High-dose methotrexate and/or leucovorin rescue for the treatment of children with lymphoblastic malignancies: do we really know why, when and how?*

Minireview

J. ŠTĚRBA[1], D. VALÍK[2], V. BAJČIOVÁ[1], V. KADLECOVÁ[1], V. GREGOROVÁ[1], D. MENDELOVÁ[1]

[1]Department of Pediatric Oncology University Hospital Brno, e-mail: jsterb@fnbrno.cz, Children's Hospital, Brno CZ 625 00, Czech Republic; [2]Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Brno CZ 656 53, Czech Republic

Received March 30, 2005

Methotrexate (MTX) remains a mainstay in the treatment of children with hematological malignancies. The availability of an antidote/rescue agent, leucovorin (LV) has allowed escalation of MTX doses to achieve enormous plasma concentrations, compared with plasma folate. However, a recent review of more than 40 trials for children with ALL concluded that the addition of high dose MTX (HDMTX) in many different doses and schedules did not improve CNS therapy and made only minor improvements in systemic therapy for children with ALL [11]. Some assessment suggested that by HDMTX benefits only limited amount of children with ALL. Recent treatment schedules vary markedly in terms of timing, dosing and scheduling of MTX and/or leukovorin, which may leave us uncertain with ideas such as "how should we best use HDMTX and LV?" or "why are we still using such by industry recomended doses of MTX?"

The answer of how best to incorporate HDMTX and/or LV into ALL treatment plans is still not known and further clinical and pharmacological studies dealing with still controversial systemic MTX issue are actual even now, after more than 5 decades of clinical experiences with the MTX in pediatric oncology.

Key words: child, acute lymphoblastic leukemia, methotrexate, toxicity, effectiveness

The development of effective therapy for children with acute lymphoblastic leukemia (ALL) is one of the biggest successes of pediatric oncology. Fifty years ago, childhood ALL was in generally fatal, but current long-term event-free survival rates are nearly 80%. Despite this improved outcome, there are still many challenges ahead. Anticancer therapy remains nonspecific, toxic, and sometimes even lethal. Treatment results reported by different cooperative groups are similar, however important differences exist in how risk groups are assigned and in the therapeutic regimens used by various treatment groups. Great deal of controversy is surrounding especially optimal CNS- directed treatment of the childhood ALL and the role of systemic i.v. methotrexate in this site, especially with respect to timing and dosing of i.v. MTX and/or leucovorin rescue.

*This work was supported by the following grants: IGA MZ ČR - NR 8448-3/2005 and the Institutional Research Grant MMCI MZ 00000209805.

Methotrexate (MTX), classic antifolate, is for decades an essential component of treatment for children with acute lymphoblastic leukemias (ALL), non-Hodgkin lymphomas (NHL), osteosarcomas. MTX achieves its cytotoxicity through the inhibition of folate-dependent enzymes [4].

Historical development

In historical, single-agent studies of MTX, performed in the 1950s under the auspices of the Acute Leukaemia Group B, MTX was administered as an age-dependent daily oral dose of 1.25–5 mg/d and, as a single agent, MTX induced a clinical remission (sustained for a median duration of 4 months) in 29% of children with acute leukemia [16]. A subsequent Acute leukemia Group B study compared the survival of children with ALL who received either daily oral (3 mg/m^2/d) or twice weekly parenteral (30 mg/m^2 per dose) MTX as monotherapy until relapse after remission induction

with vincristine and prednisolone [2]. Whereas the median duration of complete remission (CR) for children receiving the parenteral schedule was 17 months, with an associated 2-year survival rate of 20%, the median remission duration for children receiving daily MTX was 3 months, and no child survived up to 2 years. A further indication of the potential importance of MTX scheduling comes from the study of DJERASSI [13], in which 80% survival at 30 months was found for 15 children receiving post-remission induction therapy with MTX. In this study, 180–525 mg/m^2 MTX was administered as an intravenous infusion over 4 h, for two consecutive days at 3-weekly intervals. Therefore, these early clinical studies indicated that the antileukemic efficacy of MTX might be improved by either increasing the dose and or the time of exposure to the drug.

Intermediate dose of MTX (2 g/m^2 x 3) was an important novel part of the successful Dutch study ALL VI, performed between 1984 and 1988, and effectively omitting both anthracyclines and alkylating agents, while maintaining high EFS (81%) for 291 children with non-high-risk ALL. (WBC count <50x10^9/l, no mediastinal mass, no B-cell phenotype, and no CNS involvement). Such results are comparable with those, achieved by much more intensive BFM strategies. The 8 years, event-free survival (EFS) rate was 81% (SE=3%) and survival rate 85% (SE=2.9%); the median follow-up time was 7.3 years (range, 36 to 117 months). The CNS relapse rate was 1.1% of 184 patients who achieved a complete remission. The treatment protocol used 6-week induction regimen with three drugs (vincristine, dexamethasone, and asparaginase), three weekly doses of intravenous (IV) medium high-dose methotrexate (2 g/m^2 over 24 h), and 2-year maintenance therapy. High cure rate was achieved without the use of anthracyclines, alkylating agents, and cranial irradiation [42]. About at the same time HD-MTX (5 g/m^2 over 24 h) has been incorporated into BFM protocols as well [37].

The use of high dose methotrexate pulses (5 g/m^2 q2w x 4) during consolidation has been reported to be associated with improved survival, particularly for children with T-ALL by BFM group in Europe [38]. Recent Pediatric Oncology Group POG 9404 study showed that addition of HDMTX (5 g/m^2 x 4 courses) to the Dana Farber Cancer Institute (DFCI) [18] chemotherapy regimen results in improved EFS for these patients due to decreased occurrence of induction failure and CNS relapses as well [3].

In modern ALL treatment protocols, MTX use differs markedly in various protocols during remission induction, consolidation, maintenance and central nervous system (CNS)-directed therapy, in conjunction with different number of age-dependent doses of intrathecal methotrexate (different timing of systemic MTX administration is shown in Figure 1.

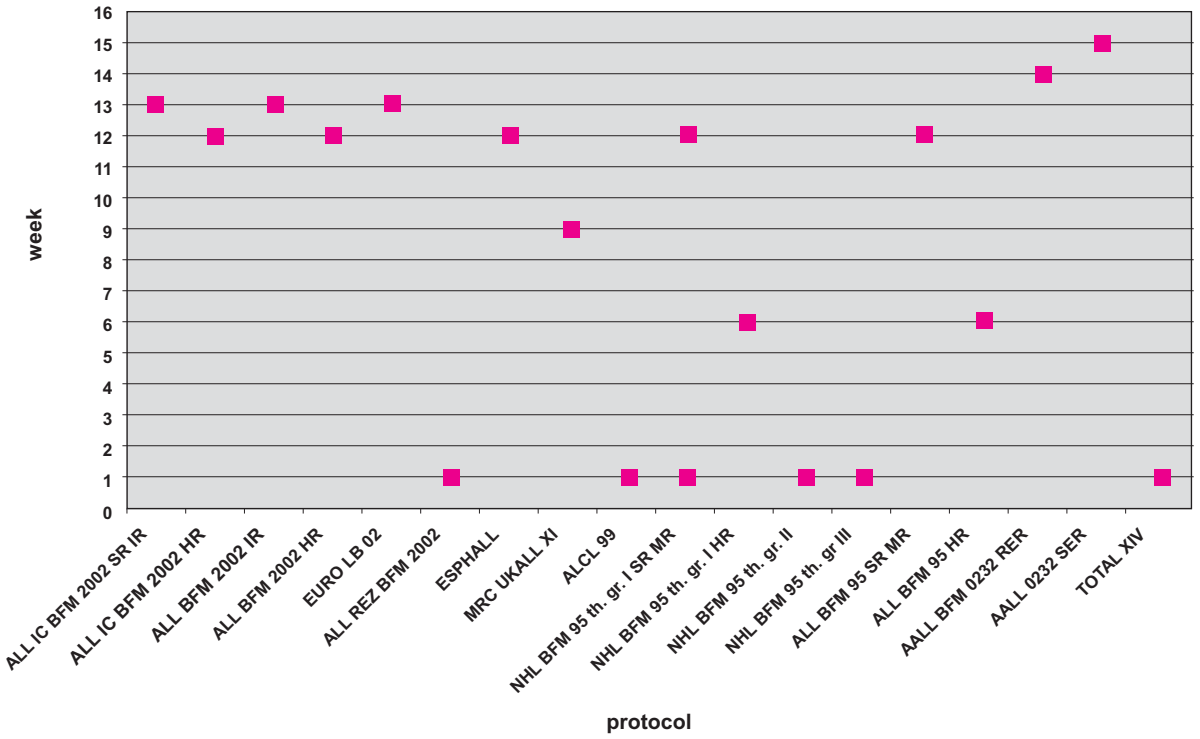

Figure 1. MTX application after the start of the therapy

Who can mostly benefit from HDMTX treatment?

An earlier assessment from St. Jude Children's Research Hospital suggested, that from HDMTX benefits only a limited amount of children with ALL (those with a white blood cell count of <25 x 10^9/l, white race, age 2–10 years, and hyperploid leukemia cells without translocations) [1]. There is growing evidence that human leukemia cells display lineage/subtype-specific differences with regard to MTXPG formation and accumulation over a wide range of methotrexate concentrations [17, 44] and that the folate pathway gene expression may differ in some subtypes of childhood ALL, even within B lineage ALL [23].

CNS-directed therapy for childhood ALL

Before the introduction of treatment for occult leukemia in the CNS, up to 75% of children with ALL relapsed in the CNS. Because of such risk, treatment designed to prevent onset of leukemia in the CNS (e.g. CNS-directed therapy, or presymptomatic CNS therapy, represented initially by radiotherapy) has become an integral part of the treatment of childhood ALL for the last 35 years [4]. However, concerns about late adverse effects of such treatment have led to the gradual reduction in radiotherapy (RT) dose and/or its omission, replacing RT mostly by systemic and intrathecal methotrexate (ITMTX). Randomized trials have shown that the combination of intensive systemic chemotherapy and regular ITMTX may obviate the need for cranial irradiation in most children with ALL [19, 33].

Critical reappraisal of real clinical contribution of different particular drugs in recent antileukemic therapies is difficult. The reasons for such difficulties are following:

– even recent treatment schemes remain often empiric or more eminent, than evidence based,

– obvious clinical efficacy of recent protocols leading to long term event free survival for about 80% of children with ALL would require large number of patients enrolled for trials in order to proof superiority of any regimen,

– there is still lack of reliable markers/predictors for many drugs in clinical use.

The contribution of particular components (e.g. methotrexate) could be assessed from the

– single leukemia cell perspective using DNA and/or RNA based and/or immunocytochemical approach,

– individual patient perspective, considering measurable effect for a particular patient (e.g. toxicity, homocysteine levels etc.),

– clinical/statistical perspective, considering clinically important endpoint variables like event free survival (EFS), time to progression (TTP), overall survival (OS), response rate (RR), in conjunction with different schedules applied.

Many recent reviews dealing with mechanisms of MTX action, including pharmacodynamic and pharmacogenetic considerations as well are published, including those dealing with genomic or single leukemic cell level of the MTX therapy issue [27, 45], including our group as well [41].

Despite very long clinical experience with antifolate therapy for children with ALL, even in most recent protocols for childhood ALL treatment there continues to be large variation regarding MTX and/or leucovorine (LV) dosing and scheduling, reaching up to 2 orders of magnitude (if the MTX dose is considered) [33, 38]. The availability of an effective antidote/rescue agent, leucovorine (LV), has allowed escalation of MTX doses to achieve enormous plasma concentrations (>1,000 μmol/l) compared with plasma folate (0.01–0.02 nmol/l). Intravenous (i.v.) intermediate (usually 0.5–2 g/m^2) or high dose (usually over 5 g/m^2) MTX appears to compensate for radiotherapy as important tool of CNS-directed therapy for childhood ALL [32]. Very high doses, up to 33.6 g were used in various protocols in an attempt to provide significant MTX exposures at disease sanctuary sites such as the CNS [5]. However, recent review of more than 40 trials for children with ALL concluded, that the addition of i.v. HDMTX in many different doses and schedules did not improve CNS therapy and made only minor improvements in systemic therapy for children with ALL reducing non-CNS relapses [11].

Interindividual and intraindividual variability

Existing pharmacokinetic data suggest wide interindividual and intraindividual variability in methotrexate levels and clearance [5, 39]. High-risk patients with lower steady state methotrexate levels seem to be at increased risk of relapse [8].

Individualized chemotherapy, including HD MTX, compared to conventional treatment based on body surface area, demonstrated a better outcome of children with B-lineage ALL treated with HD MTX dose adjustment [14]. However, there was no significant difference between treatments for patients with T-lineage leukemia.

Delayed MTX elimination following high-dose MTX (HD-MTX) treatment, could be an important problem in some circumstances, because it necessitates increased leucovorin rescue and additional hospitalization for hydration and urinary alkalinization. Risk factors for delayed excretion include:

– drug interactions (non-steroid anti-inflammatory drugs, penicillin, proton pump inhibitors, amphotericin, prior use of platinum may decrease MTX excretion),

– third spaces (pleural effusions, GI obstruction, ascites provide "sink" for MTX),

– direct nephrotoxicity of MTX itself,

– poor hydration/alkalinization,

– Down syndrome.

Overrescue concept

In 1991, BORSI et al [6], who noted a trend of better prog-

nosis, when less folinic acid was used, suggested the concept of overrescue within the clinically relevant doses of MTX. These authors recalculated the uniform folinic acid dose used according to body surface area. Their patients received 6 to 8 g/m^2 methotrexate over 24 hours, followed by 75 mg folinic acid at 36 hours, and 16 doses of 15 mg folinic acid from hour 39 to 106, for a total of 315 mg. The trend to better results was seen in the group given 158 to 315 mg/m^2 folinic acid, compared with the group given 315 to 588 mg/m^2. However, the difference in relapse rates did not reach statistical significance and there are no similar studies published more recently for children with ALL. On the other hand, similar clinical situation is well described for patients with psoriasis, where concurrent administration of folinic acid with methotrexate abolished the MTX effect [20].

MTX toxicity or effectiveness

MTX related toxicity remains to be an important issue even now, despite 2 decades of clinical experience with the high or intermediate dose systemic MTX treatment. May be even life threatening and vigorous supportive measures thus cannot be waived. Toxicities due to MTX include myelosuppression, gastro-intestinal tract mucositis, neurotoxicity, hepatotoxicity and nephrotoxicity.

While the first three types of toxicities can be prevented by leucovorin rescue, the latter two cannot. Particularly concerning have been CNS sequelae of the ALL treatment. Methotrexate and the rises of homocysteine induced by MTX have been linked to both acute and chronic neurological toxicity [36, 40].

However, the issue of possible contribution of LV timing and dosing is often overlooked [43]. Interesting recent review published by COHEN [12] raised again important questions regarding the optimal HDMTX dosing and/or the optimal LV rescue for children with ALL. Both extremes – too little and too much LV rescue – could be harmful. One may not give enough LV rescue being afraid it will reverse the efficacy of MTX. This practice may lead to more short- and long-term toxicity, neurologic particularly [30].

Although COHEN suggested that there are only few data to support that we can give too much rescue [12], important concerns remain about the antileukemic efficacy and selective rescue of a specific compartments [6]. Early models as well as clinical experience show that too much LV can reverse the antitumor effects of MTX [6, 7].

This antagonism may explain why HDMTX does not decrease the incidence of CNS relapse, e.g. the original indication for i.v. IM/HD MTX in order to omit CNS radiotherapy. MTX is poorly concentrated in the CSF compartment, and the choroid (via molecules as the multidrug resistance proteins) efficiently pumps MTX out of the CSF. In contrast, folates are actively concentrated in the CSF (the CSF: plasma ratio is 3–4:1). As little as 10 mg LV given orally can significantly raise the CNS folate level in only a few hours [24].

Thus, any potential benefit from HDMTX augmenting the amount of MTX in the CSF in lieu of intrathecal dosing could be neutralized by the need for multiple doses of LV (either orally or i.v.).

On the other hand, South American groups reported even lower dose of systemic i.v. MTX (2 g/m^2 over 24 h) as effective and non-toxic component of the therapy for B lineage ALL, omitting thus the necessity for MTX levels monitoring [9, 22].

I.v. MTX and/or leucovorin timing and dosing

Comparison of various CNS directed regimens using i.v. MTX is very difficult [11] because intrathecal MTX or LV rescue used to be given at different time points or doses in relation to i.v. HD MTX according to various treatment protocols (Fig. 1, 2, 3). Early studies pointed that cytotoxicity of MTX *in vitro* has been shown to relate more to increases in the time of exposure than to increases in the extracellular concentration of the drug. For example, for L5178Y/Asn$^-$ murine lymphoblasts, a 10-fold increase in exposure time to MTX between 3 and 42 h resulted in a 100-fold increase in cytotoxicity. In comparison, a 10-fold increase in drug concentration only resulted in a twofold increase in cytotoxicity, and this effect was lost in exposure times more than 6 h [26], underlying the importance of the MTX and LV timing. A Pediatric Oncology Group (POG) study showed that 1,000 mg/m^2 IV MTX was superior to 180 mg/m^2 given orally as 30 mg q6h x 6 with the use of the same LV rescue for both. However, outcome was only marginally improved (approximately 76% vs. approximately 80%), but there were more CNS occurrences and less toxicity in the oral MTX arm [25, 31].

Even within BFM family trials there are important differences regarding the timing of i.th. MTX in relation to i. MTX and /or LV timing and dosing. E.g. to treat relapsed patients the lower MTX dose is used compared to *de novo* diagnosed patients (1 g over 36 h is used, compared to 5 g over 24 h for new patients) (see Fig. 2, 3).

Interesting recent results from UKALL XI trial have shown that it is possible to effectively treat children with ALL at low and intermediate risk of CNS relapse (presenting WBC <50x10^9/l) without using cranial irradiation, by substituting continuing ITMTX with, or without, HDMTX. Although continuing ITMTX with three courses of HDMTX gave statistically significantly fewer isolated and combined CNS relapses than ITMTX alone, the benefit was cancelled out by an increase in hematological relapses in the HDMTX arm, which is contrary to CLARKE's review [11]. It is a bit surprising that, although the dosage of MTX used in UKALL XI (6–8 g/m^2) was higher than that used in previously reported trials, it did not produce a significant benefit in EFS. Some earlier randomized trials of moderate dose MTX infusions 0.5–1.0 g/m^2) had shown apparently reduced relapse rates [15, 35] while another trial [28] did not.

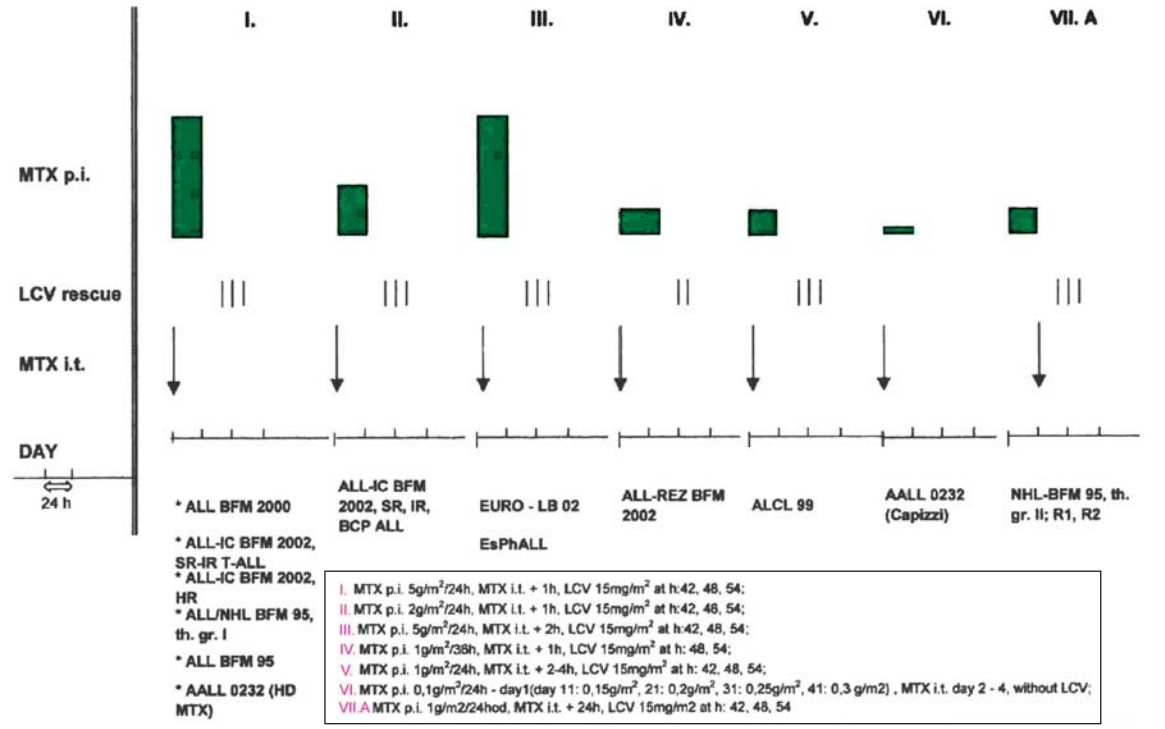

Figure 2

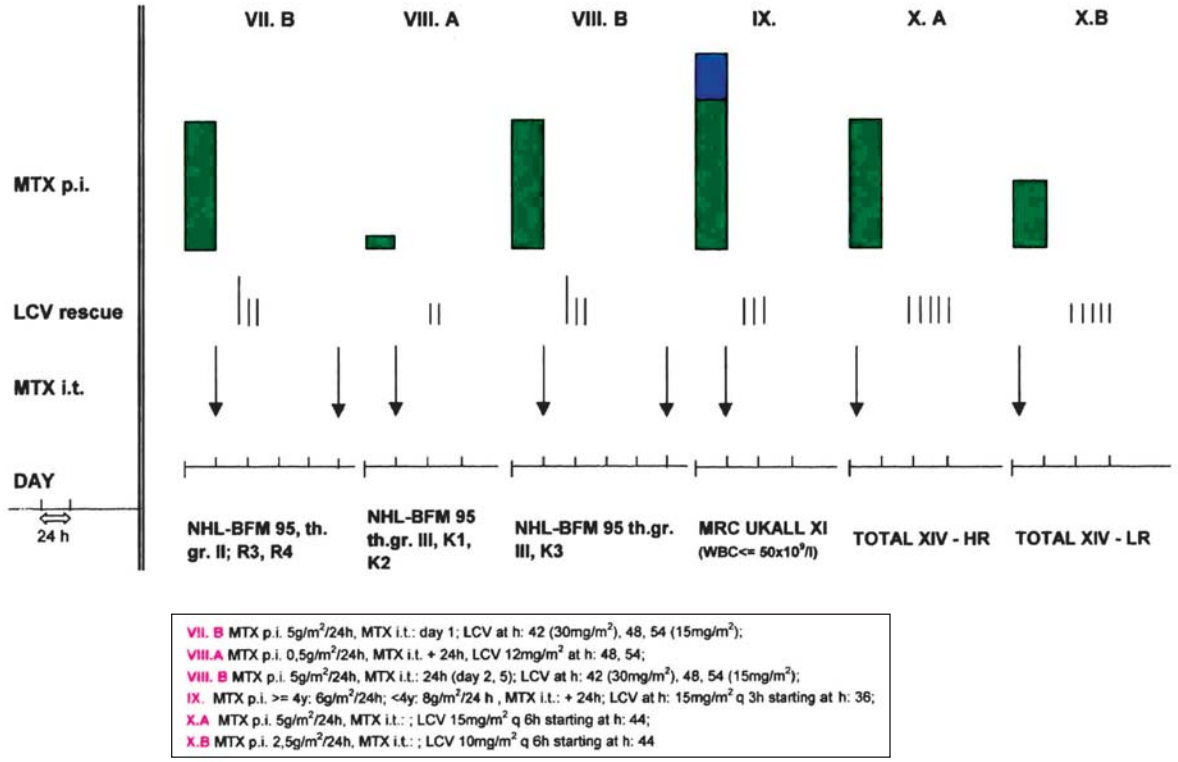

Figure 3

The leukemocidal effect of the HDMTX against CNS occult leukemia is dependent on MTX levels achieved in the CSF. Effective MTX levels in CSF samples taken at 24 h after starting the HDMTX was achieved in the majority of UKALL XI trial patients. One may speculate that late availability of MTX results may not allow to promptly adjust down folinic acid doses, which may have resulted in LV overrescue in many patients, minimizing the potential systemic benefit of MTX but not its impact on occult CNS disease, as suggested earlier by BORSI et al [6].

Why did HDMTX only marginally improve systemic therapy in the protocols that were reviewed by CLARKE, did not improve results in UKALL XI study and markedly improved results of the DFCI backbone?

MTX and 6-mercaptopurine have been the backbone of successful maintenance/continuation therapy for nearly four decades. Since treatment duration is about 2 years, children already receive over 70 courses of weekly MTX. Does substituting one or multiple courses (median four) of HDMTX (range 0.5–33 g/m^2) represent a significant increase in antifolate exposure, given pharmacologic parameters of time, dose, saturable metabolism, and the need for LV rescue? Moreover, some studies suggest that MTX may limit its own metabolism *in vitro*, so sometimes the more may not be better [21].

"Capizzi methotrexate"

Very interesting suggestions regarding the use of i.v. MTX and/or LV dosing can bring apparent clinical efficacy of the "Capizzi regimen", using completely different philosophy how to administer systemic, i.v. MTX [10, 29]. Much lower and escalating dose of i.v. methotrexate with no leucovorine rescue, followed by asparaginase, is a key element of augmented BFM therapy, which has been efficacious in the treatment of higher risk childhood ALL [34].

Clinical efficacy of Capizzi regimen and data from patients with psoriasis go well in line with original Borsi's overrescue theory.

Conclusion and questions to be answered

While considering the contribution of HD/IM MTX to overall therapeutic success in children with ALL from the single cell perspective [23], the issue is that reaction to i.v. MTX of chemotherapy naive ALL blasts (at presentation) could be different than the reaction of to chemotherapy exposed blasts on the level of minimal residual disease only, or even while in molecular remission (during the late intensification). This may complicate clinical usefulness of *in vitro* data achieved on chemotherapy naive blasts at disease presentation.

Recently it seems, that despite several decades of clinical experiences, the optimal dose and schedule of both methotrexate and/or LV is still a subject of considerable discussion. The answer how to best incorporate MTX into the treatment protocols is not yet known, and important questions regarding the timing and dosing of both MTX and LV rescue remain unanswered.

There could be burning questions raised. E.g. what is the justification for 2 weeks interval between HD MTX cycles, used in BFM and UKALL studies? E.g. similar to earlier Hodgkins studies?

What is the rationale for the i.v. MTX dose reduction for all B lineage ALLs, including TEL/AML 1+ ones, while maintaining the same leucovorine rescue in recent protocol M in ALL BFM IC 2002 study, currently in use in many countries, including our?

Given the data presented here and the nature of our assumptions backing recent treatment schemas that really can only be tested prospectively, it seems reasonable to proceed with further clinical and pharmacological studies dealing with this still controversial part of the treatment for children with ALL, despite 5 decades of clinical experience with systemic MTX treatment.

References

[1] ABROMOWITCH M, OCHS J, PUI CH,FRIRCHOUGH D, MURPHY SB, RIVERA GK. Efficacy of high-dose methotrexate in childhood acute lymphoblastic leukemia: analysis by contemporary risk classifications. Blood 1988; 71: 866–869

[2] Acute Leukemia Group B. New treatment schedule with improved survival in childhood leukemia. J Am Med Assoc 1965; 194: 187–193.

[3] ASSELIN BL, SHUSTER JJ, AMYLON M, HALPERIN E, HUTCHISON R et al. Improved Event-Free Survival with High Dose Methotrexate in T-Cell Lymphoblastic Leukemia and Advanced Lymphoblastic Lymphoma: a Pediatric Oncology Group Study. Proc Am Soc Clin Oncol 2001; 20: 367a, (abstr 1464).

[4] BALLIS MF, HOLCENBERK JC, BLANEY SM. General Principles of Chemotherapy. In: Pizzo P, Poplack D, editors. Principles and Practice of Pediatric Oncology. Lippincott, Philadelphia 2002; 237–308.

[5] BORSI JD, MOE PJ. Systemic clearance of methotrexate in the prognosis of acute lymphoblastic leukemia in children. N Eng J Med 1986; 314: 471–477.

[6] BORSI JD, WESENBERG F, STOKLAND T, MOE PJ. How much is too much? Folinic acid rescue dose in children with acute lymphoblastic leukaemia. Eur J Cancer 1991; 8: 1006–1009.

[7] BROWMAN GP, GOODYEAR MD, LEVINE MN, RUSSELL R, ARCHIBALD SD, YOUNG JE. Modulation of the antitumor effect of methotrexate by low-dose leucovorine in squamous cell head and neck cancer: a randomized placebo-controlled clinical trial. J Clin Oncol 1990; 8: 203–208.

[8] CAMITTA B, MAHONEY D, LEVENTHAL B, LAUER SJ, SHUSTER JJ et al. Intensive intravenous methotrexate and mercaptopurine treatment of higher-risk non-T, non-B acute lymphocytic leukemia: a Pediatric Oncology Group study. J Clin Oncol 1994; 12: 1383–1389.

[9] CAMPBELL M, SALGADO C, QUINTANA J, BECKER A, VARGAS

L et al. Improved outcome for acute lymphoblastic leukemia in children of a developing country: results of the Chilean National Trial PINDA 87. Med Pediatr Oncol 1999; 33(2): 88–94.

[10] CAPIZZI RL. Asparaginase-methotrexate in combination chemotherapy: schedule-dependent differential effects on normal versus neoplastic cells. Cancer Treat Rep (Suppl) 1981; 4: 115–121.

[11] CLARKE M, GAYNON P, HANN I, HARRISON G, MASERA G et al. CNS-directed therapy for childhood ALL: Childhood ALL collaborative group overview of 43 randomized trials. J Clin Oncol 2003; 9: 1798–1809.

[12] COHEN IJ. Defining the appropriate dose of folinic acid after high-dose methotrexate for childhood acute lymphoblastic leukemia that will prevent neurotoxicity without rescuing malignant cells in the central nervous system. J Pediatr Hematol Oncol 2004; 26: 156–163.

[13] DJERASSI I. Methotrexate infusions and intensive supportive care in the management of children with acute lymphocytic leukemia: a follow up report. Cancer Research 1967; 27: 2561–2564.

[14] EVANS WE, RELLING MV, RODMAN JH, CROM WR, BOYET JM, PUI CH. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. N Engl J Med 1998; 8: 499–505.

[15] FREEMAN AI, BOYETT JM, GLICKSMAN AS,BRECHER ML, LEVENTHAL BG et al. Intermediate-dose methotrexate versus cranial irradiation in childhood acute lymphoblastic leukemia: a ten-year follow-up. Med Pediat Oncol 1997; 28: 98–107.

[16] FREI E, FRIEDREICH EJ, GEHAN E, PINKEL D et al. Studies of sequential and combination antimetabolite therapy in acute leukemia: 6-mercaptopurine and methotrexate. Blood 1961; 18: 431–454.

[17] GALPIN AJ, SCHEUTZ JD, MASON E, YANISHEVSKI Y, SYNOLD TW et al. Differences in folylpolyglutamyl synthase and dihydrofolate reductase expression in human B-lineage versus T-lineage leukemic lymphoblasts: mechanisms for lineage differences in methotrexate polyglutamation and cytotoxicity. Mol Pharmacol 1997; 52: 155–163.

[18] GOLDBERG JM, SILVERMAN LB, LEVY DE, DALTON VK, GELBER RD et al. Childhood T-cell Acute Lymphoblastic Leukemia: The Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Experience. J Clin Oncol 2003; 19: 3616–3622.

[19] HILL FG, RICHARDS S, GIBSON B, HANN I, LILLEYMAN J et al. Successful treatment without cranial radiotherapy of children receiving intensified chemotherapy for acute lymphoblastic leukaemia: results of the risk-stratified randomized central nervous system treatment trial MRC UKALL XI (ISRC TN 16757172). Brit J Haematol 2004; 124(1): 33–46.

[20] HILLS R, IVE FA. Folinic acid rescue used routinely in psoriatic patients with known methotrexate "sensitivity". Acta Derm Venerol 1992; 72: 438–440.

[21] HUM M, SMITH A, LARK R, WINICK NJ, KAMEN BA. Evidence for negative feedback of xtracellular methotrexate (MTX) on ALL blasts in vitro. Pharmacotherapy 1997; 17: 1260–1266.

[22] JOANNON P, OVIEDO I, CAMPBELL M, TORDECILLA J. High-dose methotrexate therapy of childhood acute lymphoblastic leukemia: lack of relation between serum methotrexate concentration and creatinine clearance. Pediatr Blood Cancer 2004; 1: 17–22.

[23] KAGER L, CHEOK M, YANG W, ZAZA G, CHENG Q ET AL. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. J Clin Invest 2005; 111(1): 110–117.

[24] KAMEN BA, VIETTI T. Oral Leucovorine increases CSF folate concentration in children with leukemia. Br J Cancer 1989; 60: 799.

[25] KAMEN BA, WINICK N, HOLCENBERG J. Oral vs IV MTX: another opinion. J Clin Oncol 1998; 16: 2283–2284.

[26] KEEFE DA, CAPIZZI RL, RUDNICK SA. Methotrexate cytotoxicity for L5178Y/Asn lymphoblasts: relationship of dose and duration of exposure to tumour cell viability. Cancer Res 1982; 42: 1641–1645.

[27] KRAJINOVIC M, MOGHRABI A. Pharmacogenetics of methotrexate Pharmacogenomics. 2004; 7: 819–834.

[28] LANGE BJ, BLATT J, SATHER HN, MEADOWS AT. Randomised comparison of moderate-dose methotrexate infusions to oral methotrexate in children with intermediate risk acute lymphoblastic leukaemia: a Children's Cancer Group Study. Med Pediatr Oncol 1996; 27: 15–20.

[29] LOBEL JS, O'BRIEN RT, McINTOSH S, ASPENS GT, CAPIZZI RL. Methotrexate and asparaginase combination chemotherapy in refractory acute lymphoblastic leukemia of childhood. Cancer 1979; 10: 1089–1094.

[30] MAHONEY DH JR, SHUSTER JJ, NITSCHKE R, LAUER SJ, WINICK N et al. Acute neurotoxicity in children with B-precursor acute lymphoid leukemia: an association with intermediate-dose intravenous methotrexate and intrathecal triple therapy – a Pediatric Oncology Group study. J Clin Oncol 1998; 16: 1712–1722.

[31] MAHONEY DH JR, SHUSTER J, NITSCHKE R, LAUER SJ, WINICK N et al. Intermediate-dose intravenous methotrexate with intravenous mercaptopurine is superior to repetitive low-dose oral methotrexate with intravenous mercaptopurine for children with lower-risk B-lineage acute lymphoblastic leukemia: a Pediatric Oncology Group phase III trial. J Clin Oncol 1998; 1: 246–254.

[32] MARGOLIN JF, STEUBER CHP, POPLACK DG. Acute lymphoblastic leukemia. In: Pizzo P, Poplack D, editors. Principles and Practice of Pediatric Oncology. Lippincott, Philadelphia 2002: 516.

[33] NACHMAN J, SATHER HN, CHERLOW JM, SENSEL MD, GAYNON PS et al. Response of children with high-risk acute lymphoblastic leukemia treated with and without cranial irradiation: a report from the Children's Cancer Group. J Clin Oncol 1998; 16: 920–930.

[34] NACHMAN JB, SATHER HN, SENSEL MG, TRIGG ME, CHERLOW JM et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med 1998; 23: 1663–1671.

[35] PUI CH, SIMONE JV, HANCOCK ML, EVANS WE, WILLIAMS DL et al. Impact of three methods of treatment intensification on acute lymphoblastic leukemia in children: long-term results

of St Jude Total Therapy Study X. Leukemia 1992; 6: 150–157.

[36] QUINN CT, GRIENER JC, BOTTIGLIERI T, HYLAND K, TARROW A, KAMEN BA. Elevation of homocysteine and excitatory amino acid neurotransmitters in the CSF of children who receive methotrexate for the treatment of cancer. J Clin Oncol 1998; 15: 2800–2806.

[37] REITER A, SCHRAPPE M, LUDWIG WD, HIDDEMANN W, SAUTER S et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994; 84: 3122–3133.

[38] SCHRAPPE M, REITER A, ZIMMERMAN M, HARBOTT J, LUDWIG WD et al. Long-term results of four consectutive trials in childhood ALL performed by the ALL-BFM study group 1981-1995. Leukemia 2000; 14: 2205.

[39] SLANY J, GRUNDMANN M, BROZMANOVA H, BLAZEK B, STERBA J. High-dose Methotrexate for the treatment of acute lymphoblastic leukemia in children. In: Drug Resistance in Leukemia and Lymphoma II., Harwood Acad. Publishers, 1997: 183–188.

[40] SURTEES R, CLELLAND J, HANN I. Demyelination and single-carbon transfer pathway metabolites during the treatment of acute lymphatic leukemia: CSF studies. J Clin Oncol 1998; 16: 1505–1511.

[41] VALIK D, RADINA M, STERBA J, VOJTESEK B. Homocysteine: exploring its potential as a pharmacodynamic biomarker of antifolate chemotherapy. Pharmacogenomics 2004; 8: 1151–1162.

[42] VEERMAN AJP, HÄHLEN K, KAMPS WA, VAN LEEUVEN EF, DE VAAN GA et al. High cure rate with a moderately intensive treatment regimen in non-high risk childhood acute lymphoblastic leukemia: results of protocol ALL VI from the Dutch Childhood Leukemia Study Group. J Clin Oncol 1996; 14: 911–918.

[43] WABER DP, TARBELL NJ, KAHN CM, GELBER RD, SALLAN SE. The relationship of sex and treatment modalities to neuropsychological outcome in childhood acute lymphatic leukemia. J Clin Oncol 1992; 10: 810–817.

[44] WHITEHEAD VM, PAYMENT C, COOLEY L, LAUER SJ, MAHONEY DM et al. The association of the TEL-AML1 chromosomal translocation with the accumulation of methotrexate polyglutamates in lymphoblasts and with ploidy in childhood B-progenitor cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Leukemia 2001; 7: 1081–1088.

[45] WINTER-VANN AM, KAMEN BA, BERGO MO, YOUNG SG, MELNYK S et al. Targeting Ras signaling through inhibition of carboxyl methylation: an unexpected property of methotrexate. Proc Natl Acad Sci USA 2003; 11: 6529–6534.