Chemotherapy of ALL
Friend or Foe?
Neurotoxicity of Methotrexate
St. Jude Experience

Raul C. Ribeiro, MD
HEMO2016
Florianopolis 12/12/2016
Conflict of Interest Disclosure

I hereby declare the following potential conflicts of interest concerning my presentation:

• Consultancy: No
• Research Funding: No
• Honoraria: No
• Patents and Royalties: No
• Membership on an Entity’s Board of Directors or Advisory Committees: No
• Discussion of off-label drug use: No
Outline

• Background
• Historic landscape of neurotoxicity
• St. Experience
  – Acute and sub acute neurotoxicity
  – Long-term toxicity
• Future Directions
Anti-Folate Agents

Methotrexate

- MTX has been known for more than 60 years and was the first rationally designed molecule against cancer
- Methotrexate (MTX) is a folate analogue that inhibits dihydrofolate reductase (DHFR), which converts dihydrofolate (DHF) to tetrahydrofolate (THF) needed for several one-carbon transfer reactions in nucleotide synthesis
Clinical Consequences of Folate Inhibition

- Purine and pyrimidine synthesis. Essential for cell division and DNA and RNA synthesis
- Synthesis of the methyl donor S-adenosylmethionine (SAM), used in methylation reactions, including methylation of DNA (which plays a key role in gene expression)
Folic Acid Metabolism and Methotrexate
MTX Neurotoxicity: Acute Encephalopathy

In the early 70’s, several reports suggested an association between IT methotrexate and CNS toxicity in CNS leukemia.

- In 7 patients, dementia developed during treatment with MTX for CNS leukemia. Confusion, tremor, ataxia, irritability, and somnolence.
- Seizure was noted in two cases and in one case there was progression to coma and death.
- When treated with folinic and folic acid the deterioration was arrested and there has been some improvement.

Kay H et al, Archives of Disease in Childhood, 1972
MTX Neurotoxicity: Acute encephalopathy

Kay H, Archives of Disease in Childhood, 1972
MTX Neurotoxicity: Aseptic Meningitis

- Fever, headache, and vomiting, lasting 2-5 days, occurred in 61% of 39 children with acute leukemia in CR receiving CNS prophylaxis with ITMTX.
- The symptoms were accompanied by pleocytosis with lymphocytes, monocytoid cells, and neutrophils.
- There was evidence of cumulative MTX toxicity, since the toxicity occurred mostly after the third and fourth dose and did not recur with longer intervals between doses.
- Toxicity was significantly reduced by the use of Elliott’s B solution as MTX diluent, rather than water or normal saline.

Geiser et al. Blood 1975
MTX Neurotoxicity: Paraplegia

- A patient who developed paraplegia following the IT MTX
- Ten previously reported cases were reviewed. Factors associated: abnormal CSF dynamics related to the presence of CNS leukemia, and epidural cerebrospinal leakage; elevated CSF MTX concentration
- The presence of neurotoxic preservatives in commercially available methotrexate preparations and diluents; and the use of methotrexate diluents of unphysiologic pH, ionic content and osmolarity

Gagliano RG, Cancer 1976
MTX Neurotoxicity: Subacute toxicity

- An abrupt onset of focal cerebral deficits occurred in 4 of 158 patients treated for osteogenic sarcoma with combination chemotherapy, approximately 10 days after chemotherapy with HD MTX plus leucovorin.
- The syndrome was short lived. Prolonged neurological deficits remained in 2 patients.
- When similar chemotherapy was reinstituted in the 4 patients, no further neurological complications ensued.
- Possible causes include aleukoencephalopathy related MTX or an embolic cerebral vasculopathy related to necrotic tumor microemboli emanating from the lungs.

Allen JC Ann Neurol 1978
MTX Neurotoxicity: Neurocognitive deficits

Neurocognitive late effects of chemotherapy occur in 40 – 60% of acute lymphoblastic leukemia (ALL) survivors

– inhibition, which involves resistance on acting on impulses or prematurely

– working memory, which pertains to actively holding and manipulating information in mind

– cognitive flexibility, which includes effectively switching between tasks
Importance of the White Matter

• Complex cognitive processes require coordinated neural activity
• White matter which consists of glial cells and axon tracts insulated by myelin sheaths, enables speedy transfer and integration of information throughout the brain
• White matter appears to be particularly vulnerable to the impact of chemotherapy agents
Imaging Findings

• Up to 80% of ALL patients treated with chemotherapy develop chronic or transient lesions in the deep white matter identified on magnetic resonance imaging as hyper-intensities (leukoencephalopathy)

• A large cohort of childhood cancer survivors also found evidence of reduced WM volume in ALL survivors compared to healthy siblings, and these reductions in WM were significantly correlated with worse performance on measures of attention, intellect, and academic achievement
Polymorphisms of Genes Involved in MTX-Folate Metabolism

Regulatory enzyme
C677T: TT genotype associated with greater toxicity of MTX and possibly 5-FU
A1298C: Common polymorphism affecting protein function

Cell membrane

Serum folate

RFC

Drug transporter
G80A: AA genotype associated with higher MTX serum levels, and worse prognosis among MTX-treated children

Methionine synthase

Homocysteine

CBS

Cystathionine

SAH

SAM

Cysteine

CHₓ

X

DNA methylation

5-Methyl THF

Methionine

5, 10-Methylene THF

MTHFR

Pyrimidine synthesis

5-FU

dUMP

dTTP

Thymidylate synthase

Drug target
TSER polymorphism: the 2rpt/2rpt genotype responds better to 5-FU treatment, but with greater toxicity. Possible association with survival among MTX-treated patients

GART

AICARFT

GAR

AICAR

DHF

10-Formyl THF

DHFR

Methotrexate

Source: Nat Rev Cancer © 2003 Nature Publishing Group
## Polymorphisms and MTX Metabolism

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>SNP effect</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>Reduced</td>
<td>+++</td>
</tr>
<tr>
<td>DHFR ins/del 19bp</td>
<td>Increased</td>
<td>++</td>
</tr>
<tr>
<td>TS 2R/3R</td>
<td>Increased</td>
<td>++</td>
</tr>
<tr>
<td>TS UTR-6bp ins/del</td>
<td>Lower</td>
<td>++</td>
</tr>
<tr>
<td>CCND1 A870G</td>
<td>Increased</td>
<td>+</td>
</tr>
<tr>
<td>MTRR A66G</td>
<td>Reduced</td>
<td>++</td>
</tr>
<tr>
<td>SHMT C1420T</td>
<td>Reduced</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Transporters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFC1 G80A</td>
<td>Decreased</td>
<td>++</td>
</tr>
<tr>
<td>ABCG2 C421A</td>
<td>Decreased</td>
<td>+++</td>
</tr>
<tr>
<td>ABCB1 C3435T</td>
<td>Decreased</td>
<td>+</td>
</tr>
</tbody>
</table>
Drug Interaction

• MTX is bound to albumin
  – Salicylates, phenylbutazone, sulfonamides
• Renal Probenecid, amoxicillin, penicillin
• Absorption or entero-hepatic circulation
  – Tetracycline, chloramphenicol and other AB
• Hepatic
  – Azathioprine, retinoids, sulfasalazine
• Others
  – Proton-pump inhibitors, nitrous oxide, fluoroquinolone
Nitrous Oxide (N₂O)

2Co⁺ + N₂O → 2Co⁺⁺ + N₂
2Co⁺⁺ + N₂O → 2Co⁺⁺⁺ + N₂
St. Jude Study XV

- From 06/2000 to 10/2007, 498 children with ALL were enrolled on the TXV study.
- 369 had prospective MRI screening.
- 278 of 369 patients received an upfront MTX 1 g/m² assigned to 4-μ 24-hour infusion.
- Consolidation, MTX 2.5 g/m² or 5 g/m² over 24 hours, 4 doses every 2 weeks.
- Leucovorin at 5 mg/m² in the LR and 10 mg/m² SR/HR, 5 doses beginning 42 hours after initiation of MTX.
- Leucovorin doses were increased in patients with a 42-hour MTX level ≥1mol/L.
Definition of MTX-Related Toxicity

• A neurotoxic adverse event was attributed to MTX if neurologic symptoms (eg, seizure, stroke, behavioral changes, aphasia) occurred within 2 weeks of receiving MTX (intrathecal or intravenous), and other identifiable causes were reasonably ruled out.

• Patients with clinical neurotoxicity were evaluated by a pediatric neurologist.
Detection and Grading of Leukoencephalopathy

- Brain MRIs were obtained postinduction, post-consolidation; continuation week 48, and continuation week 120 (MRI4).
- Most patients with clinical neurotoxicity underwent additional imaging during or after the event.
- The protocol noncontrast MRI examinations consisted of sagittal T1, axial T1-weighted inversion recovery, axial T2, axial proton density, and axial fluid attenuated inversion recovery pulse sequences of the brain.
- Abnormal MRIs were identified by a single neuroradiologist and graded for the extent of leukoencephalopathy by a second neuroradiologist, according to radiographic criteria of CTCAE (version 4.0).
Results

• Of 369 patients, 14 (3.8%) developed MTX-related neurotoxicity
• 7 patients had seizures, six stroke-like symptoms, and one with ataxia. Most episodes were brief, but ataxia persisted in one patient
• All 12 patients with MRIs available at the time of the event had leukoencephalopathy.
• Screening MRI was available before the event for 10 patients. Existing leukoencephalopathy was evident in seven patients.
• Of 12 patients with end-therapy MRIs, leukoencephalopathy persisted in seven patients and resolved in five.
MTX Rechallenge

• 13 patients were rechallenged with high-dose MTX or ITT.
• HDMTX was substituted with MTX (40 mg/m2) in one case
• 2 patients received aminophylline before HDMTX, and five patients leucovorin 24 and 36 hours after ITT.
• Severe headache and confusion recurred in one patient when challenged with HDMTX. Further HDMTX was held, but received ITT with leucovorin rescue and experienced occasional headaches.
• The other 12 patients tolerated MTX well. One patient developed a seizure 5.5 months after his first event but was found to have a CNS thrombus likely related to asparaginase.
Risk Factors of Neurotoxicity

• Patients age > 10 years were at higher risk for neurotoxic events than those age 1 to 10 years ($P < .003$).

• Patients in the SD/HR arm were also at higher risk for clinical neurotoxicity than those treated in the LR arm ($P < .016$).

• The ratio of 42-hour MTX level to leucovorin dose not vary significantly between patients with and without neurotoxicity.

• No other risk factor retained significance in a multivariable model.
• Of 74 patients with LKE, 30 patients (40.5%), the grade of LKE improved over time, including in 17 patients (23%) in whom leukoencephalopathy resolved completely
• LKE remained stable in 33 patients (46.6%) and worsened in 11 patients (14.9%). In the majority (77%) who developed LKE, MRI abnormalities were still evident at week 120
• Higher cumulative number of ITTs, higher MTX level at 42 hours, and higher homocysteine concentration were associated with increased risk of LKE
• The ratio of 42-hour plasma MTX concentration to leucovorin dose HDMTX retained significance in a multivariable model (\(P=0038\)).
Neurocognitive and Patient-Reported Outcomes in Adult Survivors of Childhood Osteosarcoma

• To examine neurocognitive, neurobehavioral, emotional, and quality-of-life outcomes in long-term survivors of childhood osteosarcoma.

• Outcome data were collected from June 2008 to August 2014. Survivors of osteosarcoma recruited from the St Jude Lifetime Cohort Study were compared with community controls.

• Neurocognitive function, neurobehavioral symptoms, emotional distress, and quality of life. Outcomes were examined in relation to pharmacokinetic indices of methotrexate exposure and current chronic health conditions.

Edelmann M. JAMA Oncol 2016
# Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OST 72</th>
<th>OST 77</th>
<th>MIOS</th>
<th>OS86</th>
<th>OS91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>3</td>
<td>23</td>
<td>15</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Mean Dose per Course (g/m²)</td>
<td>6.2</td>
<td>4.9</td>
<td>12.1</td>
<td>12.0</td>
<td>12</td>
</tr>
<tr>
<td>Cumulative Dosage (g/m²)</td>
<td>74.9</td>
<td>46.4</td>
<td>140</td>
<td>102.6</td>
<td>104</td>
</tr>
<tr>
<td>C_{max} (µM)¹</td>
<td>292†</td>
<td>338 (93.9)</td>
<td>1,679 (302)</td>
<td>1,573 (273)</td>
<td>1,170 (201)</td>
</tr>
<tr>
<td>CL (L/h/m²)¹</td>
<td>5.2 (0.94)</td>
<td>5.0 (1.15)</td>
<td>2.8 (0.49)</td>
<td>2.8 (0.57)</td>
<td>3.3 (0.32)</td>
</tr>
<tr>
<td>AUC/course (µM*hr)¹</td>
<td>2,422 (904)</td>
<td>2,292 (820)</td>
<td>10,183 (2,030)</td>
<td>9,911 (2,247)</td>
<td>8,008 (594)</td>
</tr>
<tr>
<td>Cumulative AUC (µM*hr)¹</td>
<td>30,936 (17,765)</td>
<td>24,952 (8,198)</td>
<td>117,427 (22,298)</td>
<td>84,234 (17,711)</td>
<td>68,572 (7919)</td>
</tr>
</tbody>
</table>

¹Values: mean (standard deviation)
†Simulated
AUC = Area under the concentration curve
C_{max} = Maximum concentration
CL = Systemic clearance
Neurocognitive Toxicity

P = .18  P = .24  P = .006  P = .002  P = .67  P = .69

Age-Adjusted z Score

Reading  Attention  Memory  Speed  Fluency  Working Memory

Grade 3 or 4  Grade <3

Compared with controls, survivors of childhood osteosarcoma demonstrate reduced lower mean scores in reading skills \((P = .01)\), attention \((P = .002)\), memory \((P = .01)\), and processing speed \((P < .001)\).

Reading problems increase risk for less than full-time employment \(3.75\) and annual income less than $40 000 \(2.76\).

Slowed processing speed is associated with self-reported health problems \(0.34, P = .005\), and SF on ECHO is associated with attention problems \(P < .04\).

Plasma concentration of HDMTX during active chemotherapy is not associated with neurocognitive outcomes at nearly 25 years after diagnosis.
Summary

Long-term survivors of osteosarcoma are at risk for neurocognitive impairment, which is related to current chronic health conditions and not to original treatment with high-dose methotrexate.
Conclusion

• Friend or Foe?
  – Friend!
• MTX will continue to be used for many years to come in the treatment of childhood ALL
• MTX alone is not likely to be the only culprit for neurotoxicity
• Attention to leucovorin rescue, drug-drug interactions and polymorphism of MTX metabolism may help to decrease toxicity
Simplified Scheme of Methotrexate Inhibition

Simplified Scheme of Methotrexate Inhibition
Nucleotide biosynthesis

5, 10-methylene-tetrahydrofolate reductase (MTHFR)

5, 10-methylene-tetrahydrofolate

5-methyl-tetrahydrofolate

Homocysteine

Cystathionine

Cystathionine synthase plus vitamin B6

Methionine synthase plus vitamin B12, MSR

S-adenosyl-homocysteine

Histone/DNA methylation

Protein methylation

Lipid methylation

Methylated DNA, proteins, lipids

S-adenosyl-methionine

Methionine

Tetrahydrofolate

Folic acid receptors

Folic acid

Dietary requirements

DNA, proteins, lipids

Methyltransferases
<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Compounds</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene tetrahydrofolate reductase</td>
<td>Methotrexate and pemetrexed</td>
<td>Approved for multiple cancers\textsuperscript{14}</td>
</tr>
<tr>
<td>Thymidylate synthase</td>
<td>5-FU</td>
<td>Approved for multiple cancers, most notably colorectal cancer\textsuperscript{14}</td>
</tr>
<tr>
<td>Ribonucleotide reductase</td>
<td>Gemcitabine</td>
<td>Approved for multiple cancers, most notably pancreatic cancer\textsuperscript{14}</td>
</tr>
<tr>
<td>Polyamine synthesis enzymes</td>
<td>Various</td>
<td>Clinical trials ongoing\textsuperscript{83}</td>
</tr>
<tr>
<td>DNA methyltransferases</td>
<td>Azanucleosides</td>
<td>Approved for myeloid leukaemias\textsuperscript{72}</td>
</tr>
<tr>
<td>Histone methyltransferases</td>
<td>Various (SAM analogues)</td>
<td>Clinical trials ongoing\textsuperscript{72}</td>
</tr>
<tr>
<td>Histone demethylases</td>
<td>Various</td>
<td>Preclinical studies\textsuperscript{72}</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>DMFO</td>
<td>Clinical trials ongoing\textsuperscript{83}</td>
</tr>
<tr>
<td>S-adenosyl decarboxylase</td>
<td>MGBG and SAM486A</td>
<td>Preclinical studies\textsuperscript{83}</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; DMFO, 2-difluoromethyl ornithine; MGBG, methylglyoxal bis(guanylhydrazone); SAM, S-adenosylmethionine; SAM486A, (E)-2-(4-carbamimidoyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinecarboximidamide.
Methotrexate and Folate Metabolism
Modulation of MTX Neurotoxicity: Polymorphisms of Genes Involved in MTX-Folate Metabolism
Folic Acid

- Folic acid is a water-soluble B-vitamin
- Formation of the coenzyme referred to as tetrahydrofolate (THF)
- Essential for creating heme
- Proper formation of the brain, spinal cord, and nerve cells in the embryo.
- Closure of the neural tube in the fetus.
- Essential for synthesis of serine, methionine, ATP, GTP, thymidylate