combination with other modalities. Using this combination therapy approach, the likelihood of a favorable therapeutic outcome can be enhanced.

TREATMENT

Indications

MTX and PDX have been used to treat a wide variety of cancers. Among the cutaneous lymphomas, MTX has been used primarily to treat MF/SS and primary cutaneous CD30+ lymphoproliferative disorders (LPDs) such as lymphomatoid papulosis (LyP) and anaplastic large cell lymphoma (cALCL). PDX is FDA-approved for refractory or relapsed PTCLs. Among the cutaneous lymphomas, it has proven efficacy for advanced stages of MF/SS, including MF with large cell transformation (LCT).[16,17] It has also been used to treat other rarer forms of primary CTCLs.[18–20] Folate antagonists have not been used widely to treat cutaneous B-cell lymphomas. In fact, MTX has been associated with the development of cutaneous B-cell LPDs (sometimes related to Epstein-Barr virus), many of which regress when MTX is reduced or discontinued.[21,22]

Mechanism of Action

MTX and PDX are folic acid analogues that block cell division in the S phase.[23,24] They are competitive inhibitors of dihydrofolate reductase with an affinity for this enzyme that is several logs greater than that of its natural substrate, folate. Dihydrofolate reductase converts dihydrofolate to tetrahydrofolate, which is required for synthesis of thymidylate and purine nucleotides involved in DNA and RNA synthesis. It also inhibits the folate-dependent enzymes of purine and thymidylate synthesis such as glycinamide ribonucleotide transformylase, aminoimido-caboxyamido-ribonucleotide transformylase, and thymidylate synthase. MTX also inhibits methionine synthase, thereby reducing S-adenosyl methionine (SAM) levels. Because SAM is the principal methyl donor for DNA methyltransferases (DNMTs),[25,26] the authors propose that MTX can act as a demethylating agent by depleting DNMTs of their SAM methyl donor supply. The mechanism underlying this effect is illustrated in **Fig. 1**. Recently, the authors reported in vitro and ex vivo evidence that MTX acts as a demethylating agent for the promoter of the FAS/CD95 death receptor by blocking the synthesis of SAM.[14] When CTCL cell lines and freshly isolated leukemic CTCL cells were treated with MTX, it resulted in decreased SAM levels, decreased FAS promoter methylation, and increased FAS protein expression. This enhanced FAS expression was accompanied by a major increase in sensitivity to FAS pathway apoptosis, especially for leukemic cells. In strong support of the authors' hypothesis regarding MTX's mechanism of action, experiments using CTCL lines with high baseline FAS

Table 1
Novel combination therapies for cutaneous T-cell lymphomas involving methotrexate

Other Agent	Rationale
Interferon	In addition to effects promoting a TH1 immune response, IFN-α also upregulates Fas (CD95) by a STAT-1–dependent mechanism that is distinct from the effects of MTX on Fas. This combination is effective for advanced MF/SS; 74% complete response rate at 1 year among subjects with advanced CTCL (stage IIB–IVB).[34]
HDAC	There is well recognized interaction between DNMTs and HDACs, which collaborate to silence tumor suppressor genes. HDAC inhibitors and demethylating agents have shown synergistic reactivation of genes silenced by methylation.[49] Recent findings showed that the class III HDAC, SIRT1, is overexpressed in CTCL and that its knock-down or inhibition induced growth arrest and apoptosis.[50] Combination therapy with vorinostat is effective for controlling SS.
Photodynamic therapy	Photodynamic therapy upregulates Fas-ligand. Combined with MTX, both Fas and Fas-ligand are increased, resulting in greater apoptosis than with either agent alone (*e*PDT).[51]
UV phototherapy	Narrow-band UVB upregulates Fas-ligand. Combination therapy is effective in treating generalized patch and plaque MF.
Ionizing radiation	In addition to its direct cytotoxic effects associated with DNA and other cell damage, local radiation therapy increases Fas-ligand. Combined with MTX, it is postulated that both Fas and Fas-ligand are increased, resulting in greater apoptosis. Combination therapy induces a durable complete local response in follicular MF extensively involving both ears and external ear canals (associated with an enhanced but transient inflammatory reaction to radiation therapy).
c-CBL inhibitor	Agents that block c-CBL (an E3 ubiquitin ligase) upregulate Fas-ligand. When combined with MTX, both Fas and Fas-ligand are increased resulting in extensive apoptosis (still under investigation).[52]

Abbreviations: DNMT, DNA methyltransferase; *e*PDT, epigenetically enhanced photodynamic therapy; HDAC, histone deacetylase inhibitor; IFN-α, alpha-interferon; SIRT1, silent information regulator type-1; STAT, signal transducer and activator of transcription; TH1, type 1 helper T cell.

promoter methylation showed that the addition of SAM reversed both the decreased FAS promoter methylation and the increased FAS protein expression induced by MTX. Representative results are shown in **Figs. 2** and **3**, respectively. In aggregate, these in vitro and ex vivo data consistently support not only our hypothesis but also its clinical relevance. Other folate antagonists that we have tested (eg, pemetrexed) showed similar results. Therefore, we expect the same will be true of PDX.

In addition to its effects on DNA methylation, MTX (and related compounds) likely inhibit protein methylation because SAM is also the principal methyl donor for protein methyltransferases. For example, MTX was able to inhibit carboxyl methylation of Ras (possibly by inhibiting isoprenylcysteine carboxylmethyltransferase), thereby down-regulating Ras signaling, which is a major inducer of DNA methylation in many cancers.[13,27,28] Like acetylation, methylation of histones plays a key role in gene regulation. MTX is likely to reduce the activity of histone methyltransferases such as SETDB1 and SUV39. Histone methylation at H3K9 and H3K27 is associated with gene silencing, whereas methylation at H3K4 is associated with gene activation.[12] There is a recent report that MTX can inhibit the expression of methionine S-adenosyltransferase-1 and -2 genes.[29] This would also reduce SAM levels by blocking the conversion of methionine to SAM.

Pharmacokinetics

Therapeutic levels of MTX (1 μM) are reached at 1 to 5 hours after an oral dose of 20 mg/m^2. Levels remain greater than 0.1 μM for about 6 hours. Inhibition of DNA synthesis ends at levels below 0.01 μM. Inhibition of protein synthesis ends at levels below 0.1 μM.[30] In the plasma, about 50% to 70% of MTX is protein-bound (mainly to albumin). In the dose range generally used for cutaneous lymphomas, little MTX enters the central nervous system. There is a triphasic disappearance of MTX that depends on drug distribution, renal clearance, and the enterohepatic circulation. The mean terminal half-life is about 10 hours.

Relative to MTX, PDX has preferential uptake in cells due to its increased affinity for the reduced

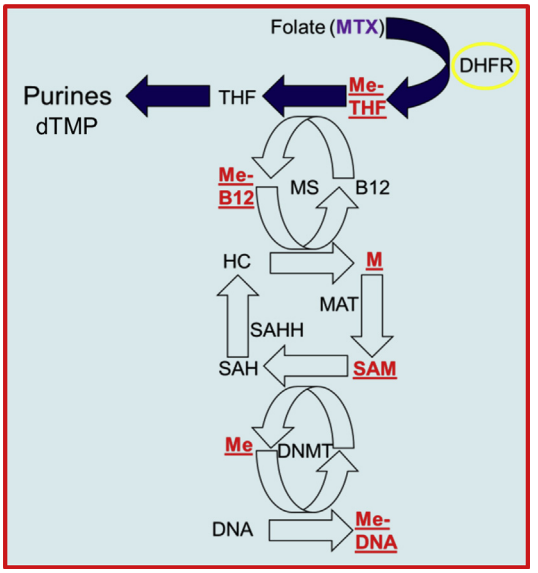

Fig. 1. MTX inhibits DNA methylation by depleting SAM. Chain of methyl group (Me) transfer is shown underlined from N-Me-tetrahydrofolate (Me-THF) to Me-vitamin B12 to methionine (M) to SAM to Me-DNA. MTX competitively inhibits dihydrofolate reductase (DHFR) which is involved in the multistep conversion of folate to Me-THF and inhibits methionine synthase (MS) and subsequent downstream methyl transfers that normally generate SAM. Other factors shown: homocysteine (HC), methionine adenosyltransferase (MAT), S-adenosylhomocysteine (SAH), SAH hydrolase (SAHH). The upper portion of the diagram is simplified and does not show that the immediate product of DHFR is THF, which is then converted into methylene THF before generation of Me-THF, which is then converted back to THF. Methylene THF is the more proximate precursor of deoxythymidine monophosphate (dTMP) and purines. DNMT, DNA methyltransferase.

folate carrier type 1 (RFC-1). This may give PDX greater selectivity for cancer cells because many tumors overexpress RFC-1. At an intravenous (IV) PDX dose of 150 mg/m^2 biweekly, the mean area under the curve (AUC) was 20.6 μM times hours, and the mean terminal half-life was 8 hours.[1] Exposure to IV PDX (AUC) is controlled with dosing based on body size. Pretreatment with folic acid and vitamin B12 can diminish the incidence and severity of mucositis while retaining drug efficacy.[31]

MTX and PDX are metabolized intracellularly into polyglutamates by folylpolyglutamyl synthetase.[16,32] These polyglutamates are preferentially retained in cells, thereby making them less susceptible to efflux-based drug resistance. The extent of polyglutamylation depends on both drug concentration and the duration of drug exposure. This process may be enhanced for PDX. Polyglutamylation is often upregulated in cancer cells, providing another potential form of relative selectivity for PDX.

Most of an MTX dose is excreted unchanged in the urine within 24 hours. A minority is metabolized during enterohepatic circulation. There are many genes that can affect the processing of folate antagonists, including RFC-1 (influx), ABCC1 and ABCG2 (efflux), adenosine receptors 1 and 2, and folate polyglutamates. For example, a single nucleotide polymorphism in exon 28 of the ABBC1 gene alters cellular efflux of MTX and affects its efficacy.[33] In addition to these variables, younger age correlates with enhanced distribution and elimination of these agents.

Typical Dosing

For CTCLs, MTX is usually administered orally once weekly at a dose of 10 to 25 mg, although higher doses have been used. The total dose is often divided into 2 or 3 portions taken 12 hours apart to enhance drug absorption and decrease gastrointestinal (GI) side effects.[30] Higher doses of MTX are sometimes used (for example, 10 mg/m^2 biweekly in combination with alpha-interferon [IFN-α]).[34] LyP is often quite sensitive to MTX and sometimes responds well to weekly doses as low as 5 mg. Oral folic acid supplementation (1–5 mg daily) ameliorates GI symptoms[35]; however, the dose or dosing of folic acid might affect efficacy.[36] When high-dose MTX (60–240 mg/m^2 IV) has been used to treat advanced MF/SS, it has been accompanied by leucovorin (folinic acid) rescue to minimize damage to normal tissues and to counteract acute toxicity.[37] Currently, such high-dose IV regimens are rarely used for cutaneous lymphomas.

Although higher and lower doses have been used successfully for various CTCLs, PDX is usually administered IV at 15 mg/m^2/wk for 3 weeks out of every 4 week cycle.[16] The total number of cycles depends on clinical response and toxicity. In addition to daily folic acid supplementation (1 mg orally), patients receive vitamin B12 supplementation (1 mg intramuscular [IM] every 8–10 weeks).

Response to Therapy

Methotrexate

Despite its longstanding and fairly common use in the management of MF/SS patients, relatively few clinical studies of MTX have been published. The response of MF/SS subjects to low-dose MTX (defined commonly as <100 mg/wk but often limited to doses ≤30 mg/wk that do not require folic acid to prevent toxicity) ranges from "definite improvement" in 9 out of 16 (using 2.5–10 mg/d)[38]

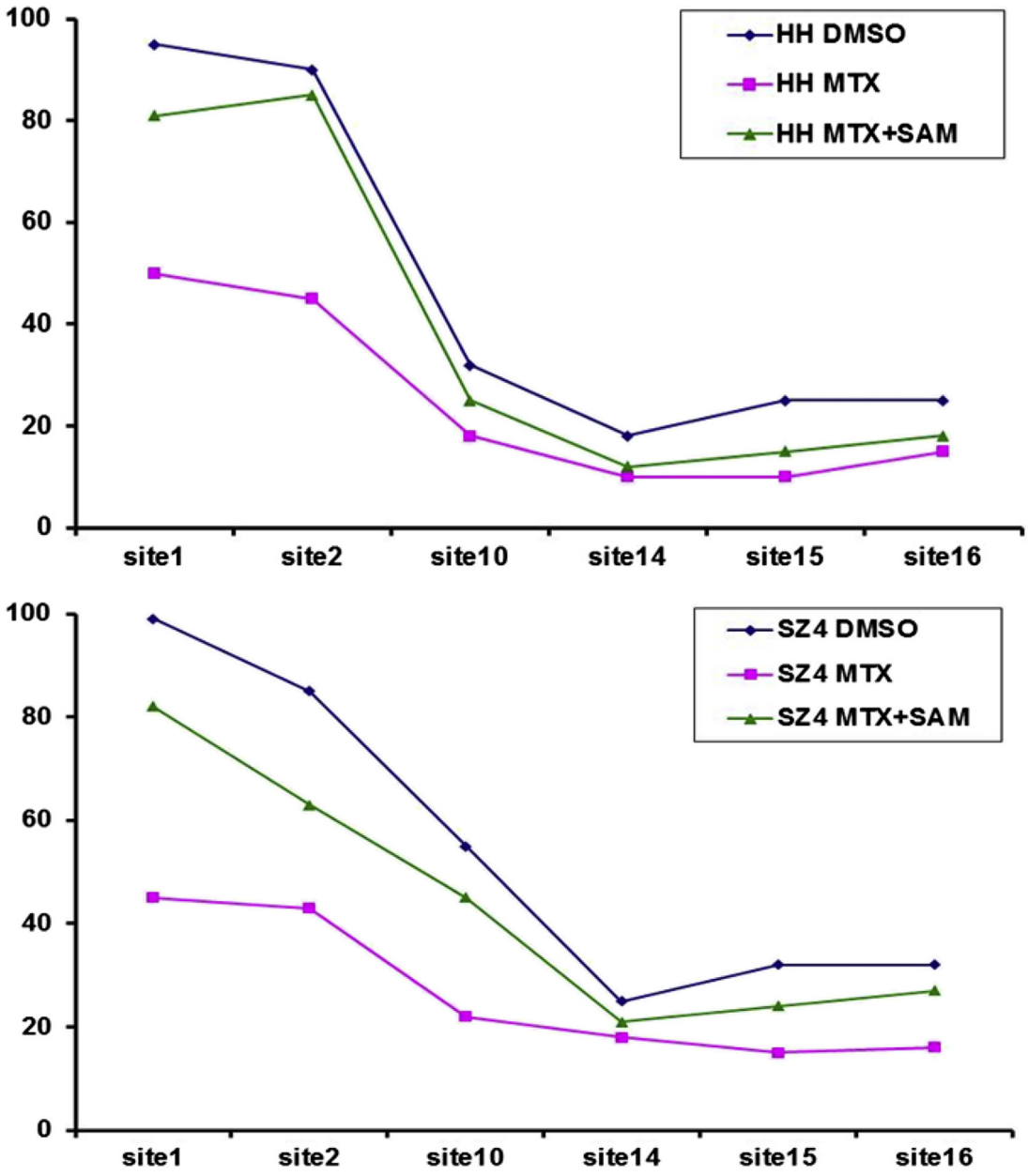

Fig. 2. MTX reduces FAS promoter methylation. SAM reverses it. Demethylation of 6 CpG sites in the FAS promoter was detected by pyrosequencing after treatment with MTX in Fas promoter methylation-high CTCL cell lines (SZ4, HH). The y-axis shows the percentage of the methylation. The upper lines are the dimethyl sulfoxide (DMSO) controls. The lower lines show the demethylating effect of MTX. The middle lines show that exogenous SAM can reverse the demethylating effect of MTX.

to an overall response (complete response [CR] plus partial response [PR]) in 17out of 29 with erythrodermic MF (E-MF) and 20 out of 60 with plaque MF (using a median dose of 25 mg/wk).[39,40] Although rarely used currently, MF/SS subjects treated with high-dose MTX (up to 240 mg/m^2 IV) showed 9 out of 11 with greater than 80% clearing including 7 of these with CR.[37] An overall response rate to MTX monotherapy in SS is difficult to estimate because of the small number of cases reported. There is one published phase I-II study of subjects with stage IA or IB MF treated topically with 1% MTX compounded in a hydrophilic gel containing laurocapram to enhance percutaneous absorption. After every-other-day application for 24 weeks, 7 out of 9 subjects showed “slight

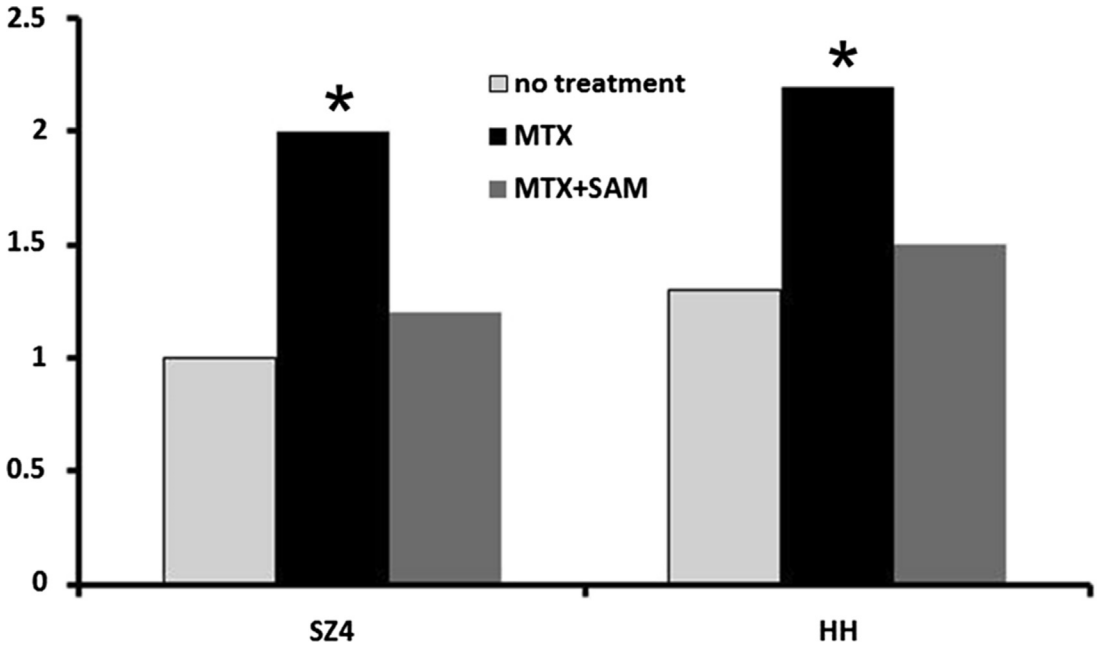

Fig. 3. MTX increases FAS death receptor protein expression; SAM reverses it. CTCL cell lines (HH, SZ4) show increased expression of FAS after treatment with MTX. Addition of exogenous SAM almost fully reverses this FAS upregulation. FAS protein detected by flow cytometry. Y-axis: fold change in mean fluorescence intensity. Asterisks represent statistically significant *t* test differences between the MTX samples and the no-treatment controls or the MTX + SAM samples (2-tailed $P<.05$ was considered statistically significant).

to moderate improvement" with statistically significant reductions in induration and pruritus without any significant toxicity.[41]

There are also studies using MTX in combination with other therapies for MF/SS. The largest involves 158 subjects with stage IIB to IVA MF/SS treated with MTX (10 mg/m^2 twice a week) plus IFN-α-2a (9 MU 3 times a week) for 6 months.[34] Those with PR continued for another 6 months on IFN and MTX and those with CR at 6 or 12 months continued only on IFN. At 6 months, there was 49% CR and at 12 months, the CR was 74%. The 10-year estimated survival was 69%. Toxicity was mild despite that there was no folic acid supplementation. The impressive clinical efficacy observed in this study is supported by the authors' own in vitro and ex vivo data that demonstrated greater MF/SS tumor cell killing with the combination of MTX and IFN-α than with either agent alone.[34] A small study of IV MTX 60 mg/m^2 IV followed by 5-fluorouracil 20 mg/kg with leucovorin rescue showed at least 80% clearing in 2 out of 2 SS subjects and 1 out of 2 E-MF subjects.[42] A single SS subject had a durable (>4 year) PR following combination therapy with MTX (10 mg/wk) and etoposide (25 mg/d).[43]

Low-dose MTX (5–25 mg/wk) is recommended by expert consensus for the treatment of primary cutaneous CD30+ LPDs, including LyP, cALCL, and intergrades. Many subjects treated with MTX for LyP have been reported in the literature; however, there is a paucity of published data regarding its overall efficacy for cALCL.[44] In the largest study of low-dose MTX for primary cutaneous CD30+ LPDs, there was an overall response rate of 87% among 45 subjects (mostly LyP).[45] A median dose of 20 mg/wk was given for 1 year followed by maintenance therapy typically at 2 wk intervals. After treatment was discontinued, about one-quarter of the responders remained disease-free for at least 2 years. From the aggregate data, it is clear that low-dose MTX is effective for controlling LyP in most cases; however, relapse off therapy is common. If the extent of relapse is clinically significant, maintenance therapy may be needed for an extended period. There is a case report of LyP responding to local application of topical MTX.[46]

Pralatrexate Regarding treatment of advanced MF/SS with PDX, there is a dose deescalating study in which the starting dose was 30 mg/m^2/wk IV for 3 weeks out of every 4-week cycle.[16] The dose was progressively reduced to find the optimal balance between efficacy and toxicity. Overall, 54 subjects were treated, 29 with the optimized schedule of 15 mg/m^2/wk IV for 3 of 4 weeks (median of 4 cycles). The overall response among these 29 cases

was 45%, mostly PRs with 2 CRs. Another study of subjects with relapsed or refractory PTCLs included 12 subjects with MF and LCT.[20] The overall response rate was 58% by investigator assessment and 25% by central review.

PDX therapy for cALCL or other rare types of CTCL is not well reported. However, a few cases are contained in larger studies of PTCLs and showed some objective responses, including CRs.[16,18–20] In addition, 17 subjects with the systemic CD30+ cALCL showed an overall response of rate of 35%.[20]

Trimetrexate Finally, there is a study of trimetrexate (another MTX-related folate antagonist) administered IV at a dose of 200 mg/m^2 biweekly. There was a 47% overall response rate among 15 MF/SS cases (most with LCT).[47]

Adverse Effects

MTX side effects include GI (nausea, vomiting, stomatitis, ulcers, diarrhea), bone marrow (leukopenia, anemia, thrombocytopenia), liver (increased transaminases, hepatitis, fibrosis, cirrhosis; the latter 2 related to cumulative dose), lung (pneumonitis, fibrosis), pregnancy (abortifacient, teratogen), and miscellaneous (alopecia, anaphylaxis, oligospermia, photosensitivity, radiation recall, reactivation sunburn). Several MTX-induced LPDs have been reported, including in a patient with SS.[48]

PDX has a toxicity profile similar to MTX; however, in the dose ranges commonly used, PDX side effects tend to be more common and severe, thereby limiting its use to more advanced stages of MF/SS and other aggressive types of CTCLs. The most common PDX side effects ($\geq$10%) include mucositis (17% grade 3), fatigue, nausea, vomiting, anorexia, skin toxicity, epistaxis, and anemia.[16]

Toxicity of MTX and PDX can be enhanced by interactions with other folate antagonists (dapsone, sulfonamides, trimethoprim), hepatotoxins (ethanol, retinoids), preexisting liver disease and other conditions or drugs (eg, nonsteroidal antiinflammatory drugs, probenecid) that result in increased blood levels (reduced renal excretion, displacement from binding proteins).

PEARLS TO HELP MANAGEMENT USING METHOTREXATE AND PRALATREXATE

It is important to remember that LyP does not need to be treated if mild. In fact, it is useful to titrate the dose of MTX close to the point at which a few small lesions will still occur. In this way, excessively large doses can be avoided. If oral MTX induces problematic GI side effects, it can be administered intramuscularly instead. This also results in a higher, more prolonged level of MTX in the serum relative to the same dose given orally. The IM route may also be useful in those patients who are suspected of poor uptake of MTX from the GI tract and may be used to enhance compliance when delivered at a physician's office. When administering IV PDX, the dosing schedule can be modified by delaying or reducing doses as needed to help manage severe adverse events such as mucositis. Side effects of both MTX and PDX can be ameliorated by avoiding concomitant use of the many other drugs that can potentiate their toxicity.

SUMMARY

This article reviews the use of MTX and its more recently developed analog, PDX, for the treatment of cutaneous T-cell LPDs. Although traditionally regarded principally as proliferation inhibitors that block the S phase of the cell cycle, folate antagonists are now known to inhibit DNA methylation by depleting cellular stores of S-adenosylmethionine, the main methyl donor for DNMTs. This has led to their novel use as agents that can derepress silenced tumor suppressor genes. Furthermore, recent mathematical modeling of cancer cell mutational dynamics provides a rationale for the use of all anticancer agents as part of combination therapy regimens rather than as monotherapies. A strategic advantage of combination regimens is the ability to attack multiple cell signaling pathways simultaneously, thereby preventing the emergence of drug-resistant tumor clones. Together, these recent advances hold new promise for the use of folate antagonists in combination with other modalities such as IFNs, histone deacetylase inhibitors, photodynamic therapy, narrow band UVB phototherapy, and ionizing radiation (see **Table 1**). Clinical validation for this approach comes from the impressive 74% CR at 1 year observed among subjects with advanced stage CTCL who were treated with a combination of low-dose MTX and IFN-α-2b.[34] In aggregate, these advances are ushering in a new era for the use of folate antagonists in the management of cutaneous lymphomas.

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