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Mathematical Modelling of Folate Metabolism

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Abstract

Folate metabolism is a complex biological process that is influenced by many variables including transporters, co-factors and enzymes. Mathematical models provide a useful tool to evaluate this complex system and to elucidate hypotheses that would be otherwise untenable to test *in vitro* or *in vivo*. Forty years of model development and refinement along with enhancements in technology have led to systematic improvement in our biological understanding from these models. However, increased complexity does not always lead to increased understanding, and a balanced approach to modelling the system is often advantageous. This approach should address questions about sensitivity of the model to variation and incorporate genomic data. The folate model is a useful platform for investigating the effects of antifolates on the folate pathway. The utility of the model is demonstrated through interrogation of drug resistance, drug-drug interactions, drug selectivity, and drug doses and schedules. Mathematics can be used to create models with the ability to design and improve rationale therapeutic interventions.

Folate is an indispensable water-soluble vitamin and reduced folate is a cofactor essential for transfer and redox processing of one-carbon units including metabolism of nucleotides and certain amino acids.¹ Mathematical models of folate metabolism have existed in various forms since the 1970s with a common theme that the biology behind the computation is a complex, non-intuitive, non-linear system.^{2–5} Experimental studies of folate metabolism largely focus on a single reaction or small portion of the pathway. Mathematical models of the folate pathway in conjunction with the advent of genomic data and tracer based metabolomics allow us to investigate the full system that would be difficult by *in vitro* methods alone.

Models of folate metabolism have great utility including: testing hypotheses about mechanisms that are difficult to study experimentally; understanding the complex inhibitory effects drugs such as methotrexate (MTX) and fluorouracil (5-FU) have on the folate network and the outcomes of the network such as *de novo* pyrimidine and *de novo* purine synthesis; and explaining regulatory and homeostatic mechanisms that allow large fluctuations in one part of the network without affecting critical functions elsewhere in the network. The addition and availability of genomic data adds potentially new information that can help quantify modelling details. The goal of this focus article is to evaluate the use of the folate metabolism model system as a tool to explore and understand the mechanism of action of treatments or genetic polymorphisms that perturb this pathway. This process

includes how the inclusion of genomic data can help in the parameterization and sensitivity analyses of the models and how the models can be used to reveal therapeutic targets, predict drug sensitivity, and design combination treatments.

FOLATE PATHWAY DESCRIPTION

The well understood dynamics of the folate metabolism pathway make it a good system to model mathematically. While each of the models defined in the literature vary from one another they all follow a similar structure. One general description of the folate metabolic processes that are being described by these models is shown in Figure 1 with terms described in Table 1.⁶ The arrows in the diagram describe the enzymatic process of converting the various substrates (e.g. DHF to THF by the enzyme DHFR) and are usually modelled as a Michaelis-Menten process. Allosteric inhibitory effects are indicated with the lines with bars. These either describe internal feedback (e.g. the inhibitory effects of SAM on MTHFR) or the inhibitory effects of antifolates (MTX in this example). The goal of all the studies has been to use mathematical and systems biology tools to describe this pathway and to deploy these models to test hypotheses about the system.

Modelling Assumptions

Assumptions are inherent in the development of models of the folate metabolism pathway. It has been pointed out that these assumptions are no different than those made when developing an *in vitro* experiment. For example, Nijhout *et al.*⁴ note that in an *in vitro* experiment one may assume that an inhibitor has no other effects other than its intended target and in a model one would typically only model the effects of the inhibitor on the term describing its known target.

The assumption that the folate metabolism system is at steady-state is used in most studies. While this is reasonable when describing an *in vitro* experiment with cells actively proliferating, it is not necessarily appropriate where only a portion of cells are proceeding through the cell-cycle. This is due to the large fluctuations in activity of some of the folate metabolism enzymes during different phases of the cell-cycle (e.g. DHFR and TYMS).⁷ Another common assumption is that most of the enzyme reactions are modelled using Michaelis-Menten kinetics. Inherent in this assumption is that the enzyme is working at capacity and therefore the occupancy rate is approximately constant. Other model assumptions commonly made are that substrates other than folate components are considered constant. For example, in the conversion of DHF to THF by DHFR it is typically assumed that NADPH is constant.

One of the more difficult challenges with the folate metabolism model is its parameterization, so it is important to understand the assumptions that are involved in determining these parameters. Typically parameters for these models come from multiple diverse sources which are not always from the same species. For example, parameters for a single model have been derived from L1210 murine leukemia cells, L5178Y murine leukemia cells, Ehrlich ascites carcinoma cells, chicken liver, and beef liver.³ In addition, some are determined in whole cell assays, some with isolated enzymes. It has been noted that this approach to defining parameters from multiple diverse sources for larger systems is consistent with current systems biology approaches.⁸ However, the validity of the assumption that one can combine parameters from these various sources should be addressed. Several approaches that could be used include a study of the sensitivity of the model to changes in these parameters, an analysis of how these parameters may be more refined using genomic data, and the incorporation of tracer based approaches to describe fluxes through the system.

Model Variations and Complexity

Mathematical models to describe the folate metabolism pathway have been developed and analysed by many groups and the form and complexity of these models vary depending on the goals of the studies. Some variations to the main components of a basic folate metabolism model³ include the inclusion of folate polyglutamation^{9, 10}, allosteric interactions¹¹, methionine cycle¹², glutathione synthesis¹³, and subcellular compartments such as the mitochondria.¹⁴ This increasingly complex group of models leads us to the questions: do more complex models yield more accurate/robust results, or are more parsimonious models a better option? One approach to addressing these questions is through sensitivity analysis. For example, if the main objective of a study is to determine the inhibitory effects of an antifolate on DNPS, then sensitivity analysis methods can be used to determine whether the inclusion of additional components to a basic folate metabolism system, such as the methionine cycle, significantly affects the dynamics of DNPS inhibition. Another approach to addressing complexity is through reduction techniques.¹⁵ These provide a method to analyse the mathematical system for redundant terms. Depending on the intent of the model and the availability of appropriate parameters, these redundant terms may or may not be appropriate to keep in a model.

An interesting example of the function of a very reduced model is from a study of changes in homocysteine levels due to MTX.¹⁶ This indirect response model only explicitly considered the formation (by SAM, SAH) and elimination (by MTR) of homocysteine along with the effects of MTX on these dynamics. This model was sufficient to quantify changes in homocysteine as a function of MTX and the effects of leucovorin (THF) rescue on the homocysteine dynamics. However, a higher level version of the model would be justified to address details of other mechanisms that may be involved in homocysteine dynamics.

FOLATE METABOLISM MODEL PARAMETERIZATION

Folate pathway model parameterization is one of the more challenging aspects of designing models that accurately represent the system dynamics and current models do not address the variability in parameters within populations. Genomic data and tracer based approaches are two techniques that can help address these challenges.

Genomic Data

The abundant genomic data made available over the past several years is one source of information that can be used to address these issues.^{8, 17, 18} By using genomic data across individuals and assuming there is a correlation between gene expression of an enzyme and its activity, one can define how individual patient's parameter values differentiate from a set of base parameters. For example, Radivoyevitch¹⁷ used leukemia blast gene expression data from frontline ALL studies^{19, 20} to determine relative changes in V_{MAX} parameters in a folate model (based on the model from Morrison and Allegra⁹). This approach gave very similar predictions on the flux of DNPS and *de novo* thymidylate synthesis relative to direct empirical analysis of gene-gene correlations. One advantage of using this model is that it can help one understand the mechanisms behind these correlations. In addition, with a model appropriately parameterized to a specific study (such as frontline ALL), more study specific hypothesis testing can be accomplished.

The idea of using gene expression data to help quantify enzyme and transporter kinetics has also been studied in the context of physiologically based pharmacokinetic modelling. Meyers et al.¹⁸ used gene expression data to approximate tissue specific protein abundance for drug metabolizing enzymes and transporters. Tissues with higher expression were modelled with relatively higher kinetic parameters. This approach was shown to provide better model performance compared to models that did not consider expression data.

These approaches give a basis for how the parameterization of the folate pathway model can be individualized using genomic data. However, such approaches do not consider the potential effects on protein function of a missense coding SNP, which can alter catalytic activity of the encoded protein without altering the overall level of mRNA expression or abundance (e.g., TPMT SNPs alter function but not mRNA expression). Additionally, transcript levels may not correlate with protein levels complicating interpretation of mRNA based measures of the folate pathway. Models that incorporate both the potential for genomic variation to alter enzyme activity and the possible disconnect between mRNA and protein levels may have increased ability to more robustly characterize biological systems influencing folate metabolism.

Tracer Based Approaches

Tracer based approaches have been used to determine the flux through network pathways in a variety of systems.^{10, 21–27} The general idea is to radio-label a compound and follow its progression through the network pathway. Several examples of where these approaches have been used with the folate pathway include using ¹⁴C histidine and ³H methionine to follow changes in oxidative and reductive carbon flux in the presence of MTX²¹, and using the conversion of ¹⁴C formate to ¹⁴C adenine and ¹⁴C guanine to follow changes in DNPS in the presence of MTX.²⁷ These tracer based approaches provide another method to improve the parameterization of folate pathway models.

MODEL PARAMETER SENSITIVITY

Methods for analysing the sensitivity of the system to both small and large perturbations are necessary to address the following questions: are enzyme kinetic parameters derived from isolated enzyme studies sufficient to describe whole cell folate pools;⁹ and, what are potential therapeutic targets in the folate pathway? Many studies have evaluated some form of model sensitivity analysis. However, they usually use a local approach where they perturb one or two terms in the system and observe how it affects the system as a whole. For example, Ulrich *et al.*⁷ used a folate model to test hypotheses about the effects of both known and yet unidentified polymorphisms. They highlight the robustness and sensitivity of the folate system to genetic variation. In particular, they showed that allosteric interactions (e.g. SAM inhibits MTHFR and BHMT and activates CBS) tended to have a stabilizing effect on the DNA methylation rate when there were large fluctuations in the methionine input.

With the numerous parameters in the system, most of which were defined independently in isolated enzyme studies, it is reasonable to consider a more complex global approach to determining sensitivity.^{28, 29} One general method is to use a Monte Carlo sampling approach such as Latin hypercube sampling to efficiently sample the parameter space multiple times and then use a partial rank correlation coefficient or Fourier amplitude sensitivity test to quantify how changes in individual model parameters or groups of model parameters affect the system while accounting for the variability in the remaining parameters.²⁸ This approach typically assumes that the model parameters are independent, though correlation structures can be included. For example, a biologically based method to define the distribution of and correlation pattern in the folate model parameters is to leverage the microarray gene expression data and LD structure in genetic polymorphisms. The use of this approach has the potential to yield a more robust and complete understanding of how sensitive the system is to biologically plausible perturbations such as those resulting from polymorphisms in folate pathway genes.^{7, 30} An example of the correlation structure of folate related gene expression is described by Kager *et al.*³¹ They showed that expression levels of DHFR, TYMS, MTHFD1, MTHFD2, ATIC, GART, and RUVBL2 are strongly correlated with each other. This suggests an appropriate approach to investigating the sensitivity of the

model is to vary this group of enzymes (parameters) in the same correlated manner. As noted earlier, this assumes that expression level correlation translates to correlation in enzyme activity.

In these studies, genetic variation was modelled as a change in $V_{\max}$. What has not been considered are variations in K_m . Since these parameters are usually estimated based on *in vitro* assay data, it is also important to consider how sensitive the system is to perturbations in them. The challenge here is to determine how this group of parameters should be varied.

The traditional approach to address parameter sensitivity is to perturb the system from its steady-state and observe the change in that steady-state. A different question is, how do continuous large fluctuations in the system parameters (e.g. due to cell-cycle fluctuations in DHFR or TYMS) influence the folate metabolism system. One approach, proposed by Nijhout et al.¹¹, is to use fluctuation theory. Expansion of this and similar ideas has the potential to give us a better understanding of the variability in the folate metabolism system.

FOLATE METABOLISM MODELS AND CHEMOTHERAPY

One important use of folate metabolism models is to investigate effective approaches for antifolate therapy. These models are particularly useful for this task as they can effectively test hypotheses on drug sensitivity/resistance, drug-drug interactions, drug selectivity, or pharmacokinetic dose/schedule. The models are useful both retrospectively---they can help interpret results from an experimental study, or prospectively---they can help determine which experimental study or clinical trial will more likely be successful.

Drug Sensitivity and Resistance

Models of folate metabolism have been helpful in understanding the dynamics of antifolate drug resistance. Some of the earliest studies by Jackson and Harrap³ used folate models to describe potential mechanisms of MTX resistance. Their model showed that increases in DHFR activity, decreases in drug transport, and elevated levels of TYMS had varying effects on MTX inhibition. Since these early studies by Jackson *et al.*^{2, 3, 32–36} there have been a variety of modelling and experimental studies that investigated differences in folate related enzymes and transporters as related to drug resistance. These studies have helped explain differences in intracellular MTX accumulation and pharmacodynamic effects of MTX in leukemia cells and support many of Jackson *et al.* hypotheses on antifolate drug resistance.

Several studies investigated differences in the reduced folate carrier (RFC) in pediatric ALL blasts.^{31, 37} These have shown that RFC (SLC19A1) expression is significantly higher in B-lineage hyperdiploid (BHD) ALL cells and lower in E2A-PBX1 ALL cells compared to other subtypes of ALL. In a similar manner, the expression of the efflux transporters MRP1 (ABCC1) and BCRP (ABCG2) are higher in T-lineage ALL and TEL-AML1 ALL cells respectively (Figure 2). This translated to higher MTXPG accumulation in BHD and lower in E2A-PBX1, T-lineage, and TEL-AML1 ALL cells (Figure 3). Because of the relationship between higher MTXPG accumulation, greater depletion of ALL blasts, and inhibition of DNPS,^{27, 38, 39} these results support the hypothesis made by Jackson that drug transport was a factor involved in antifolate drug resistance.

Changes in DHFR and TYMS activity have also been predicted to have a significant impact on antifolate drug resistance. An early *in vitro* study showed that the MTX resistance murine leukemia cell-line L5178YR had 300-fold higher DHFR levels compared to the parental cell-line L5178Y. This translated to an greater than 100,000-fold increase in resistance to MTX.⁴⁰ There are also *in vivo* data to support this model derived hypothesis. Specifically,

the *in vivo* antileukemic response to MTX (measured as a relative change in circulating leukemia cells from day 0 to day 3 of MTX treatment) was negatively associated with TYMS and DHFR activity. Furthermore, TYMS, DHFR, and *in vivo* response to MTX were significantly related to ALL treatment outcome (i.e., 5 year DFS).³⁹

By retrospectively considering *in vivo* studies, one can develop additional hypotheses on drug sensitivity and resistance. For example, Zaza *et al.*⁴¹ showed that several genes involved in purine metabolism were differentially expressed (lower) in the TEL-AML1 ALL subtype and this subtype had lower DNPS activity. In addition, this subtype had lower MTXPG accumulation (Figure 3) and Kager *et al.*⁴¹ described how genes involved in folate transport, metabolism, and polyglutamylation were differentially expressed in different ALL subtypes, including TEL-AML1 (Figure 2). Data from these results can help validate folate pathway model simulations and help develop and test hypotheses on why TEL-AML1 ALL has a favourable prognosis.

The above clinical studies show at least qualitative support for some of the early hypotheses made with the folate metabolism models. By leveraging additional clinical and genomic data, one can perhaps develop more quantitative models that better define inter- and intra-individual variability. These models can then be used to guide one to a more complete understanding of resistance to antifolates such as MTX and help determine effective strategies to overcome these resistance mechanisms. For example, could adjusting the MTX dose, based on MTX transporter expression, DHFR, and TYMS activity (similar to how dose is already adjusted based on ALL subtype and other prognostic features) be an effective approach to overcome the observed differences in MTXPG accumulation, acute *in vivo* response to MTX, or ultimately treatment outcome?

Drug-Drug Interactions

Early studies considered the effects of combinations of MTX with 5-FU³, 6-MP³², and Ara-C² in both mathematical models of folate metabolism and in *in vitro* studies. These studies showed the advantage of using a mathematical model to investigate drug-drug interactions. Unlike empirical methods for defining drug interactions and determining synergy or antagonism (e.g. response surface modelling⁴²), the folate metabolism models can be used to explore the possible mechanisms of these interactions. For example, model simulations of the MTX and Ara-C combination predicted either synergistic or antagonistic interactions depending on TYMS activity, where lower TYMS activity related to a more synergistic effect.² Sensitivity analysis along with the availability of genomic data makes studies of drug combinations even more practical.

Drug Selectivity

Differences in enzyme activity can explain activity and selectivity of drugs. For example, it has been hypothesized that higher TYMS activity in cancer cells may lead to the selectivity of MTX.² This supports the idea of the cell-cycle selectivity of MTX (and similar drugs). Specifically, TYMS and DHFR activity in cells not in S-phase is about 1 to 10% of their activity when in S-phase. These cell-cycle effects have been included in several folate metabolism models^{9, 10, 43, 44} and can be leveraged to address questions on antifolate selectivity.

There have also been recent genome wide analyses that have described the association of the folate transporter SLCO1B1 with MTX PK and GI toxicity indicating that drug transport is also involved in modulating the effects of antifolates.^{45, 46} Specifically, SNPs in SLCO1B1 showed slower MTX clearance and lower gastrointestinal toxicity (thought to be due to

lower concentrations of MTX in either the gastrointestinal tract or enterocytes due to lower SLC01B1 transport from blood to bile due to the functional SNP).

Pharmacokinetics and Defining Effective Doses and Schedules

Most early studies (both experimental and modelling) considered the effects of antimetabolites on folate metabolism in the *in vitro* setting. In several frontline pediatric ALL studies both plasma and intracellular MTX and its active metabolites, MTXPG, were serially measured.^{47–49} Using these data, a pharmacokinetic model was developed that allowed characterization of the accumulation of MTX/MTXPG in ALL cells. These results helped elucidate determinants of variability in MTX accumulation. Specifically, the analysis showed that model estimated parameters including those related to FPGS activity, MTX active transport, and GGH activity were significantly different across pediatric ALL subtypes and could explain a significant portion of the variability in MTXPG accumulation. Furthermore, this study also related differences in accumulation of intracellular MTX/MTXPG to differences in folate related pharmacodynamic effects of DNPS inhibition by linking the pharmacokinetic model of the plasma and intra-cellular MTX/MTXPG to a model of the folate pathway.⁴⁴ The parameters used for the folate pathway model were those defined in previous studies.^{9, 44} Although these parameters were from various independent sources and variability in these parameters was not considered in the model, the results of these simulations were informative. For example, the simulations showed that groups with lower accumulation of intracellular MTXPGs (e.g. T lineage or shorter MTX infusion length) had less inhibition of DNPS (Figure 4). This combined MTX pharmacokinetic and folate pathway model allowed us to predict the differences in DNPS inhibition due to the variability in intracellular MTXPG pharmacokinetics and various doses and schedules of MTX.

Conclusion

Our understanding of the biological, genetic and pharmacological basis for differences in and perturbations of folate metabolism by either endogenous or exogenous means is certainly enhanced by mathematical models of the folate pathway. One of the main strengths of these models is their use for hypothesis testing. The ability to model the language of nature in mathematics allows for rapid prototyping and exploration of many different types of systems at a much lower cost. Specifically, mathematical models of folate metabolism have been used to address a wide variety of questions including the relative sensitivity or resistance to drugs and drug selectivity. Perhaps one of the more tangible benefits of these mathematical models is the increased understanding of drug-drug interactions that would be impossible to otherwise discern without understanding the fundamental interactions in the underlying networks and pathways of drug targets. These modelling studies along with the *in-vitro* and *in-vivo* data suggest a dynamical approach to addressing these therapeutic questions similar to the learn-and-confirm paradigm commonly used in pharmacokinetic drug development.⁵⁴ First the folate pathway model is used to test a hypothesis (learn) then experimental data is used to confirm the hypothesis. This is an adaptive process where depending on the outcome of the confirmation step the model structure or parameterization may be altered to better describe the system.

In an effort to expand these existing folate models to improve the design of therapeutic interventions, several limitations of the current models and approaches to hypothesis testing are being addressed. First, population and individual specific parameterizations that adequately represent the variability in the model parameters, especially the more sensitive parameters, is needed. Sensitivity analysis helps prioritize, in a rational sense, the parameters that require more robust prediction while genomic data and tracer based approaches allow one to fine-tune these model parameters for specific study populations.

Second, as the ability to model more parameters increases, the benefits of this increased complexity and detail should be considered. Do these increases in complexity help or hinder the ability to rationally explain the pharmacodynamics of medications and determine effective schedules and doses of medications?

In the end, a balance must be struck between increased model complexity and the increased ability of that model to reflect nature. Mathematical modelling of complex biological systems including folate pathways is more than an exercise in annotation and description; it has practical benefits on one's ability to design rationale therapeutic interventions.

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Computation/software tools

There are many tools for modelling biological systems *in silico*, ranging from point and click systems to more in depth low level programming.⁵⁰ One common format for representing systems biology models is **SBML**⁵¹ (www.sbml.org) which is used in many modelling software packages.

Matlab (The MathWorks, Inc.) is a flexible computing environment and with the addition of the SimBiology toolbox, it provides an environment for building models either by importing a SBML coded model, by using its graphic editor, or by explicitly coding the system of model equations. It provides tools to analyse the models including options for simulations and sensitivity analysis. In addition, it provides tools for model parameter estimation including nonlinear least squares and nonlinear mixed effects approaches.

The **R computing environment** (www.R-project.org) provides an open source option for analysing systems biology models. In addition, the R package Bioconductor⁵² provides an interface for microarray analysis tools and SBMLR⁵³ adds an interface with SBML coded models along with systems biology analysis tools.

MoBi (Bayer Technology Services), like the Matlab toolbox SimBiology, provides a GUI interface for developing models and simulation tools for both individual and population simulations. It also provides the option to include physiologically based pharmacokinetics through PK-Sim (Bayer Technology Services) and to link its models to either Matlab or R so one can take advantage of the features of those tools.

Figure 1. Folate metabolism pathway also depicting the inhibitory effects of methotrexate. The abbreviations are described in Table 1. [Copyright: PharmGKB Reprint permission given by PharmGKB and Stanford University.]

Figure 2.

Gene expression of FPGS and 3 transporters with known MTX transport capacity in ALL subtypes. Box plots with medians, quartiles, and ranges excluding outliers (circles) of log mRNA expression are depicted for folylpolyglutamate synthetase (FPGS) (A), reduced folate carrier (RFC, or SLC19A1) (B), multidrug resistance-associated protein 1 (MRP1, or ABCC1) (C), and breast cancer resistance protein (BCRP, or ABCG2) (D). Data from 197 patients were plotted (BHD, $n = 42$; BNHD, $n = 58$; E2A-PBX1, $n = 21$; T-ALL, $n = 35$; TEL-AML1, $n = 41$). P values were determined by the Kruskal-Wallis test. The red boxes indicate subtypes in which gene expression was significantly higher, whereas the green boxes indicate subtypes with significantly lower expression of the gene depicted.

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Figure 3.

Box plot of intracellular concentration of total methotrexate polyglutamates (MTXPG₂₋₇) according to ALL subtypes, following in vivo treatment with 1 g/m² MTX infused over 24 hours. MTXPG accumulation (picomoles per 10⁹ bone marrow ALL cells) is shown for hyperdiploid B-lineage ALL (BHD, n = 19), nonhyperdiploid B-lineage ALL without defined molecular genetic abnormalities (BNHD, n = 39), ALL with E2A-PBX1 fusion (E2A-PBX1, n = 5), T-ALL (n = 14), and ALL with TEL-AML1 fusion (TEL-AML1, n = 24). Medians, quartiles, and ranges excluding outliers (circles) are depicted. P values are from pairwise comparisons using the Wilcoxon rank sum test after adjustment for multiple testing. [Reproduced from Kager et al.³¹ The journal of clinical investigation by

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Figure 4.

Simulations of the effects of ALL lineage and MTX dosage on model estimated DNPS activity. The curves represent the median and the shaded regions represent the quartiles of the results from the respective simulated patient populations. (A) Simulated percent change in DNPS vs time after a 1 g/m² dose of MTX. Solid curve and Red shading: B-lineage Hyperdiploid; Dashed curve and Blue shading: T-lineage. (B) Simulated percent change in DNPS at 42 hrs vs Dose. Solid curve and Red shading: 24 hr MTX infusion; Dashed curve and Blue shading: 4 hr MTX infusion. [Reproduced from Panetta *et al.*⁴⁸]

Table 1

Folate metabolism pathway abbreviations.

Abbreviation	Full Name
10-CHO-THF	10-Formyltetrahydrofolate
5,10=CH-THF	5-10-Methenyltetrahydrofolate
5,10-CH ₂ =THF	5-10-Methylenetetrahydrofolate
5-CH ₃ -THF	5-Methyltetrahydrofolate
5-CHO-THF	5-Formyltetrahydrofolate
ADA	adenosine deaminase
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
BHMT	betaine homocysteine methyltransferase
CBS	cystathionine-beta-synthase
DHF	dihydrofolate
DHFR	dihydrofolate reductase
DNPS	<i>de novo</i> purine synthesis
FPGS	folylpolyglutamate synthase
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase
GGH	gamma-glutamyl hydrolase
MTHFD1	methylenetetrahydrofolate dehydrogenase (NADP ⁺ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase
MTHFR	methylenetetrahydrofolate reductase
MTHFS	5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
PPAT	phosphoribosyl pyrophosphate amidotransferase
SHMT1	serine hydroxymethyltransferase 1
THF	tetrahydrofolate
TYMS	thymidylate synthetase

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