Acute Lymphoblastic Leukemia

Amsacrine combined with etoposide and high-dose methylprednisolone as salvage therapy in acute lymphoblastic leukemia in children

In a retrospective analysis of 24 children with refractory or multiply relapsed acute lymphoblastic leukemia (ALL), a salvage regimen comprising amsacrine, etoposide, and high-dose methylprednisolone AEP achieved a significant treatment response in 11 of 19 evaluable patients (8 complete and 3 partial remissions). Five of 9 AEP-responsive patients who underwent subsequent stem cell transplantation are alive (median follow-up: 43 months; range 10 to 91). The load of minimal residual disease prior to transplantation appears to be predictive for outcome in this very poor-prognosis subgroup of ALL.

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The success of allogeneic hematopoietic stem cell transplantation (HSCT) in patients with high-risk acute lymphoblastic leukemia (ALL) critically depends on the pretransplant burden of leukemic cells.[1] Particularly in refractory or multiply relapsed ALL it is a challenging task to repress the leukemic cell population to as little submicroscopic disease as possible until consolidating HSCT can be performed. Various drug resistance mechanisms and considerable co-morbidity of these often heavily pretreated patients have to be faced. Here we report results of an institutional, non-randomized, single-arm protocol comprising amsacrine, etoposide, and high-dose methylprednisolone as a salvage treatment for multiply relapsed and/or refractory ALL in children. According to institutional guidelines, written informed consent was obtained from patients or their guardians before initiation of therapy. The patients' characteristics are presented in Table 1.

To assess the response to treatment cytomorphological examinations were complemented by the assessment of minimal residual disease (MRD) based on real-time polymerase chain reaction (PCR) of T-cell receptor or immunoglobulin heavy chain gene rearrangements.[2] Patients received amsacrine 100 mg/m^2/day i.v. for 2 days, etoposide 500 mg/m^2/day i.v. for 2 days, and methylprednisolone 1 g/m^2/day i.v. or p.o. for 3 days (AEP). A total of 24 children were eligible for the retrospective analysis of the efficacy and feasibility of AEP salvage therapy of recurrent/refractory ALL (14 c-ALL, 4 pre-B-ALL, 1 pro-B-ALL, 5 T-ALL). Among the patients with B-precursor ALL four were identified as carrying a Bcr-abl rearrangement. In all, ten patients presented with disease refractory to remission induction either primarily (n=2) or after the first (n=6) or subsequent relapses (n=2). The remaining patients to be reinduced (n=9) had a median of two relapses. Following treatment with AEP, 11 out of 19 evaluable patients (58%) achieved a significant response with 8 patients (42%) entering a complete cytomorphological remission and 3 patients achieving a partial remission (Table 2).

Refractory disease could be overcome in 4 of 10 patients. There was no AEP-related mortality after a total of 34 treatment cycles in 24 patients. The overall morbidity was acceptable (n=9 infectious diseases; n=2 mucositis). Myelotoxicity was moderate with a median time of 24 days (range: 12-28) to proceed from AEP to a subsequent treatment element. Nine of 11 responding patients underwent HSCT, four of whom (patients 18, 17, 6, 19) are alive and free of disease 10, 13, 61, and 91 months from the start of AEP. The median overall survival after AEP for remission induction and HSCT is 12 months (range 5.3 to 91). Among six AEP non-responders fludarabine-based regimens[3] improved the remission status in three patients (patients 7, 14, 16) resulting in two partial and one complete remission. The combined use of topotecan, vinorelbine, thiotepa, dexamethasone, and gemcitabine (TVTG) 4 achieved a partial remission in a patient with primarily refractory T-ALL not responding to either AEP or idarubicin, fludarabine, cytarabine and granulocyte colony-stimulating factor (ida-FLAG). The overall survival of patients not responding to AEP was 4 months (range 1.6 to 18). MRD analyses could be performed in 17 of 24 patients on the AEP protocol. Four of six patients (patients 17,18,19,24) with a low or negative level of MRD ($<10^{-3}$) pre-HSCT are alive without evidence of disease.

By contrast, only one (patient 21) of six patients carrying a high level of MRD ($>10^{-3}$) into HSCT has survived and is event-free. These observations are in line with an earlier study on ALL defining MRD as a strong prognostic predictor for outcome before HSCT.[5] The efficacy of AEP in the HSCT-setting appears to be similar to that of the recently introduced TVTG-regimen for relapsed or refractory acute leukemia.[4] Compared to the latter regimen AEP resulted in fewer adverse events with faster hematopoietic recovery and fewer infectious complications without any treatment-related deaths. A further dosage escalation of amsacrine up to a total dose of 500 mg/m^2 may be feasible according to former studies on childhood acute myeloid leukemia although potentially at the expense of an increased treatment-related morbidity and a higher risk of secondary acute myeloid leukemia.[6] In the present study etoposide and amsacrine may have complemented each other in their topoII-poisoning activities.

A synergy between amsacrine and etoposide can be at least partially explained by the difference in structure of cleavable complexes, which may account for the retained activity of amsacrine in etoposide-resistant cells and vice versa.[7] Also, amsacrine exerts its cytotoxicity by binding both topoII isoforms α and β equally, whereas etoposide predominantly targets topoIIβ.

Table 1. Characteristics of patients treated with AEP (n=24).

Pt	Diagnosis	Age (y) at diagnosis/ at AEP	Sex	Prior therapy	Disease status at enrollment
1	c-ALL	13.5/14.5	M	CoALL 89; early relapse (BM): ALL REZ BFM 90	1st rel, early; ref
2	c-ALL	14.9/19.1	M	CoALL 89; late relapse (BM): ALL REZ BFM 90	2nd rel, early
3	Pre-B ALL	10.1/10.9	F	CoALL 92; very early relapse (BM): ALL REZ BFM 96	2nd rel, very early
4	c-ALL(Ph+)	3.9/4.8	F	CoALL 92; BMT (MRD); early relapse (BM); VCR, ADR	1st rel, early
5	c-ALL	4.4/10.9	M	CoALL 97; late relapse (BM): ALL REZ BFM 96	2nd rel, very early; ref
6	Pro-B ALL	0.6/1.0	M	Interfant-99; early relapse (BM); OCTADD	1st rel, very early; PR
7	c-ALL (Ph+)	14.3/15.5	M	ALL BFM 95; very early relapse (BM): ALL REZ BFM 99 (pilot); HU, AraC, VCR	2nd rel, very early; ref
8	T-ALL	2.2/3.9	M	CoALL 97; early relapse (BM); Ida,VCR, Dex	1st rel, early; ref
9	T-ALL	3.6/3.8	M	CoALL 03; ALL BFM 2000	primarily ref
10	c- ALL	2.4/5.8	F	CoALL 97; early relapse (BM): ALL REZ BFM 96	2nd rel, very early
11	Pre-B ALL (Ph+)	7.2/8.5	M	CoALL 97; BMT (MUD); very early relapse (BM); VCR, DNR, ASP	1st rel, very early (ref)
12	c-ALL	7.5/10.5	F	CoALL 97; late relapse (BM): ALL REZ BFM 96	2nd rel, very early
13	Pre-B ALL	13.1/14.3	M	CoALL 97; very early relapse (combined, BM+CNS): ALL REZ BFM 96	2nd rel; very early
14	c-ALL	3.7/5.6	M	CoALL 97; early relapse (BM): ALL REZ BFM 96; Dex, VCR, HD-AraC, ASP	1st rel, early; (ref)
15	c-ALL	3.5/5.2	F	CoALL 97; early relapse (BM): ALL REZ BFM 96	1st rel, early; (ref)
16	T-ALL	3.9/4.5	F	ALL BFM 2000 HR; very early relapse (BM)	1st rel, very early
17	c-ALL	8.0/12.1	M	CoALL 97; late relapse (BM): ALL REZ BFM 2002	1st rel, late; ref
18	T-ALL	7.7/9.2	M	CoALL 97; very early relapse (EM, CNS): ALL REZ BFM 2002; i.th. MTX, AraC, Pred	3rd rel, very early (CNS)
19	T-ALL	10.5/10.9	M	CoALL 97	primarily ref
20	c-ALL	3.4/8.2	F	CoALL 92; late relapse (BM): ALL REZ BFM 96	3rd CR
21	c-ALL (Ph+)	4.4/4.8	F	CoALL 97; NHL-BFM 95	1st CR
22	c-ALL	9.5/13.6	M	MB 91; late relapse (BM): ALL REZ BFM 96; DNX, HD-AraC, VCR, Pred	3rd CR
23	c-ALL	2.4/4.5	M	CoALL 97; early relapse (BM): ALL REZ BFM 2002 pilot	2nd CR
24	Pre-B ALL	11.2/11.5	M	CoALL 97; early relapse? (BM)	1st CR or 1st rel (?)

Rel: relapse; BM: bone marrow; EM: extramedullary; combined, BM and EM; Ref, refractory disease; (Ref), refractory to a single course of intensive induction treatment; CR, complete (cytomorphological) remission; PR, partial remission; Ph+, Philadelphia chromosome (Bcr-abl rearrangement); CoALL, Cooperative ALL Study Group9; ALL BFM, Berlin-Frankfurt-Münster ALL Study Group; ALL-REZ BFM10; Interfant 99, international infant ALL study group (unpublished); MB91, Moscow-Berlin ALL protocol ; VCR, vincristine; ADR, doxorubicin; DNR: daunorubicin; DNX, liposomal daunorubicin; Ida, idarubicin; HD-AraC, high dose cytarabine; Dex, dexamethasone; Pred, prednisolone; MTX, methotrexate; HU: hydroxyurea; ASP: asparaginase.

Table 2. Response to salvage therapy and outcome.

Pt	Response to AEP	AEP cycles	Additional salvage therapy after AEP (response)	Time salvage to SCT (days)	SCT	Status at last follow-up (time after AEP)
1	Ref	1	–	–	–	DOD (1.6mo)
2	Ref	2	–	–	–	DOD (6mo)
3	PR	2	–	20	MUD	DOD (11mon)
4	PR	2	HAM (PR), 2 DLI (CR)	–	HSCT prior to AEP	DOD (33mon)
5	CR	1	n.d. (3rd rel)	(89)	MMRD	DOTRM (7.7mon)
6	CR	2	–	28	MUD	EFS (61mon)
7	Ref	2	Ida-FLAG (PR)	41	MUD	DOD (18mon)
8	Ref	1	–	–	–	DOD (2mon)
9	Ref	1	Ida-FLAG (NR); TVTG (PR)	27	MRD + IF-Rx	EFS (3.5mon)
10	CR	2	DNX-FLAG (CCR)	–	–	DOTRM (2mon)
11	Ref	1	HAM (Ref);DLI (Ref); Imatinib (CR)	–	HSCT prior to AEP	DOD (4mon)
12	CR	1	–	35	MRD	4th rel (d872; OS 46mon)
13	Ref	1	–	39	MUD	DOD (4mon)
14	PR	1	DNX-FLAG (CR)	31	MRD	DOTRM (5.3mon)
15	CR	2	–	35	MRD	DOD (29mon)
16	Ref	1	DNX-FLAG (PR)	–	–	DOD (4.5mon)
17	CR	1	DNX-FLAG (CCR)	34	MRD	EFS (13mon)
18	CR	2	–	38	MUD	EFS (10mon)
19	CR	2	–	37	MUD	EFS (91mon)
20	CCR	1	VCR/ASP (CCR)	29	MUD	DOTRM (26mon)
21	CCR	1	–	27	MUD	EFS (69mon)
22	CCR	2	DNX/VCR/AraC/Pred; VCR/CPM/Pred	29	Haploident	DOD (12mon)
23	CCR	1	–	35	MRD	DOD (13mon)
24	CCR(?)	1	–	26	MUD	EFS (15mon)

Ref, refractory disease; C(C)R, complete (continuous) remission; PR, partial response; MRD, matched related donor; MMRD, mismatched related donor; MUD, matched unrelated donor; EFS, event-free survival: OS, overall survival; DLI, donor lymphocyte infusion; DOD, dead of disease; DOTRM, dead of treatment-related mortality; FLAG, fludarabine, cytarabine, GCSF; DNX, liposomal daunorubicin; Ida, idarubicin; HAM, high-dose cytarabine + mitoxantrone; TVTG: topotecan, vinorelbine, thiotepa, dexamethasone, gemcitabine; CPM, cyclophosphamide; IF-Rx, involved field lymph node irradiation; -, not applicable; n.d., additional salvage between AEP and HSCT not documented (patient 5).

Importantly, hyperphosphorylation of topoIIβ may compensate for hypophosphorylation of topoIIβ to maintain normal topoII function during proliferation making the topoIIβ isoform an equally important drug target.[8] In conclusion, AEP may be considered as one element of a rescue strategy in refractory or multiply relapsed ALL of childhood. Larger, prospective studies

are warranted to define its role more clearly. MRD-monitoring is a prerequisite to control rescue efficacy precisely.

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