Table 1 Phenotypic characteristics of T cells in B-CLL patients

		$CD4^+$ T cells (%)		$CD8^+$ T cells (%)		
T cell marker	*Group*	*Median*	*(P25–75)*	*Median*	*(P25–75)*	*P-value*
CD54	NP	13.1	(3–49.6)	51.2	(8–71.6)	<0.05
	P	30.3	(18–34.5)	44.1	(5.7–87.8)	NS
	NC	8.5	(1.5–15.7)	27.9	(9.9–60.7)	<0.01
TCR ζ chain	NP	88.8	(66–95.8)	81.3	(56.9–95.1)	NS
	P	75.6	(44.9–90.9)	68.1	(42.6–93.5)	<0.05
	NC	92.4	(76.3–99.2)	90.7	(71.2–99)	NS
CD28	NP	74.6	(35.9–95.5)	44	(24.5–86.3)	<0.01
	P	71.5	(25.1–90.3)	26.6	(5.7–95)	<0.05
	NC	94.5	(64.5–100)	50.1	(19.6–82.2)	<0.001
sCTLA-4	NP	0.8	(0–3.5)	0	(0–2.9)	<0.05
	P	10	(5.5–38)	8.6	(3.2–35.6)	NS
	NC	0.1	(0–2.4)	0.1	(0–0.8)	NS
cCTLA-4	NP	11.4	(0–28)	2	(0–11.5)	<0.05
	P	15.7	(7.5–33)	6.2	(1–8.7)	<0.05
	NC	4.3	(0.5–6.4)	0.5	(0–2.1)	<0.05

sCTLA-4, on the cell surface; cCTLA-4, intracellular expression; NP, B-CLL patients with nonprogressive disease, P, B-CLL patients with progressive disease, NC, normal controls; NS, not significant; P, percentile.

patients.[5] T cells with an enhanced expression of CD54 and a high IL-4 content were found in patients with asthma.[8] The biological significance and mechanism of upregulation of CD54 on T cells in B-CLL need, however, further clarification.

This study showed that several critical activation/interaction molecules on T cells in B-CLL are abnormally expressed, and more pronounced by advanced stage. These defects may contribute to immune dysfunction responsible for immunological abnormalities and an impaired regulation of the leukemic clone. Therapeutic attempts might be instituted to restore T-cell functions, those interventions may be crucial when developing immunotherapeutic concepts, especially tumor vaccines.

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Lack of asparagine depletion in the cerebrospinal fluid after one intravenous dose of PEG-asparaginase: a window study at initial diagnosis of childhood ALL

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TO THE EDITOR

L-Asparaginase (ASP) plays a well-established role in the treatment of children with acute lymphoblastic leukemia (ALL). ASP is postulated to exert its antitumor activity by hydrolyzing asparagine (Asn) to aspartic acid and ammonia, thereby depleting the leukemic cells from Asn, leading to impaired protein synthesis and leukemic cell death. ASP may

also act by depleting glutamine. Different preparations and ways of administering ASP result in different plasma activities. The half-life of Erwinia-ASP is shorter than that of *Escherichia coli*-ASP, which is in turn shorter than that of the polyethylene glycol (PEG)-conjugated form of ASP.[1] PEG-ASP shows less immune response and a prolonged half-life of 5.7 days compared to 1.3 days for native ASP. These variations are related to differences in the extent of Asn depletion.[2]

Pharmacokinetics and pharmacodynamics of PEG-ASP are not well characterized in the cerebrospinal fluid (CSF). The ASP activity in the CSF is less than 1% of the corresponding plasma activity using native *E. coli*-ASP. Yet, ASP is believed to play a role in the prevention of meningeal leukemia probably by depleting the pool of Asn in the CSF. It is not known whether an incomplete depletion of Asn in the CSF results in a suboptimal antileukemic effect. In Rhesus monkeys and in a number of adult patients, Riccardi *et al*[3] demonstrated that, after native *E. coli*-ASP, CSF-Asn levels were depleted to $<0.2\ \mu$M. Several groups showed that a plasma *E. coli*-ASP activity >100 IU/l leads to an Asn depletion of $<0.2\ \mu$M in the plasma and CSF.[2,4] Müller *et al*[5] demonstrated that after one dose of 1000 IU/m^2 PEG-ASP intravenously (i.v.) as second-line treatment, the plasma ASP activity was still $\geqslant 100$ IU/l after 14 days in 44/66 patients. Unfortunately, no CSF levels were measured in their study.

Recently, Avramis *et al*[6] showed that PEG-ASP (1×2500 IU/m^2) intramuscularly (i.m.) reached an ASP plasma activity >100 IU/l accompanied by Asn levels $<3\ \mu$M during 3–14 days in 95% of the patients. CSF-Asn concentrations fell to $0.6\ \mu$M at day 28. In the present paper, we report on 24 newly diagnosed children with ALL treated in our center with a single dose of PEG-ASP (Oncaspar™) 1000 IU/m^2 i.v., 5 days before starting induction chemotherapy according to the ALL-9 study of the Dutch Childhood Leukemia Study Group (DCLSG).

The bone marrow, blood and CSF were obtained at diagnosis. From day -5 till day 0, peripheral blood samples were collected daily, and later on twice a week. At day 0, a second lumbar puncture (LP) was performed at the start of combination induction therapy (dexamethasone, vincristine, and intrathecal triple therapy during the first 4 weeks, daunorubicine only in case of initial high-risk criteria). A third CSF sample was drawn at day 15.

The quantification of ASP activity was performed by incubating the samples with an excess amount of L-aspartic acid β-hydroxamate (AHA) at 37°C. ASP hydrolyzed AHA to L-aspartic acid and hydroxylamine, which was detected at 710 nm after condensation with 8-hydroxychinoline and oxidation to indooxine. This method allowed the quantification of 2.5 IU/l ASP in human serum with coefficients of variation for intra- and interday variability of 1.98–8.77 and 1.73–11.0%, and an overall recovery of 101 ± 9.92%.[7] Asn levels in the plasma and CSF were measured using the RP-HPLC technique following precolumn derivation with *o*-phthaldialdehyde and fluorescence detection according to Lenda and Svenneby.[8] The lower limit of detection (LOD) was $0.2\ \mu$M.

All patients reached an ASP activity $\geqslant 100$ IU/l for at least 10 days (Figure 1). A peak level of 744 ± 132 IU/l (mean$\pm$s.d.) was reached 1 h after the PEG-ASP infusion, declining to 483 ± 101 IU/l (mean$\pm$s.d.) after 5 days and to 212 ± 66 IU/l (mean$\pm$s.d.) on day 7, 12 days after the PEG-ASP administration; on day 15 of the treatment schedule, 20 days after the PEG-ASP infusion, ASP activity had declined to 39 ± 28 IU/l (mean$\pm$s.d.). Avramis showed that the mean peak of PEG-ASP activity was 1000 IU/l when measured 5 days after a dose of 2500 IU/m^2 i.m. was given to children with ALL, declining to about 100 IU/l on day 24.[6]

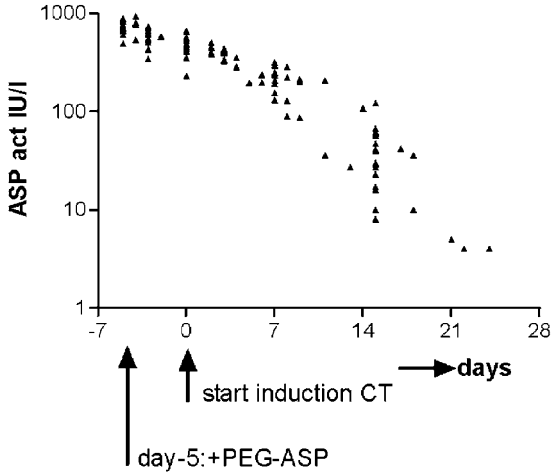

Figure 1 Sequential analysis of plasma asparaginase activity (IU/l) after 1000 IU/m^2 of PEG-ASP i.v. given 5 days before starting combined induction chemotherapy (CT).

We also analyzed all CSF samples for ASP activity and detected no activity above the limit of quantification (2.5 IU/l).

In all patients, plasma Asn levels declined below the LOD of $0.2\ \mu$M (Figure 2a). From the point of view of peripheral treatment intensity, the results of Asn plasma concentrations after 1000 IU/m^2 PEG-ASP indicate a treatment intensity comparable to that observed with native unpegylated ASP[2] (10 000 IU/m^2).

Riccardi *et al*[3] showed that Asn depletion in CSF was only achieved after plasma Asn depletion. However, no complete Asn depletion in the CSF occurred in our patient group (Figure 2b): pretreatment starting levels at day -5 ranged from 3.5 to $8\ \mu$M (mean $5.1\pm 1.1\ \mu$M), decreasing to a mean concentration of $1.58\pm 0.66\ \mu$M at day 0 just before starting induction chemotherapy. At day 14 (19 days after the administration of PEG-ASP), the mean CSF Asn concentration was $2.2\pm 0.67\ \mu$M. The Asn concentration never dropped below the LOD ($0.2\ \mu$M). We also spiked CSF samples with ASP, incubated the samples at 37°C and analyzed them for Asn by HPLC. No Asn was detected in the spiked samples; thus, we are sure that we determined Asn in the CSF samples. CSF-Asn concentrations in the study of Avramis fell from a median pretreatment level of 2.3–1.1 μM on day 7 and $0.6\ \mu$M on day 28,[6] demonstrating that i.m. PEG-ASP also does not fully deplete CSF Asn. These Asn levels are still high above the detection limit of $0.01\ \mu$M in their study. The study of Avramis differs from our study in terms of the dose of PEG-ASP (2500 *vs* 1000 IU/m^2), the route of PEG-ASP (i.m. *vs* i.v.), and concomitant chemotherapy (prednisone p.o., vincristine i.v., and intrathecal cytarabine/methotrexate *vs* none in the first 5 days). The influence of concomitant antileukemic treatment on the pharmacokinetic and pharmacodynamic effects of ASP is not clear.

It has been suggested that the CSF-ASP activity never exceeds 0.2% of the ASP activity in plasma.[3] One explanation for the lack of Asn depletion in CSF may be that the pegylated form of ASP results in CSF-ASP levels that are even less than 0.2% of the plasma activity. We also analyzed all CSF samples for PEG-ASP activity and detected no activity above the limit of quantification (2.5 IU/l). Another explanation could be that the central nervous system is capable of synthesizing Asn locally despite the depletion of the systemic Asn pool. To maintain sufficient amino acids in the CSF, a net amino-acid entry from blood to

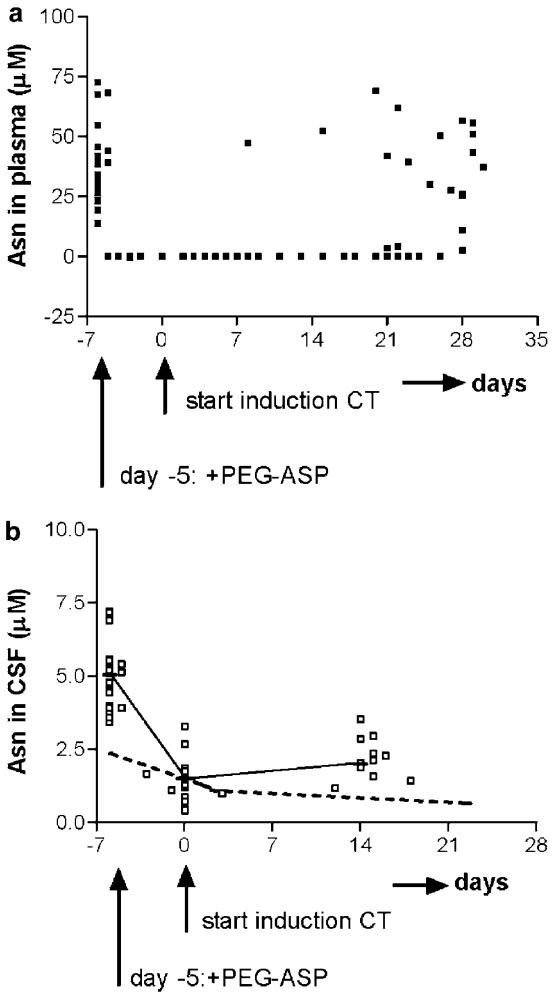

Figure 2 (a) Sequential analysis of plasma (■) asparagine levels (μM) after 1000 IU/m^2 of PEG-ASP i.v. given 5 days before starting combined induction chemotherapy (CT). (b) Sequential analysis of CSF (□) asparagine levels (μM) after 1000 IU/m^2 of PEG-ASP i.v. given 5 days before starting combined induction chemotherapy (CT). The dotted line depicts the results of Avramis after 2.500 IU/m^2 PEG-ASP given i.m. and combined with induction chemotherapy during the first 5 days.

CSF against a concentration gradient has been demonstrated in sheep.[9]

So, despite the fact that a dose of 1000 IU/m^2 PEG-ASP results in plasma Asn levels <0.2 μM, the human body is still capable of maintaining the CSF Asn levels. Although PEG-ASP may have the advantage of fewer injections, this might be counterbalanced by less effective killing of blasts in the CSF. It is yet unknown whether an impaired depletion of Asn in CSF by PEG-ASP has an effect on the incidence of CNS relapse in this group of patients.

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