

Cancer chemotherapy: targeting folic acid synthesis

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Abstract: Antifolates are structural analogs of folates, essential one-carbon donors in the synthesis of DNA in mammalian cells. Antifolates are inhibitors of key enzymes in folate metabolism, namely dihydrofolate reductase, β -glycinamide ribonucleotide transformylase, 5'-amino-4'-imidazolecarboxamide ribonucleotide transformylase, and thymidylate synthetase. Methotrexate is one of the earliest anticancer drugs and is extensively used in lymphoma, acute lymphoblastic leukemia, and osteosarcoma, among others. Pemetrexed has been approved in combination with cisplatin as first-line treatment for advanced non-squamous-cell lung cancer, as a single agent for relapsed non-small-cell lung cancer after platinum-containing chemotherapy, and in combination with cisplatin for the treatment of pleural mesothelioma. Raltitrexed is approved in many countries (except in the United States) for advanced colorectal cancer, but its utilization is mainly limited to patients intolerant to 5-fluorouracil. Pralatrexate has recently been approved in the United States for relapsed or refractory peripheral T-cell lymphoma. This article gives an overview of the cellular mechanism, pharmacology, and clinical use of classical and newer antifolates and discusses some of the main resistance mechanisms to antifolate drugs.

Keywords: antifolates, cancer, molecular pharmacology, pemetrexed, methotrexate, folate metabolism

Introduction

Folates are essential, one-carbon donors in the synthesis of purines, pyrimidines, serine, and methionine, all critical to de novo synthesis of DNA in mammalian cells, as they lack the capacity to synthesize folates and require these anionic hydrophilic molecules to be transported into the cells via sophisticated transport systems (reduced folate carrier, RFC). After folate was discovered to be vital to many cellular processes, the antifolates aminopterin and methotrexate (MTX) were synthesized in the early 1940s.¹ In 1948, aminopterin was the first drug to induce temporary remissions in childhood leukemia.^{1,2} Only 10 years later, MTX was part of a therapy regimen that was first shown to cure some selected solid tumors, namely choriocarcinoma.³ MTX is still used in the treatment of a variety of tumors, including acute lymphocytic leukemia,⁴ breast cancer,⁵ osteosarcoma,⁶ primary central nervous system lymphoma,⁷ and head and neck cancer.⁸ Above all, it is also used in certain autoimmune diseases, such as rheumatoid arthritis or psoriasis. Recently, the newer antifolate pemetrexed or multitargeted antifolate (MTA) has been established in the first-line treatment of mesothelioma⁹ and non-squamous, non-small-cell lung cancer (NSCLC).¹⁰ An important task for the future is treatment individualization, eg, by identifying genetic variations in drug pathway-associated genes with an important impact on clinical outcome in patients receiving

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antifolates^{11–13} or the use of therapeutic drug monitoring (TDM), eg, with MTX, enabling adequate drug exposure in all patients.^{14,15}

Cellular folate metabolism

Folates (pteroylglutamates) belong to the family of B₉ vitamins that are essential to mammalian cells. They form a family of cofactors based on the structure of folic acid (2-NH₂-4-OH-pteroylglutamic acid). Folic acid undergoes intracellular reduction first to dihydrofolate and then to tetrahydrofolate (THF). Both reduction steps are mediated by dihydrofolate reductase (DHFR). The major dietary form of folates is 5'-methyl-THF (5'-MTHF). Together with homocysteine, MTHF is converted to methionine and THF, a vitamin-B₁₂-dependent step that is mediated by methionine synthase. THF is the main substrate for folylpolyglutamate synthetase (FPGS), which progressively adds glutamates at the γ -carboxyl residues. The resulting folate polyglutamates are polyanionic molecules that can no longer be transported through the lipophilic cell membrane. These folate polyglutamates are the biologically active form of folates, as they serve as one-carbon donors in de novo synthesis of purines, thymidylate, and polyamines. Furthermore, folates are required for the synthesis of S-adenosyl methionine, which promotes methylation of DNA, histones, lipids, and neurotransmitters.¹⁶

Antifolate drug metabolism

As structural analogs of folates, antifolates use the same transport systems to enter the cells, namely the reduced folate receptor (RFC), folate receptors (FR), and the recently discovered proton-coupled folate transporter (PCFT) or soluble carrier 46A1 (SLC46A1).¹⁶ The RFC plays a dominant role in cellular uptake of antifolates, as it has low affinity to its main endogenous ligand MTHF. Its affinity to antifolates varies from low for MTX to high for talotrexin (PT-523).¹⁷ The RFC works as an anion exchanger that utilizes the gradient built up by an asymmetrical distribution of organic phosphates across cell membranes.^{18,19} The RFC is expressed both in tumor cells and normal tissue,²⁰ limiting the tolerability of antifolates. On the contrary, the folate receptors FR- α and FR- β are overexpressed at the surface of some tumor cells, making these tumors vulnerable to antifolate drugs.^{21,22} The FR family consists of two cell-surface receptors (FR- α and FR- β) and a constitutively secreted isoform (FR- γ).^{23,24} FR- α is expressed in a number of normal epithelial cells as well as in a number of carcinomas, with the exception of carcinomas of the head and neck.²¹ FR- β serves as a myeloid

differentiation marker and is overexpressed in a variety of nonepithelial malignancies,^{21,25} whereas the expression of FR- γ is restricted to hematopoietic tissues.^{24,26} In contrast to the high-capacity and low-affinity RFC, transport by FR- α and FR- β is by low-capacity and high-affinity endocytosis.²⁷ After antifolate transport to the endosomal compartment, transport to the intracellular compartment involves the PCFT.^{28,29} Accordingly, mutations in the gene encoding for PCFT have been shown to cause rare hereditary folate malabsorption.²⁹ In addition to its role in folate endocytosis, PCFT also serves as a high-affinity folate-proton symporter that is important for the intestinal absorption in the proximal small intestine.³⁰ Besides these specific folate transporters, a number of other transport systems have been described to be involved in the efflux of antifolates, including the multidrug resistance-associated proteins MRP1–5 and the breast cancer resistance protein (BCRP or ABCG2).^{31,32}

Intracellularly, the classical antifolates undergo polyglutamation by FPGS, resulting in effective intracellular retention and increased affinity of the antifolates to their target enzymes.^{33–36} The nonclassical lipophilic antifolates such as talotrexin or trimetrexate (TMQ) are not substrates of FPGS and do not require activation by polyglutamation for anticancer activity.³⁷

Cellular targets: TYMS, DHFR, GARFT, and AICARFT

Antifolates are inhibitors of key enzymes in folate metabolism, namely DHFR, β -glycinamide ribonucleotide transformylase (GARFT), 5'-amino-4'-imidazolecarboxamide ribonucleotide transformylase (AICARFT), and thymidylate synthetase (TYMS). GARFT and AICARFT are two key enzymes of the de novo purine biosynthesis. In a first step, GARFT enables the formation of the purine imidazole ring of purines. The substrate for this reaction is 10'-formyl-THF, which is synthesized from THF and formate, a step mediated by 10'-formyl-THF synthetase. In a second step, AICARFT generates inosine monophosphate, which serves as the precursor for purine nucleotides adenylate (AMP) and guanylate (GMP). DHFR catalyzes the reduction of DHF to 5',6',7',8'-THF, which is converted to 5',10'-methyltetrahydrofolate (5',10'-MTHF), the substrate for TYMS.³⁸ TYMS catalyzes the initial step in DNA synthesis, in which deoxythymidine monophosphate (dTMP), a precursor of DNA synthesis, is generated from deoxyuridine monophosphate (dUMP), resulting in the oxidation of MTHF to DHF. This metabolic step is essential for de novo synthesis of thymidine nucleotides for DNA synthesis. DHFR was the first enzyme to be

identified as a cellular target for the antifolates aminopterin and MTX.³⁹ The latter exhibits its anticancer effect by almost irreversible inhibition of DHFR, with subsequent disruption of purine and pyrimidine synthesis.⁴⁰ Newer antifolates have been designed with a higher affinity toward DHFR, eg, talotrexin, with a 15-fold increased affinity when compared with MTX.³⁷

Mechanisms of resistance

Antifolate resistance might result from impaired cellular influx or increased efflux, impaired polyglutamation, increased expression, or mutation of cellular targets, or intracellular accumulation of THF cofactors. Various transport-resistant phenotypes have been described in MTX-resistant cell line models, some of them resulting from mutations of the *RFC* gene,^{41–43} and others from *RFC* overexpression.^{44–46} A genetic polymorphism within the *RFC* gene (80G > A) results in replacement of arginine in position 27 with histidine,⁴⁷ and is associated with a worse clinical outcome in children with acute lymphoblastic leukemia (ALL) receiving MTX.⁴⁸ In osteosarcoma, which is known for its intrinsic resistance to conventionally dosed MTX, mutations at the 3'-UTR and promoter methylation of the *RFC* were described.⁴⁹ The role of *FR* is less well characterized and more controversial. Although overexpression of *FR- α* was found to predict resistance to platinum-based chemotherapy in ovarian cancer patients,⁵⁰ suppression of *FR* expression by gene methylation was also found as a potential mechanism of resistance.⁵¹ Similarly, hypermethylation of the *PCFT* gene (*SLC46A1*) was found in a resistant HeLa cell line.⁵² Multidrug resistance-associated proteins (*MRP* or *ABCC*) 1–4 confer the efflux of MTX and have been shown to potentially confer resistance to MTX in cell line models.^{53,54} However, MTX polyglutamates have low affinity toward the *ABCC* transporters, which is why this type of resistance might not be clinically relevant. However, breast cancer resistance protein (*BCRP* or *ABCG2*) also transports polyglutamates out of the cell, and mutations within the *ABCG2* gene (at amino acid position 482) have been shown to confer resistance to various antifolates.^{55,56} Overexpression of P-glycoprotein (*MDR1*) is suggested to be important for antifolate resistance in the presence of a defective *RFC* or in case high doses of MTX are administered.⁵⁷ Impaired polyglutamation is another mechanism that is of special importance for the classical antifolates that undergo extensive polyglutamation to be active. Finally, amplification of the gene encoding for DHFR has been identified in ALL,⁵⁸ ovarian cancer,⁵⁹ and soft-tissue sarcoma⁶⁰ as a potential mechanism of resistance

to MTX, but the clinical relevance of such amplifications is unclear at present.⁶¹

Specific substances

Classical antifolates

The classical antifolates have a similar structure to MTX, utilize the *RFC* for entering human cells, and are subject to intracellular polyglutamation.

MTX

MTX is one of the earliest anticancer drugs and is extensively used in lymphoma, acute lymphoblastic leukemia, and osteosarcoma. The drug competitively inhibits DHFR and, to a lesser extent, *GARFT*, *AICARFT*, and *TYMS*. Although thymidylate depletion is the main cytotoxic driver of MTX, inhibition of *GARFT* and *AICARFT* also results in impaired purine synthesis. As a result of their inability to synthesize DNA and RNA, the malignant cells are unable to proliferate and cause further damage, resulting in cell apoptosis.

Pharmacology

7-Hydroxymethotrexate (7-OH-MTX) is the main metabolite in serum following MTX infusion,⁶² and it contributes to activity and toxicity. The concentrations of 7-OH-MTX exceed those of the parent compound in plasma shortly after the infusion.⁶³ Both MTX and 7-OH-MTX exhibit first-order pharmacokinetics.⁶² MTX is eliminated by renal excretion involving passive glomerular filtration and active tubular reabsorption and secretion. 7-OH-MTX is also renally cleared but more slowly than MTX. Renal elimination is prolonged in patients with renal impairment or third-space fluid collections, due to slow redistribution of MTX from these extravascular compartments.⁶² MTX is prone to drug–drug interactions, especially nonsteroidal antirheumatics (NSARs).⁶⁴ The uptake of MTX into the cell is primarily mediated by the *RFC* and, to a lesser amount, by the *FR- α* . Intracellularly, MTX undergoes extensive γ -polyglutamation by *FPGS*, and these negatively charged polyglutamates are retained intracellularly. Polyglutamates can also undergo hydrolysis by γ -glutamyl hydrolase (*GGH*, also known as folic polyglutamate hydrolase or *FPGH*) into short-chain polyglutamates.^{65,66} The MTX pentaglutamate moiety is most active, with K_i values 100 times below K_i values of the nonglutamated compound.

High-dose MTX

MTX at doses ≥ 1 g/m² is the backbone for treating diseases such as primary central nervous system lymphoma (PCNSL),

osteosarcoma, or ALL. Careful patient selection, adequate hydration and urinary alkalization, avoidance of drug interactions, drainage of third-space fluids, and TDM with appropriate adjustment of leucovorin (LV) rescue make HDMTX a well-tolerated treatment option most of the time. LV rescue starts 24 h after the start of MTX infusion at a dose of 15 mg/m² IV push every 6 h for 3 days and should be continued until serum MTX concentrations drop below 0.05 µmol/L. Despite supporting measures, acute renal failure is seen in ≤2% of patients receiving HDMTX, as a consequence of the precipitation of MTX and 7-OH-MTX in the kidney tubules.⁶⁷ Because of considerable interpatient variability, TDM is essential to identify patients at high risk for severe toxicity, and the need for increased hydration or prolonged LV rescue. Before TDM with supplemental LV rescue was incorporated into HDMTX regimens, toxicity was substantial, including a 6% fatality rate;⁶⁸ 80% of these fatalities were attributed to severe myelosuppression, which resulted in either sepsis or hemorrhage, and 20% were attributed to renal failure. Conventional treatment for HDMTX-induced renal dysfunction includes prompt escalation of LV rescue and adequate hydration and urine alkalization, provided adequate urine output can be maintained. MTX removal by hemodialysis is of potential value in refractory cases. Finally, carboxypeptidase-G2 (CPDG2), a recombinant bacterial enzyme that hydrolyzes MTX to the inactive metabolite 2,4-diamino-N10-methylptericoic acid (DAMPA), is another option in refractory cases of MTX-associated renal dysfunction. CPDG2 lowers plasma concentrations of MTX within 15 min of administration by roughly 99%.⁶⁹ More recent studies suggest individual exposure to MTX to be an important predictor of a favorable treatment response in patients with PCNSL,^{14,15} but this awaits prospective validation.

Raltitrexed

Raltitrexed is a selective and direct TYMS inhibitor. As an analog of the THF cofactor, it cannot be incorporated into DNA, and cellular accumulation of dUMP does not result in resistance to raltitrexed.⁷⁰ Its long-lasting inhibition of TYMS allows a convenient 3-weekly schedule of administration. Raltitrexed is approved in many countries (except the United States) for advanced colorectal cancer, but its utilization is mainly limited to patients who are intolerant to 5-fluorouracil (5-FU). Although raltitrexed proved to be equally active to 5-FU/LV in advanced colorectal cancer, there were some raltitrexed-associated deaths due to combined gastrointestinal and hematologic toxicity.⁷¹ Combining the phase III

MCRC trials, raltitrexed-related mortality (1.6%–4%) was greater than with 5-FU (1.2%–2.8%).⁷² This occurred in spite of a significantly lower all-cause serious toxicity rate with raltitrexed and has been attributed to administration of raltitrexed after a toxic event or in the presence of renal impairment.⁷³ Patient education, monitoring of renal function, and supportive measures are essential in the management of patients receiving raltitrexed.⁷⁴

Pharmacology

Raltitrexed predominantly enters the cell by RFC and then undergoes polyglutamation, with the polyglutamated form again being more potent than the parent compound. With repeated administration at 3-weekly intervals, no clinically significant accumulation of raltitrexed was found in patients with normal renal function.⁷⁵ Raltitrexed is contraindicated in patients with severe renal or hepatic impairment and/or clinically significant cardiac arrhythmias requiring drug therapy. The importance of dose reductions in patients with reduced creatinine clearance is critical in preventing subsequent severe toxicity. In patients after accidental overdosing or those suffering from severe toxicity, LV rescue is of potential value.

Pralatrexate

Pralatrexate (PDX; 10'-propargyl 10'-deazaaminopterin) is a newer antifolate that was rationally designed to have a high affinity for the RFC, resulting in increased cellular internalization.⁷⁶ In a phase II lymphoma study, PDX demonstrated some activity against peripheral T-cell lymphoma (TCL).⁷⁷ Subsequently, the multicenter confirmatory phase II PROPEL (Pralatrexate in Relapsed or Refractory Peripheral T-cell Lymphoma) trial has led to the approval of PDX in the United States for relapsed or refractory TCL.^{78,79} Treatment response in a total of 109 evaluable patients in the PROPEL trial was 29%, with 12 patients (11%) achieving a complete response.⁷⁹ The median duration of response was 10.1 months. Mucosal inflammation was seen in >70% of patients but was usually mild to moderate. Hematological toxicity consists of severe thrombocytopenia in a third of patients and severe anemia in 16% of patients. Febrile neutropenia was noted in 5% of cases. Patients should receive supplementation with B₁₂ and folic acid to avoid severe toxicity.

PDX was rationally designed to have high affinity for the RFC, which leads to better cellular internalization of the drug and a greater antitumor effect when compared with MTX.⁸⁰ The structural difference between PDX and MTX is based on the presence of a propargyl group substitution at

carbon 10 instead of the methyl group in MTX. The basis of increased efficacy of PDX *in vitro* is based on its increased affinity for the RFC, but whether this is enough to overcome MTX resistance is unknown. PDX effectively binds to and inactivates DHFR, depleting intracellular reduced folate stores and blocking DNA synthesis.

Lometrexol

Lometrexol (LMTX) is a potent and selective inhibitor of GARFT, with broad antitumor spectrum. GARFT catalyzes the formation of purines from the reaction of 10'-formyltetrahydrofolate (10'-FTHF) to THF. Its inhibition results in a depletion of intracellular purine levels. LMTX enters the cell via the RFC and undergoes extensive polyglutamation, with a slow elimination of polyglutamates. Without folic acid supplementation, severe cumulative myelosuppression and mucositis are likely.⁸¹ At present, LMTX is not approved as an anticancer agent.

Edatrexate

Edatrexate (EDX) is a classic antifolate with a more efficient intracellular polyglutamation compared with MTX.⁸² EDX polyglutamates are potent inhibitors of DHFR but less potent inhibitors of TYMS.⁸³ EDX exhibits saturable, nonlinear Michaelis–Menten pharmacokinetics, with $\leq 55\%$ of EDX undergoing renal excretion as unchanged parent compound.⁸⁴ As EDX was associated with severe stomatitis, toxic dermatitis, and even fatalities,⁸⁵ clinical development was halted.

Nonpolyglutamable classical antifolates

Talotrexin

Talotrexin (PT-523) is a newer antifolate and potent antagonist of DHFR. Talotrexin combines characteristics of both the classical and nonclassical antifolates. As talotrexin does not contain a glutamic acid side chain, it is not converted to intracellular polyglutamates, with a potential advantage for drug safety and less bone marrow toxicity.⁸⁶ The drug binds tightly to DHFR, with an inhibitory constant (K_i) of 0.35 pmol/L, 15-fold lower than for MTX. Talotrexin exhibits linear pharmacokinetics with a rapid initial disposition phase.⁸⁷ Patients with relapsed or refractory leukemia or MDS received talotrexin for five subsequent days, together with supplemental folic acid and vitamin B₁₂.⁸⁷ Dose-limiting toxicity was stomatitis, and talotrexin 0.6 mg/m²/day for 5 days every 3 weeks was recommended for phase II studies, where evaluation in patients with refractory ALL is ongoing.⁸⁷

Nonclassical antifolates

Nonclassical antifolates do not contain glutamic acid and are not polyglutamable; they are more lipophilic than the classical antifolates and enter cells by passive diffusion.

TMQ

TMQ is a nonclassical, lipophilic quinazoline derivative with a high inhibitory potency toward DHFR.⁸⁸ Because of its lipophilicity, TMQ can rapidly enter cells by a non-energy-dependent process. Unlike most antifolate drugs, TMQ is mainly eliminated by hepatic metabolism instead of renal excretion, with a terminal elimination half-life of 15–20 h.⁸⁸ Cell lines resistant to MTX because of deficiencies in folate transport generally retain their sensitivity to TMQ. As TMQ lacks the glutamic acid side chain, it cannot be polyglutamated and is not retained within the cell for prolonged periods of time.⁸⁹ Although TMQ has undergone broad phase II testing in solid tumors,⁹⁰ results were disappointing and there is no current indication in oncology.

Piritrexim

Piritrexim (PTX) is an oral lipophilic antifolate that is not a substrate of the active folate transport systems but enters cells via an energy-independent process and is effective against cancer cells resistant to MTX because of transport defects.⁹¹ PTX is not polyglutamated by FPGS, but it is a potent inhibitor of DHFR and TYMS. Oral absorption of PTX is rapid, with an overall bioavailability of 75%–95%.⁹² The terminal half-life following oral administration is 4.5–5.6 h,⁹² with hepatic metabolism being the primary route of drug clearance. Despite the potential as an oral antifolate, PTX did not show any therapeutic advantage over more established antifolates.

Nolatrexed

Nolatrexed (Thymitaq, TM) is a nonclassical, lipophilic antifolate and a noncompetitive, high-affinity TYMS inhibitor. TM causes extensive dTMP depletion and dUMP accumulation, causing thymineless cell death. TM is not dependent on the cell cycle, as high concentrations of TM failed to induce S-phase arrest but still resulted in apoptosis.⁹³ Although TM itself is lipophilic, it can be administered via intravenous infusion as a water-soluble dihydrochloride salt. Due to its lipophilicity, TM enters cells by passive diffusion and does not undergo polyglutamation.⁹⁴ TM was granted orphan drug status for the treatment of unresectable hepatocellular carcinoma by the US Food and Drug Administration (2001) and the European Medicines Agency (2007), based on a randomized phase III Asian study comparing TM with

doxorubicin, and two North American phase II studies.⁹⁵ TM has never gained widespread use in oncology.

MTA

Pemetrexed

Pemetrexed has been approved in combination with cisplatin as a first-line treatment for advanced non-squamous-cell lung cancer,¹⁰ as a single agent for relapsed NSCLC after platinum-containing chemotherapy,⁹⁶ and in combination with cisplatin for the treatment of pleural mesothelioma.⁹ Important is histotype-selective activity of pemetrexed, with a significant benefit seen only in patients with non-squamous-cell lung cancer,¹⁰ potentially as a consequence of increased TYMS expression in tumors of squamous histology.⁹⁷ Supportive treatment with oral folic acid 0.5 mg/day and intramuscular vitamin B₁₂ 1 mg every 9 weeks is routinely used, as it has been shown to reduce the incidence of potentially fatal myelosuppression.⁹⁸ Pemetrexed is a cell-cycle-dependent antifolate with a 6–5-fused pyrrolopyrimidine-based nucleus,⁹⁹ and it inhibits TYMS, DHFR, GARFT, AICARFT, and C1-THF synthase, which catalyzes the incorporation of a formyl group into 10'-FTHF for purine synthesis, and the incorporation of a methylene group into 5',10'-MTHF for thymidylate synthesis. Mechanisms of resistance include diminished accumulation of pemetrexed polyglutamates due to decreased activity of FPGS,¹⁰⁰ increased enzymatic cleavage of pemetrexed poly- γ -glutamates by high intracellular GH activity, and TYMS amplification.¹⁰¹ Inhibition of TYMS leads to intracellular accumulation of dUMP and subsequent efflux of deoxyuridine (dUrd) into the circulation, which can be used as a pharmacodynamic marker of in vivo TYMS inhibition following administration of pemetrexed.¹⁰²

Pharmacology

Pemetrexed is transported into cells mainly by the RFC and undergoes rapid intracellular transformation by FPGS to the more potent polyglutamate derivatives.¹⁰³ Pemetrexed has a small steady-state volume of distribution of about 15 L and is rapidly eliminated from plasma with a terminal elimination half-life of between 2 and 5 h at doses of 525–700 mg/m². Pemetrexed undergoes mainly urinary excretion as an unchanged parent compound. Furthermore, pemetrexed exhibits dose-proportional increases in plasma concentration and no signs of accumulation in patients with normal renal function. Third-space accumulation seems not to play a clinically significant role.¹⁰⁴ As pemetrexed is often combined with potentially nephrotoxic cisplatin, adequate monitoring of renal function is important. Recommendations

for the management of pemetrexed toxicity in the presence of renal failure have not been established, but treatment options with LV, folate, thymidine, carboxypeptidase, or hemodialysis are all possible.¹⁰⁵ Homocysteine is a marker for overall folate status in the body and predicted severe pemetrexed-associated toxicity in a clinical study.¹⁰⁶

Summary

The approval of pemetrexed for the first-line treatment of non-squamous-cell lung cancer, second-line treatment of NSCLC, and first-line treatment of malignant pleural mesothelioma has substantially added to the clinical importance of antifolates in oncology. Treatment individualization ever since has played an important role in the development of antifolate drugs. Although LV rescue and TDM are standard for HDMTX regimens, and folate and B₁₂ supplementation is standard for pemetrexed to increase MTD, new strategies will include pharmacogenetic markers such as tumoral TYMS expression for further improvement of antifolate treatment.

Disclosure

The authors report no conflicts of interest in this work.

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