Methotrexate-associated toxicity in children with Down syndrome and acute lymphoblastic leukemia during consolidation therapy with high dose methotrexate according to ALL-BFM treatment regimen

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Supplemental information

Recruitment periods

Patients were recruited from three consecutive multicenter ALL-BFM trials (ALL-BFM 95, ALL-BFM 2000 and AIEOP-BFM ALL 2009) who were diagnosed between 13th of January 1996 and 6th of September 2016 for DS-ALL and between 11th of April 1995 and 4th of May 2007 for NDS-ALL patients. In studies ALL-BFM 95 and ALL-BFM 2000, detailed data on treatment and toxicity were routinely collected by report forms for all patients; in contrast, no routine toxicity recording was performed during consolidation in the AIEOP-BFM ALL 2009 trial. By using the ALL-BFM 95 and ALL-BFM 2000 data, we were able to establish a representative large cohort of NDS-ALL patients for our HD-MTX toxicity study but the number of DS-ALL patients was still limited. Therefore, study centers who had treated DS-ALL patients according to AIEOP-BFM ALL 2009 were also contacted to provide toxicity data for these patients in order to enlarge the DS-ALL cohort.

Stratification and treatment in ALL-BFM trials

Treatment stratification into the high risk (HR) groups in ALL-BFM trials 95, 2000 and AIEOP-BFM ALL 2009 based on high risk molecular genetic markers (e.g. *BCR-ABL, KMT2-AFF1*) and on poor response to treatment. Patients were stratified into the HR group if they had a poor response to the prednisone prephase (prednisone poor response), by morphological non-remission in bone marrow after induction and by high minimal residual disease (MRD) after induction and consolidation phases (for details see supplemental references[1-2], clinicaltrials.gov *NCT01117441*). As MRD results after consolidation were available only after several weeks, in patients not

stratified to HR before, treatment in consolidation phase protocol M was started and then switched to HR treatment if MRD post consolidation qualified for the HR group.

HD-MTX consolidation therapy protocol M

HD-MTX consolidation *protocol M* is an 8-week therapy element in which patients receive 4 courses of *i.v.* HD-MTX (5 g/m^2 each) every second week in addition to age-adapted intrathecal MTX and daily oral 6-MP (25 mg/m^2/d). *i.v.* MTX was given as a loading dose (10% of total dose) within 30 min, the remaining 90% were infused over the following 23.5 h. MTX plasma levels were measured at 24, 42 and 48 h after start of each MTX infusion. Each HD-MTX course was followed by an *i.v.* LCV rescue (15 mg/m^2) at 42, 48 and 54 h after start of the MTX infusion. For high MTX plasma levels at 42 or 48 h (≥ 1.00 µmol/L and ≤ 0.40 µmol/L, respectively) the LCV rescue was performed with a higher LCV dose every six hours until MTX plasma levels were < 0.25 µmol/L. Some patients enrolled in the ALL-BFM 95 trial were randomized to receive *protocol MCA* instead of *protocol M* (DS-ALL n= 5, NDS-ALL n= 219). In *protocol MCA* patients were treated as in *protocol M* but additionally received a cytarabine infusion (200 mg/m^2) over 24 h starting one day after each MTX administration. No differences in occurrence of toxicities between *protocol M* and *MCA* patients were observed.

DNA and allelic discrimination assay

DNA from DS-ALL patients was prepared from blood or bone marrow samples (QIAmp DNA Blood Midikit, Qiagen, Hilden, Germany), from blood or bone marrow smears (ChargeSwitch Forensic DNA purification Kit, Invitrogen) or were provided by M. Stanulla[3]. For most patients remission samples were used for DNA isolation. Genotyping of the *SLC19A1* rs1051266 80G>A polymorphism was performed by using a TaqMan-based allelic discrimination assay (Custom TaqMan SNP genotyping assay, Applied Biosystems, Foster City, CA, USA; AssayID "AHUAP0W").

Primer/Probe	*Modification*	*Sequence 5' -> 3'*
forward	none	GCCTGACCCCGAGCTC
reverse	none	CATGAAGCCGTAGAAGCAAAGGTA
probe 1 (A allele)	VIC/NFQ	ACACGAAGGTGCCGCC
probe 2 (G allele)	FAM/NFQ	ACGAGGCGCCGCC

Toxicity grading based on CTCAE Version 2.0

TABLE S1: Toxicity grading based on CTCAEv2.0

Toxicity	Grade	Definition
Leukopenia	0	≥ 4.0 Gpt/L
	1	3.0 – < 4.0 Gpt/L
	2	2.0 – < 3.0 Gpt/L
	3	1.0 – < 2.0 Gpt/L
	4	< 1.0 Gpt/L
Thrombocytopenia	0	≥ 100 Gpt/L
	1	75 – < 100 Gpt/L
	2	50 – < 75 Gpt/L
	3	10 – < 50 Gpt/L
	4	< 10 Gpt/L
Infection	0	no infection
	1	mild infection
	2	i.v. antibiotics, no pathogen identified
	3	i.v. antibiotics, pathogen identified
	4	septic shock
Stomatitis	0	no stomatitis
	1	erythema, painless ulcerations
	2	painful ulcerations, able to eat
	3	painful ulcerations, not able to eat
	4	requirement of total parenteral nutrition
Nephrotoxicity (serum creatinin)	0	within age-specific reference range (N)
	1	> N – 1.5x N
	2	> 1.5 – 3.0x N
	3	> 3.0 – 6.0x N
	4	> 6.0x N
Hepatotoxicity (serum GOT/GPT)	0	within age-specific reference range (N)
	1	> N – 2.5x N
	2	> 2.5 – 5.0x N
	3	> 5.0 – 20.0x N
	4	> 20.0x N
Bilirubinemia (bilirubin)	0	within age-specific reference range (N)
	1	> N – 1.5x N
	2	> 1.5 – 3.0x N
	3	> 3.0 – 10.0x N
	4	> 10.0x N

TABLE S2. *SLC19A1* rs1051266 80G>A allele combination frequencies and comparison of allele combinations with regard to grade 3/4 toxicities in DS-ALL after the 1st HD-MTX course.

Allele combinations	GGG			GGA			GAA			AAA		
°3/4 toxicities[b] after 1st MTX course	**total**[a] n= 21/95 (22.1%)	**0.5 g/m²** n= 10/21 (47.6%)	**5 g/m²** n= 9/21 (42.9%)	**total** n= 36/95 (37.9%)	**0.5 g/m²** n= 19/36 (52.8%)	**5 g/m²** n= 17/36 (47.2%)	**total**[a] n= 27/95 (28.4%)	**0.5 g/m²** n= 14/27 (51.9%)	**5 g/m²** n= 11/27 (40.7%)	**total** n= 11/95 (11.6%)	**0.5 g/m²** n= 5/11 (45.5%)	**5 g/m²** n= 6/11 (54.5%)
leukopenia	12/21 (57.1%)	3/10 (30.0%)	8/9 (88.9%)	11/35 (31.4%)	4/18 (22.2%)	7/17 (41.2%)	7/27 (25.9%)	3/14 (21.4%)	4/11 (36.4%)	1/10 (10.0%)	1/5 (20.0%)	0/5 (0%)
thrombocytopenia	7/21 (33.3%)	1/10 (10.0%)	4/9 (44.4%)	8/34 (23.5%)	2/18 (11.1%)	6/16 (37.5%)	5/27 (18.5%)	3/14 (21.4%)	2/11 (18.2%)	3/10 (30.0%)	1/5 (20.0%)	2/5 (40.0%)
infection	1/19 (5.3%)	1/9 (11.1%)	0/9 (0%)	3/35 (8.6%)	0/18 (0%)	3/17 (17.6%)	2/26 (7.7%)	1/13 (7.7%)	1/11 (9.1%)	0/10 (0%)	0/5 (0%)	0/5 (0%)
stomatitis	9/21 (42.9%)	2/10 (20.0%)	5/9 (55.6%)	8/35 (22.9%)	2/18 (11.1%)	6/17 (35.3%)	9/26 (34.6%)	2/13 (15.4%)	6/11 (54.5%)	4/9 (44.4%)	3/5 (60.0%)	1/4 (25.0%)
hepatotoxicity	6/21 (28.6%)	3/10 (30.0%)	3/9 (33.3%)	3/33 (9.1%)	3/17 (17.6%)	0/16 (0%)	6/26 (23.1%)	3/14 (21.4%)	2/10 (20.0%)	1/10 (10.0%)	0/5 (0%)	1/5 (20.0%)
nephrotoxicity	0/21 (0%)	0/10 (0%)	0/9 (0%)	0/35 (0%)	0/18 (0%)	0/17 (0%)	0/26 (0%)	0/14 (0%)	0/10 (0%)	0/10 (0%)	0/5 (0%)	0/5 (0%)
bilirubinemia	0/21 (0%)	0/10 (0%)	0/9 (0%)	0/33 (0%)	0/17 (0%)	0/16 (0%)	0/27 (0%)	0/14 (0%)	0/11 (0%)	1/10 (10.0%)	0/5 (0%)	1/5 (20.0%)

[a]In the GGG and GAA carrier subgroups some patients received an intermediate MTX dose (0.551 – 4.499 g/m^2). [b]Toxicity grading for individual toxicities was missing in some patients, therefore in some cases the denominator deviates from the total number of patients of the respective subgroup.

Supplemental References:

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