Role of methotrexate in juvenile idiopathic arthritis: where we have been and where we are going

Methotrexate is a cornerstone of therapy worldwide for juvenile idiopathic arthritis, yet there remains vast variability in drug dosing and administration, as well as unpredictable outcomes on the drug. Recent efforts through worldwide organization and collaboration have resulted in large registries from which we can now collect clinical data, attempts to standardize therapeutic management with methotrexate and ongoing goals to further individualize drug therapy. Recent studies in juvenile idiopathic arthritis have begun to predict outcomes on the drug by investigating clinical, genetic and cellular biomarkers in children. Pediatric rheumatology as a field has grown from extrapolating data from adult studies to overcoming the barriers to conduct needed investigations in children; however, there remains much to learn and discover about this commonly used drug in juvenile idiopathic arthritis. This nonsystematic review will briefly discuss the history of methotrexate use in juvenile arthritis, the current clinical challenges practitioners face in the variability of drug utilization and drug outcomes, and highlight recent research that has focused on investigating factors that contribute to this variability.

KEYWORDS: individualized therapeutic, juvenile idiopathic arthritis, methotrexate, pediatric rheumatology, pharmacogenomic

Mara L Becker
Children’s Mercy Hospitals & Clinics, Division of Pediatric Rheumatology & Clinical Pharmacology & Medical Toxicology, MO, USA
mlbecker@cmh.edu

History of juvenile idiopathic arthritis

Although the observation of arthritis in children was first described in the late 19th century by the well-known pathologist George Fredrick Still amongst others [1–3], it took time to widely accept that juvenile arthritis was an entity distinct from adult rheumatoid arthritis (RA). Centers for childhood rheumatic diseases were established in England (Taplow) and Germany (Garmish-Partenkirchen) in the mid-20th century [4]; however, there remained a lack of formal recognition of juvenile arthritis by major organizations such as the International League Against Rheumatism (ILAR) and the American Rheumatism Association (ARA) until the 1970s. When the first ARA Council on Pediatric Rheumatology met at Park City (UT, USA) in 1976, there were no more than 30 identified pediatric rheumatologists in the USA at that time [4].

Not surprisingly, therapeutic advancements in children with rheumatic disease have been historically slow, secondary to many factors including the elusive etiology and pathophysiology of these rare diseases, a paucity of physicians dedicated to treat these conditions in children and the fact that children in general have been termed ‘therapeutic orphans’ largely ignored by pharmaceutical companies in the quest for more lucrative adult markets not plagued with the difficulties of studying a vulnerable and developmentally dynamic population of patients [5–7].

Fashioning after adults with RA, the earliest identified therapies for juvenile rheumatoid arthritis (JRA), or juvenile chronic arthritis (JCA; old terminology largely determined by what continent one practiced upon), included salicylates, glucocorticoids and NSAIDs. However, the efficacy and safety of even NSAIDs in children was largely unknown until studies were performed in the late 1970s spearheaded by the Pediatric Rheumatology Collaborative Study Group (PRCSG) [8]. These initial therapies targeted the known excessive inflammation; however, further advancements were made with the development of ‘disease-modifying antirheumatic drugs’ (DMARDs) in the 1980s, specifically methotrexate (MTX), which, although slow acting, appeared to be effective and better tolerated than other drugs in its class.

Evolution of therapy with MTX in JIA

After MTX was approved for the treatment of RA, pediatric rheumatologists soon explored its use in the pediatric population. Several small uncontrolled descriptive studies supported the effectiveness of MTX in children with
JRA (9-13). Additionally, one of the few double-blind, randomized, placebo-controlled studies in pediatric rheumatology that required worldwide collaboration to complete, supported the efficacy and safety of low-dose MTX (10 mg/m²/week) in children with JRA with at least four active joints resistant to NSAIDs [14]. This evidence was enough to solidify MTX as a safe and effective option in the small armamentarium of therapies used to treat childhood arthritis at the time, and justified its use to clinicians desperately in need of improved therapeutics for children with arthritis.

With this valiant initial global effort and now decades of extensive clinical experience with the drug, methotrexate has an official US FDA indication for use in only polyarticular JRA (the subtype most closely resembling adult RA); however, it does not carry a similar indication from the EMA. Newer biologic agents, by contrast, have benefited from recent US pharmaceutical company incentives supported by the FDA through the Best Pharmaceuticals for Children Act (BPCA) and Pediatric Research Equity Act (PREA) to extend their labeling to include children. Therefore, newer biologic agents that target TNF-α (etanercept and adalimumab), IL-6 (tocilizumab) and CTLA-4 (abatacept) have subsequently been approved for use in JIA over the last decade by both the FDA and EMA, but with the exception of tocilizumab, generally require failure of nonbiologic DMARDs such as MTX before approval for use, and often coadministration of both MTX and biologic agent is optimal. It is an interesting position for clinicians to be in to have older drugs with years of extensive experience not officially indicated for use in JIA; however, newer more costly biologic options that have an official indication cannot be used unless there is failure of an initial (often nonindicated) DMARD drug.

MTX is traditionally considered to be the first-choice second-line therapeutic option for children who fail NSAID therapy; however, with the recent trends for early and aggressive therapy for JIA, utilizing MTX as a first-line option, or as an anchor drug with a biologic DMARD, has been shown to result in rapid clinical remission in some JIA patients [15]. Furthermore, over the last two decades, and even in recent trials such as the Trial of Early Aggressive Therapy (TREAT) in polyarticular JIA study [15], outcomes on MTX have remained plagued with variability in efficacy and toxicity to the drug and there remain ongoing debates about optimal dosing and route of administration.

### Exploring variability in MTX therapy in clinical practice

In a recently published manuscript by Beukelman et al., nearly three-quarters of all JIA patients in the Childhood Arthritis and Rheumatology Research Alliance (CARRA) registry have received MTX at some point in their treatment course (1939 of 2748 JIA children total) [16], yet despite this vast use, there remains variability in several areas with regards to MTX therapy in JIA. Treatment practices, including the initial dosing strategy of MTX, the time to initiate MTX and even when to stop MTX have no clear guidelines from which to make these decisions, although strides are being made to answer these questions.

Optimal dosing and route of administration has been an ongoing debate in children. Although the original 1992 randomized controlled trial comparing two doses of MTX (5 mg/m²/week vs 10 mg/m²/week) with placebo supported efficacy of the 10 mg/m²/week dosing regimen, increased doses have been suggested, based on the fact that many patients did not reach remission on low-dose MTX and children generally require higher doses per body surface area than adults. In a retrospective cohort study investigating two different MTX dosing preferences (high vs low) in two pediatric centers in close proximity, there appeared to be no significant therapeutic advantage observed with higher initial MTX doses, although elevated liver transaminases occurred more frequently and severely in the higher dosed group [17]. This study is, of course limited, by its retrospective design, unable to control for confounders as in a prospective randomized study. Data supporting an increase in MTX dose for nonresponders to conventional dosing is mixed. Early reports supporting the safety and efficacy of higher dosing regimens (25–30 mg/m²/week) [18] have been followed with studies that do not support additional gains with higher doses [19]. However, these studies targeted some of the most severely affected JRA patients and were mostly descriptive in nature. A multicenter prospective study by Ruperto et al. investigated a randomized increase in MTX dose to intermediate (15 mg/m²/week) and high dose (30 mg/m²/week) in JIA patients who were nonresponders to initial low dosing after 6 months, and found an additional 60% clinical improvement in both intermediate and high-dose groups, but no significant improvement gained from the high-dose group [20]. Adverse events were not significantly different between intermediate and high-dosing groups, and

[19]
dropout rates were not different between the initial low-, intermediate- or high-dose groups [20]. Utilizing 15 mg/m²/week dosing has become standard in recent trials [15], and the recently published 2011 ACR Recommendations for Treatment of JIA assumes MTX dosing to be 15 mg/m²/week administered via the parenteral route [23].

Route of administration, however, also continues to be debated. With higher doses of MTX there are alterations in the oral bioavailability of the drug, and earlier work suggested the increased effectiveness of subcutaneous MTX administration in patients who did not respond or tolerate initial oral dosing [22]. Although the ACR recommendations for treatment of JIA assume the parenteral route of administration [21], and a majority of the MTX users in the CARRA registry (74%) had received MTX via the subcutaneous (sc.) route [16], oral administration of the drug is still quite prevalent. In the recently published large observational German Methotrexate Registry of a subgroup of patients who remained on an uninterrupted route of administration for 6 months or longer, over half of the patients (63%) received oral MTX exclusively, and this group had similar rates of ACR Pediatric (Pedi) 30, 50 and 70 response as well as toxicity to subjects treated with sc. dosing [23]. Although this is a retrospective observational study at risk for confounding by indication, it certainly brings the issue of route of administration to the forefront yet again. To fully answer this question, there will likely need to be a large prospective randomized controlled study comparing MTX outcomes on the two routes of administration, and additional pharmacogenomic studies investigating drug absorption and distribution, taking into account the ontogeny and function of MTX transporters in the GI tract and liver to better understand route-related differences.

Utilization of MTX and the time to initiate MTX is also variable based on the subtype of JIA. In the recently reported CARRA registry, JIA patients with oligoarticular JIA (53%) and enthesitis-related arthritis (ERA; 63%) were the least likely to ever receive nonbiologic DMARDs such as MTX, compared with RF+ polyarticular (91%) or extended oligoarticular JIA patients (89%) [16]. The evidence and consensus-based ACR recommendations also utilize MTX differently by JIA subtype, with most subtypes using a ‘step-up’/escalation approach incorporating MTX after initial NSAID and/or intra-articular corticosteroid failure [21]. The exception is with JIA patients with high disease activity, poor prognostic or systemic features, where MTX (or biologics for systemic JIA) are recommended to be used earlier, even first line [21]. The recent TREAT trial investigated two aggressive treatment arms to induce clinically inactive disease in the early ‘window of opportunity’ in polyarticular JIA patients, by randomizing to either MTX 0.5mg/kg/week plus etanercept 0.8 mg/kg/week plus prednisolone 0.5 mg/kg/day tapered to 0 over 4 months; or MTX 0.5 mg/kg/week SC (maximum 40 mg weekly) [15]. Interestingly, by month 4, 71 versus 44% (p = 0.01) of JIA patients achieved ACR Pedi 70, respectively, and by month 6, 40 versus 23% (p = 0.09) reached clinically inactive disease (CID) on medications [24], and even fewer remained in remission by 12 months on therapy [19]. While there was no statistically significant difference in CID at 6 months between groups, the trial illustrated two important points; there remains significant variability in clinical response both to biologic and nonbiologic DMARDs over time and there is a subset of patients who can reach early excellent outcomes on MTX therapy alone. Identifying these optimal responders remains the challenge, but may permit the avoidance of costly medications in a subgroup of patients who may not need them.

Once disease is well controlled, there is very little guidance on when to discontinue the medication, and in doing so, one must decide whether the risk for disease flare with drug discontinuation outweighs the risk for potential medication toxicity with unnecessary drug exposure. Studies report that remission is only sustained in approximately 50% of patients who discontinue the medication [11,24], and there have been very little data to guide the timing of discontinuation of therapy in JIA [25] until recently [26]. The myeloid-related protein (MRP) 8 (S100A8) and MRP 14 (S100A9) heterocomplex (calprotectin or MRP8/14), an endogenous activator of toll-like receptor-4 [27], secreted by activated phagocytes at local sites of inflammation [28], has been shown to be a marker of subclinical disease activity in JIA [25,29]. In a study by Foell and colleagues, MRP8/14 was used as a predictive marker for disease flare in children in remission who had MTX discontinued randomly at 6 and 12 months [26]. Levels of MRP8/14 at the time of MTX discontinuation were significantly higher in patients who subsequently developed flares, compared with those who remained...
in stable remission (715 ng/ml [interquartile range: 320–1110] compared with 400 ng/ml [interquartile range: 220–800]; \( p = 0.003 \)) [26]. Subjects who had MRP8/14 levels lower than the cutoff 690 ng/ml, had a low risk of flare in the subsequent 3 months (area under the receiver operating characteristic curve 0.76 [95%CI: 0.62–0.90]) [26]. Utilizing biomarkers to identify subclinical inflammation that indicates inadequately treated disease or an increased risk for future clinical flare is invaluable when faced with the decision of whether to stop remission-inducing medications that have potential risks and toxicities. Unfortunately, biomarkers in pediatric rheumatology have been notoriously difficult to discover and utilize due to several challenges including: a low prevalence of disease in children resulting in expensive and logistically difficult studies across centers; few validated clinical outcome measures to correlate with potential biomarkers; age-dependent changes in physiology that affect drug utilization and clinical disease expression; and ethical challenges with studying a vulnerable population [5,30].

### Exploring variability in MTX outcomes

Although the collective clinical experience with MTX is extensive, like in adults, considerable interindividual variability in clinical response and adverse reactions exists with few predictors for efficacy or toxicity [20,31–32]. It is inadequate in achieving remission in 40% or more of adults and children treated for arthritis [20,31]. Gastrointestinal (GI) toxicity was traditionally reported in up to 15% of JIA patients receiving MTX [33] and evidence of hepatotoxicity in 4–17% [17]; however, recent evidence suggests that the prevalence of MTX intolerance is much higher, and may even differ between route of administration. A conditioned response to MTX has been shown to occur in addition to the physical GI side effects in JIA [34]. The concept of MTX intolerance has been explored more closely by Butatovic et al., who developed a ‘Methotrexate Intolerance Severity Score’ (MISS) to identify the prevalence of drug intolerance including GI side effects and behavioral symptoms occurring after MTX intake, before MTX intake (anticipatory symptoms) and/or when thinking of taking MTX (associative symptoms; [Figure 1]) [35]. MTX intolerance based on the MISS was defined as a score of ≥6 with at least one point on anticipatory and/or associative and/or behavioral symptoms, and in a cohort of 297 patients, 50% had MTX intolerance [35]. The MTX intolerant patients were on slightly higher doses of MTX, had longer durations of disease and therapy, and were treated with parenteral MTX, although in a multivariate model, only the route of administration remained a significant predictor of MTX intolerance (\( p = 0.046 \)) [35]. Although one must interpret these results with caution, as the parenteral route may be required in children who do not initially tolerate oral dosing, or require higher doses of MTX due to disease severity, thus subject to confounding by indication, this is the first study to confront the issue of anticipatory and associative GI adverse effects, and quantify the high degree of these symptoms in the JIA population. Clinically, these frequently uncharacterized symptoms can result in MTX dose adjustment, and nonadherence leading to untimely interruption or termination of therapy. Therefore, the results are interesting and useful as we strive for more sophisticated measures of drug response and toxicity.

Recent studies have investigated predictors of response to MTX to better understand what clinical features may contribute to variable drug outcomes. Post hoc analysis of the Ruperto et al. study [30] revealed that longer disease duration (>1.3 years), negative ANA, high Childhood Health Assessment Questionnaire disability index and bilateral wrist involvement were associated with poor response to MTX, defined as being an ACR Pedi 70 nonresponder by 6 months [36]. Additionally, in a retrospective study by Albers et al. investigating clinical and genetic predictors to MTX response in a cohort of 128 JIA patients, responders started MTX earlier, had higher disease activity at baseline on the physician’s global assessment (PGA) and received a lower starting dose of MTX than nonresponders [37]. Studies such as these certainly support the notion of early identification and treatment of JIA. Furthermore, they bring up the possibility that ideal responders are not dependent on higher doses of MTX but possibly intrinsic differences between patients that may contribute to the variability seen clinically. An optimal dose of MTX for an individual patient has yet to be determined, and remains further challenged by the fact that the mechanism of action of MTX is not fully understood, nor are the effects of ontogeny or development upon drug distribution and response known. Recent attempts have been made to understand what factors may contribute to interindividual variability in drug outcomes, focusing on genetic and cellular biomarkers that...
Mechanisms of action of MTX
MTX acts as a folate antagonist, absorbed via the proton-coupled folate transporter (PCFT/SLC46A1) in the gut and entering the cells primarily through the reduced folate carrier (RFC/SLC19A1) [38], and folate receptors (FOLR) 1 and 2 [39]. Once intracellular, MTX is bioactivated to a polyglutamated (MTXGluₙ) form by folylpolyglutamyl synthase (FPGS), which enhances the pharmacological activity and intracellular retention of MTX [40]. The enzymatic addition of glutamate residues to the MTX molecule in vivo (polyglutamation/MTXGluₙ) is thought to be critical for pharmacologic activity by inhibiting drug efflux from the cell thus increasing the intracellular concentration of the drug, as well as increasing its affinity for its enzymatic therapeutic targets [40–42]. The initial target of MTX to be identified was dihydrofolate reductase (DHFR), which forms tetrahydrofolate, a precursor required for one carbon donation for synthesis of thymidylate, purines, methionine and serine, and provision of methyl donors for remethylation of homocysteine to form methionine, and multiple methyltransferase enzymes [40]. Additionally, MTX inhibits thymidylate synthetase (TYMS), both directly and indirectly via depletion of tetrahydrofolate, leading to inhibition of pyrimidine (thymidylate) biosynthesis with a resultant antiproliferative effect [41]. Subsequently, the list of MTX target genes has been extended to include aminoimidazole carboxamide ribonucleotide (AICAR) transformylase (gene name ATIC), which inhibits de novo purine synthesis and promotes the accumulation of AICAR that inhibits AMP deaminase and results in a build up of intracellular AMP, with subsequent increase in extracellular adenosine [43–46]. Adenosine has

<table>
<thead>
<tr>
<th></th>
<th>No complaints</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stomach ache</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My child has a stomach ache after taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child has a stomach ache several hours to 1 day before taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child has a stomach ache when thinking of MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My child is nauseous after taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child is nauseous several hours to 1 day before taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child is nauseous when thinking of MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Vomiting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My child vomits after taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child vomits hours to 1 day before taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Behavioral complaints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My child is restless when taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child cries when taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child is irritable when taking MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>My child refuses to take MTX</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

Figure 1. Methotrexate Intolerance Severity Score.
MTX: Methotrexate.
Figure 2. Intracellular folate pathway. Both folate and MTX are represented separately. Red dotted lines and squares denote known enzymes inhibited by MTX. MTX acts as a folate antagonist, entering the cells through the reduced folate carrier (SLC19A1). Once intracellular, MTX is bioactivated to MTXGlu by FPGS, which enhances the intracellular retention of MTX. No or low glutamation, facilitated by the degradating enzyme GGH, leads to the efflux of MTX by the ABC family of transporters. MTX’s initial enzymatic target was identified as DHFR, important in the formation of THF. The list of target genes has been extended to include AICAR transformylase (gene name, ATIC) and TYMS. Additional endogenous enzymes in the folate pathway include MTHFD1, MTHFR, MTR, MTRR, SAM, SAH, GART, SHMT and FOLH1. Folate isoforms and their polyglutamated states are represented as: THF-Glu, 10-formyl-THF-Glu, 5,10-methenyl-THF-Glu and 5-methyl-THF-Glu. The dotted rectangle represents the mitochondrial folate pathway, which produces a formic acid for utilization in de novo purine synthesis. SLC25A32 is a mitochondrial specific folate transporter. The bifunctional MTHFD2 and MTHFD1L in mitochondria replicate the function of cytosolic MTHFD1.

5,10-methyl-THF-Glu: 5,10-methyltetrahydrofolate; 5,10-methylene-THF-Glu: 5,10-methylene-tetrahydrofolate; 5-methyl-THF-Glu: 5-methyl-tetrahydrofolate; 10-formyl-THF-Glu: 10-formyl-tetrahydrofolate; ABC: ATP-binding cassette; AICAR: Amino-imidazole carboxamide ribonucleotide; DHFR: Dihydrofolate reductase; FPGS: Folylpolyglutamyl synthase; FOLH1: Folate hydrolase 1; GART: Glycinamide ribonucleotide transformylase; GGH: γ-glutamyl hydrolase; MTHFD1: Methylene tetrahydrofolate dehydrogenase; MTHFD1L: Methylene tetrahydrofolate dehydrogenase 1-like; MTHFD2: Methylene tetrahydrofolate dehydrogenase 2; MTHFR: Methylene tetrahydrofolate reductase; MTR: Methionine synthase; MTRR: Methionine synthase reductase; MTX: Methotrexate; MTXGlu: Methotrexate polyglutamate; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SHMT: Serine hydroxymethyltransferase; THF: Tetrahydrofolate; THF-Glu: Tetrahydrofolate; TYMS: Thymidylate synthetase.

Reproduced with permission from [72].

been thought to be a large contributor to the site specific anti-inflammatory effects of MTX [45,47,48], and furthermore, pharmacogenomic studies in RA and JIA have provided additional support for the importance of the purine synthesis and adenosine pathways with MTX response [46,49,50]. γ-glutamyl hydrolase (GGH), the enzyme responsible for glutamate removal from MTX, transforms MTX into a form that can be effluxed from the cell by the ATP-binding cassette (ABC) family of transporters (Figure 2) [39].

Predictors of variability/ individualization of therapy: is it possible?

In an attempt to enhance the prediction of clinical outcomes on MTX, and even individualize therapy, the quest for pharmacogenomic associations and cellular biomarkers has evolved over the last several years.

Genetic predictors of variability

The effect of genetic variation within the folate pathway upon drug response has been a focus in adult RA and several review papers have discussed the vast work and the potential influence of genotype upon response to the drug [51,52]. However, studies thus far have remained inconclusive owing to several reasons including: variable MTX treatment regimens, outcome measurements and folate supplementation; as well as small sample sizes; the yet unclear role of MTXGlu and folate upon drug metabolism; and the unknown functional impact of the genetic polymorphisms [51].

In children, pharmacogenomic studies related to MTX are even more difficult to conduct due to the rarity of rheumatic diseases in this population. Recently, however, a candidate gene study from the Sparks CHARMS cohort found that folate pathway single-nucleotide polymorphisms (SNPs) in ATIC SNP rs12995526 and SNP rs4673990, as well as ITPA SNP rs2295553 were associated with poor response to MTX [53]. One of the two ATIC SNPs trended towards significance (p = 0.07) in a validation cohort from the USA, but there were no proxies (r² > 0.8) available for testing the ITPA SNP in the validation cohort [53]. The SNPs in genes closely involved in the purine synthesis pathway (such as ATIC and ITPA) may have functional consequence upon the production of adenosine, and hence result in altered anti-inflammatory effects of MTX. Furthermore, in a longitudinal Dutch cohort of 287 JIA patients on MTX, multivariate analysis corrected for the disease duration prior to therapy, the dose of MTX and the PGA at baseline revealed that SNPs in two transporter genes (ABCB1 SNP rs1045642 or ABCC3 SNP rs4793665) increased the likelihood of achieving an ACR Pedi 70 response within the first year, and the presence of a SNP in SLC19A1 SNP rs1051266 diminished the likelihood two- to three-fold [54]. The membrane transporter SLC19A1 transports MTX into the cell and has been shown to have an effect upon response in RA [55,56]; however, this association was not seen with JIA in prior studies [49,55], and in the current work by de Rotte et al., the association did not remain significant after Bonferroni correction, thus requiring further replication and validation despite a potential physiologic explanation. Although there are variable reports supporting or refuting the association of these SNPs and drug effect in the literature [54], efflux transporters ABCB1 and ABCC3 could affect MTX response by resulting in cellular retention.
of MTX resulting in higher intracellular drug levels. These findings require replication in future cohorts of patients, but begin to illustrate the effect that genetic variation may have upon drug response phenotype.

In an attempt to develop a pharmacologic model of nonresponders to MTX (defined as patients who did not satisfy the ACR Pedi 70 response criteria at least two out of three visits within the first year of therapy), Bulatovic and colleagues reported a prediction model combining ESR (≥12 mm/h) and four SNPs in: (MTTR) SNP rs1801394, ABCB1 SNP rs1045642, ABCC1 SNP rs35592, and proton-coupled folate transporter (PCFT) SNP rs2239907 [57]. If none of the predictors listed were present, the probability of nonresponse to MTX was 0.98, if all were present, the probability of nonresponse was 0.42 [57]. The authors transformed regression coefficients to create a score ranging from zero to 11 (11 being the highest probability of nonresponse), and found that a cutoff of ≥3 provided the highest sensitivity (correctly identifying future nonresponders) and reasonable specificity (avoiding misidentification of MTX responders). However, using this prediction model with cutoff ≥3, sensitivity in the derivation cohort was 78% and specificity was 49%, and within the validation cohort, sensitivity was 79% and specificity was 26% [57]. Although work such as this attempts to make pharmacogenomic evaluation practical and useful to the clinician, there is obviously much more to explore and validate before models such as these are ready for prime time clinical use.

Little is known about the ontogeny of SNPs in the folate pathway in humans, but there are some data to show developmentally expressed folate enzymes in human fetal liver [58], as well as fetal rabbit [59], rat [60] and sheep [61] tissues. The expression of folate genes may also be variable to adapt to changing DNA needs throughout growth and development. This frontier of investigation has not been fully explored, however, with the similarity in MTX doses between children and adults, despite vast differences in size, understanding development-related differences in drug disposition will likely contribute to the understanding of the vast variability we see clinically with drug response to MTX.

### Cellular predictors of variability

To date, there has been no correlation between serum MTX pharmacokinetics and efficacy or toxicity in JIA or RA [62,63], making serum drug levels useless for clinical management. The polyglutamated forms of MTX (MTXGlun) are important for cellular retention of the drug and target enzyme inhibition, and are more stable than rapidly cleared serum levels of MTX, thus have been explored as more reliable cellular MTX biomarkers [40]. Although an association between red blood cell (RBC) long-chain MTXGlun concentrations and the effectiveness of MTX in RA has been reported [46,64,65], these findings have not been consistently replicated in RA or JIA [49,66,67]. In approximately 100 JIA patients on stable doses of MTX, RBC MTXGlun concentrations and proportions were found to vary 40–100-fold. Dose, route and duration of MTX treatment were clinical variables that contributed to the observed variability, with higher concentrations of long-chain MTXGlun in patients dosed subcutaneously, and higher concentrations of short-chain MTXGlun observed in patients dosed orally [68]. Although an association with route of administration was noted, there was no association between MTXGlun distribution and active arthritis, in contrast to adult reports [46,65]. However, these studies were cross-sectional in nature, thus associations with drug efficacy are difficult to make, and complicated by concurrent medications and varying amounts of time on MTX. Although there were no significant differences in RBC MTXGlun concentrations or patterns in subjects with or without arthritis, long-chain polyglutamate concentrations were approximately 30% higher in subjects who had elevated liver transaminases at the time of their visit (mean 173.0 [±162.9] nmol/l vs 111.8 [±85.5] nmol/l; p = 0.03) and in subjects who reported GI side effects at the time of their visit (mean concentrations 159.2 [±134.4] nmol/l vs 107.7 [±85.2] nmol/l; p = 0.013) [49]. To further investigate the effect that genetic variability had upon patterns of MTX polyglutamation, the multifactor dimensionality reduction (MDR) method was applied to clusters of the raw concentrations of MTXGlun, revealing the combination of ATIC SNP rs4673990 and ADORA2a SNP rs3761422 SNPs differentiated subjects in the cluster with the highest concentrations compared with the lowest concentrations of MTXGlun [49]. Understanding the genetic influence upon MTXGlun is only important, however, if MTXGlun plays an important role either as a contributor or biomarker for patient outcomes.

When unraveling the importance of biomarkers such as MTXGlun, one must
remember that MTX does not work in isolation; in fact it acts upon the endogenous folate pathway. Intracellular folates have also been shown to have effects upon target enzymes [69,70]. Therefore, understanding the effect of MTX upon its target folate environment may add to the understanding of how patients respond to MTX differently. Interestingly, some studies have made the observation that lower intracellular folate (measured as total folate) have been associated with an improved level of disease control [64,71]; however, the utilization of folic acid supplementation to combat GI side effects is commonplace. Striking a balance between achieving optimal drug efficacy and minimizing drug toxicity will likely need to include a better understanding of the folate environment in which the drug works.

In children with juvenile arthritis, folate status is variable and distinct folate phenotypes are present. In a subgroup of 93 juvenile arthritis patients not currently receiving MTX, RBC intracellular concentrations of 5-methyl-tetrahydrofolate (5-CH$_3$-THF) and 5,10 methenyl-tetrahydrofolate (5, 10-CH$_3$-THF), varied approximately 20- and 80-fold, respectively. RBC folate concentrations in twelve subjects with historic MTX toxicity were significantly lower than the rest of the group (p = 0.003), despite no significant differences in age, gender, folate supplementation, active arthritis, inflammatory markers, NSAID or steroid use [72]. This raises the question, could patients with lower cellular folate status or those incapable of adapting to drug perturbation be more intolerant of MTX? The potential contributors to variability in folate homeostasis are vast, including several genes in the folate pathway that may alter the pathway’s function. In a multivariate regression analysis of clinical and genetic predictors of intracellular 5-CH$_3$-THF and 5,10-CH$_3$-THF in the abovementioned JA cohort, only variation in SLC25A32 SNP rs17803441 (p < 0.0001) remained significantly associated with 5-CH$_3$-THF concentrations and variation in MTHFR SNP rs1801131 (p = 0.0006) and MTR SNP rs1805087 (p = 0.015) remained significantly associated with 5,10-CH$_3$-THF concentrations [72]. SLC25A32 is a cellular mitochondrial transporter responsible for supplying tetrahydrofolate to the mitochondrial folate pathway, which eventually supplies purine synthesis (Figure 2). MTHFR is a well-studied enzyme in the folate pathway and has been variably associated with MTX response in RA [52] and JIA [73], and MTR is integral in the one carbon donation from 5-CH$_3$-THF for conversion of homocysteine to methionine (Figure 2). Although most studies investigating the role of genetic variation in cellular folate concentrations [26,36] or homocysteine concentrations [37] have focused on MTHFR, these data imply that genetic control may be exerted at many additional levels.

Folate, like MTX, is polyglutamated within the cell, and the polyglutamated forms of folate are also important for biological activity and cellular retention [40], and can provide additional insight into cellular folate status. In the 93 JA patients reported above, hierarchical clustering of the polyglutamated form of 5-CH$_3$-THF (5-CH$_3$-THFGlu$_n$) revealed two distinct groups (Figure 3). ‘Long-chain’ folate polyglutamates clustered as 5-CH$_3$-THFGlu$_6$–8 (group 1), and ‘short-chain’ folate polyglutamates as 5-CH$_3$-THFGlu$_4$+5 (group 2). In animal studies, lower cellular folate isoform concentrations and higher concentrations of long-chain polyglutamates...
were observed in rats fed a folate-depleted diet for 25 weeks [74], and we have observed a similar phenomenon in our work in preliminary in vitro experiments (data not shown). Applying this knowledge, group 1 then may display a polyglutamation pattern consistent with a relative folate deficient state (low 5-CH₃-THF=Glu₅ and high 5-CH₃-THF=Glu₇) compared with group 2 (high 5-CH₃-THF=Glu₇, low 5-CH₃-THF=Glu₅) [72]. Understanding the interplay between intracellular folate and MTX in relation to genetic predisposition and disease status will be necessary to mechanistically understand the variability of drug response in an individual patient. Future studies will likely need a systems-based approach to better understand the relationship between these variables. Translational studies incorporating hypotheses generated from in vitro systems applied to patients with more complex systems will hopefully further delineate both the mechanisms of action of MTX and the contributors to interpatient variability seen in the clinical realm.

**Conclusion**

MTX remains an important cornerstone of therapy for JIA and is used extensively both as mono- and combination therapy worldwide. Despite this widespread use, there remains much to be learned about its mechanisms of action and its clinical variability. This has been a challenge particularly in pediatrics, as many older drugs have not benefited from newer legislation that encourages pediatric specific indications in pharmaceutical drug development. Additionally, unique to pediatrics, is the effect of development upon drug disposition and response that is often ignored or simply overlooked. Regardless, MTX has a long history of use, has well-known side effects, is affordable and available, and deserves our attention and investigation to optimize its use. Guideline development has occurred recently to guide clinicians on therapeutic recommendations for JIA, although these recommendations were developed on scant evidence and expert opinion and consensus. Implementation of comparative effectiveness studies is underway to study outcomes on developed consensus treatment plans and may begin to standardize therapy across centers. Despite this, however, there remains a vast amount of variability in outcomes on the drug. Pharmacogenomic studies, despite known limitations, have identified some genetic contribution to MTX response, although the impact of these studies in the clinical realm is not yet fully realized. Cellular biomarkers to better define outcomes on therapy are also being actively sought, and continue to further define a phenotype of response that can be utilized in future investigations and eventually in the clinical realm. If we can identify optimal responders to MTX, or patients at risk of developing side effects and toxicity, we have the opportunity to individualize therapy and maximize MTX use in the patients who will benefit most, while targeting biologic therapies in patients who will respond poorly to MTX.

**Future perspective**

Over the next 5–10 years, work will focus on the complex balance of standardization of care for children with rheumatic disease and individualization of therapeutic interventions. International collaborative efforts in standardization of care will include the development of validated outcome measures to assess the impact of interventions; improved characterization of the clinical disease course, including risks of flare and likelihood of disease remission; and development of future comparative effective studies to begin to study response to therapy in the ‘real world’ generalizable clinical environment. Organizations including Childhood Arthritis and Rheumatology Research Alliance, Pediatric Rheumatology InterNational Trials Organization and the Pediatric Rheumatology Collaborative Study Group are paving the way to enhance collaboration to allow the successful investigation of these rare diseases in children. However, no matter how hard one standardizes, there remain ‘outliers’ who may respond differently to interventions. Individualization of therapy can then be an enhancement to standardization of care. The major benefits of pharmacogenomic strategies will be seen with the a priori identification of patients who will represent the extremes of response (both lack of efficacy and higher risk of severe toxicity). Striving to expand phenotypes of disease and response to include not only clinical features, but also cellular and biochemical biomarkers will aid in establishing correct genotype/phenotype relationships. Additionally, recognition of biological changes that occur with growth and development will enhance drug investigations within the pediatric population. As the field grows and develops, the incorporation of all of these approaches will be necessary to identify the contributors to individual variability in MTX response and this information will
Role of methotrexate in juvenile idiopathic arthritis

enhance and optimize treatment regimens for children with JIA.

Financial & competing interests disclosure
ML Becker’s work was supported by the Katharine B Richardson Grant, the Children’s Mercy Young Investigator Award, the Paul Henson Immunology Research Award, the Kansas City Area Life Sciences Award, the PhRMA Foundation Award and the ACR REF Rheumatology Investigator award. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Methotrexate as a standard therapeutic option in juvenile idiopathic arthritis
- Methotrexate (MTX) is the widely agreed upon first-choice second-line agent to treat juvenile idiopathic arthritis (JIA), up to 75% of JIA patients in recent registries have used MTX at some time in their disease course.
- One of the few randomized placebo-controlled trials conducted in pediatric rheumatology patients supported the safety and efficacy of this drug in children with JIA.
- MTX acts as a folate antagonist and interrupts the synthesis of purines and pyrimidines, as well as the remethylation of homocysteine to form methionine, and multiple methyltransferase enzymes. A downstream effect of MTX is the buildup of adenosine, an anti-inflammatory agent.

Variability in MTX clinical treatment practices
- There remain ongoing questions about optimal dose, route of administration and time to discontinue MTX in JIA patients currently in remission.
- Utilization of biomarkers such as MRP8/14 may be a useful adjunct to predict successful discontinuation of MTX in JIA patients in remission.
- ACR guidelines for the treatment of JIA were recently released in 2011, and utilized the best available data and expert consensus to develop treatment guidelines for JIA.

Variability in MTX outcomes
- Clinical response to MTX has been variable in JIA, with up to 40% of patients having suboptimal response, and variable reports of gastrointestinal side effects and liver toxicity.
- Recognition of anticipatory and associative symptoms with tools such as the methotrexate intolerance severity score, have revealed a possibly higher amount of drug intolerance than previously recognized.

Clinical & genetic predictors of MTX response
- Clinical predictors of MTX response have consistently supported disease duration as an important predictor of MTX response, along with other clinical variables including baseline physician global assessment and baseline Childhood Health Assessment Questionnaire.
- Pharmacogenomic studies have been utilized to investigate the genetic contribution to variability in response, and thus far, have suggested that single-nucleotide polymorphisms within folate pathway genes may have an impact on drug outcomes, but these findings require further validation.
- Very little is known about the ontogeny of genes responsible for MTX pharmacologic activity.

Cellular predictors of MTX response
- MTX polyglutamates have been investigated in JIA and thus far appear not to be correlated to drug response, as has been reported in rheumatoid arthritis.
- However, preliminary evidence has revealed the association of long-chain MTX polyglutamates with gastrointestinal and liver toxicity.
- Intracellular folate concentrations may also contribute to a better understanding of the pharmacologic effects of MTX upon the folate pathway.

Conclusion
- Methotrexate remains an important cornerstone of therapy for JIA.
- Strides towards guideline development and implementation have occurred recently to guide clinicians on therapeutic recommendations for JIA, and begin to standardize therapy.
- Despite standardization of therapy, there remains a vast amount of variability in outcomes on the drug, thus future studies investigating genetic and cellular predictors of this variability will be useful to further tailor therapy for patients.

References
2 Diamantberger M. [From rheumatism nodosa in children to idiopathic juvenile arthritis or from Diamantberger to the Durban criteria]. Arch. Pediatr. 7(2), 121–124 (1891).
Role of methotrexate in juvenile idiopathic arthritis

Becker


31 Lambert CM, Sandhu S, Lochhead A, Hurst NP, Mcrote E, Dhillon V. Dose escalation of parenteral methotrexate in active rheumatoid arthritis that has been unresponsive to conventional doses of methotrexate: a randomized, controlled trial. Arthritis Rheum. 50(2), 364–371 (2004).


Rheumatoid arthritis


