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Multi-centre pilot study of 2-chlorodeoxyadenosine and cytosine arabinoside combined chemotherapy in refractory Langerhans cell histiocytosis with haematological dysfunction

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Abstract

The aim of this study was to assess the efficacy and adverse effects of 2-chlorodeoxyadenosine (2-CdA) and cytosine arabinoside (Ara-C) in children with refractory Langerhans cell histiocytosis (LCH) and haematopoietic dysfunction. Ten patients, with a median age at diagnosis of 0.5 years, were enrolled in this study. Treatment comprised at least two courses of Ara-C (1000 mg/m^2/d) and 2-CdA (9 mg/m^2/d) administered for 5 d every 4 weeks; subsequent median follow-up was 2.8 years (range 0.03–6.4 years). Among the 7 patients who received at least two courses of therapy, disease activity decreased in 6 patients, and control of disease was achieved in all patients after a median delay of 5.5 months. All patients suffered World Health Organisation (WHO) grade 4 haematological toxicity. Two septic deaths occurred shortly after administration of the first course of 2-CdA/Ara-C; a third patient was withdrawn from the trial after the first course and subsequently died following haematopoietic stem cell transplantation. This series is small, but we conclude that 2-CdA and Ara-C combined chemotherapy probably has major activity in childhood refractory Langerhans cell histiocytosis.

Keywords: Langerhans cell histiocytosis; Haematopoietic dysfunction; Thrombocytopaenia; 2-CdA; Ara-C; Pilot study

1. Introduction

Langerhans cell histiocytosis (LCH) is a rare disease resulting from the clonal proliferation and accumulation of pathological Langerhans cells (LCH cells) and accompanying inflammatory cells, with variable clinical manifestations and prognosis. Patients with multi-system disease and organ dysfunction, especially so-called 'haematopoietic dysfunction', are usually treated with combination chemotherapy including vinblastine and corticosteroids, but the prognosis of patients with

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disease progression during 'standard' chemotherapy is extremely poor [1–4].

The deoxyadenosine analogue, 2-chlorodeoxyadenosine (2-CdA, cladribine, Leustat, Ortho Biotech, New Jersey, USA) is phosphorylated *in vivo* by the enzyme deoxycytidine kinase (dCK), leading to the formation of 2-CdA mono-, di- and tri-phosphate (2-CdATP), which inhibit DNA synthesis. 2-CdA exerts an antiproliferative effect on histiocytes and lymphocytes [5]. Many studies have reported that 2-CdA as a single therapeutic agent can control recurrent or refractory localised LCH [6,7], but disease control is rarely observed in patients with marrow involvement or haematological dysfunction [7–12].

Cytosine arabinoside (Ara-C), a cytostatic drug also phosphorylated by dCK, has been used successfully in combination with vincristine and prednisolone in small numbers of children with disseminated LCH and organ dysfunction [13]. Furthermore, treatment with 2-CdA results in increased accumulation of Ara-CTP (the active form of Ara-C) in circulating blasts from adult patients with relapsed and refractory acute myelogenous leukaemia [14,15]. These results suggest that an approach combining 2-CdA and Ara-C chemotherapy may be useful in the treatment of refractory LCH. To date, there have been only two published reports of patients who have received combined 2-CdA and Ara-C therapy, with success in one case, and a poor response in the other [16,17]. Here, we report the results of a nationwide pilot study in which combined 2-CdA and Ara-C chemotherapy was used for the treatment of refractory or relapsed LCH with haematopoietic dysfunction in 10 children.

2. Patients and methods

2.1. Database and enrolment

The diagnostic criteria for LCH in this study were based on those previously proposed by the Histiocyte Society, including morphological features and either demonstration of CD1a antigenic determinants on the surface of lesional cells, or detection of Birbeck granules by electron microscopy [18].

All 34 French pediatric haemato-oncology centres participated in the study. Patients who received the 2-CdA and Ara-C regimen represent nine out of 21 patients identified in the French register with haematopoeitic dysfunction between 1997 and 2002. Of these 21 patients, 12 were considered to have LCH refractory to conventional chemotherapy. Three patients were not included in this study; 1 received other chemotherapy as salvage therapy and died, whilst two received haematopoïetic stem cell transplantation as a salvage therapy and 1 died [26]. In addition, 1 patient from a Belgian centre was enrolled. All cases treated by this regimen both in France and in Belgium, identified through the network of paediatric haemato-oncology units in these two countries, are reported here.

2.2. Inclusion criteria

Patients were eligible for the study if they had documented 'haematopoietic dysfunction', defined by the need for blood cell or platelet transfusions, and if disease activity had progressed after at least one course of multiagent chemotherapy including vinblastine and corticosteroids. The criteria used to define disease progression are those used in the Histiocyte Society protocols [1]. Patients could be included during the initial phase of the disease, soon after diagnosis, or after relapse.

2.3. CdA and Ara-C therapy

Ara-C was administered at a dose of 1000 mg/m^2/d by two 2-h intravenous infusions, and 2-CdA at 9 mg/m^2/d infused intravenously continuously, for five consecutive days. 2-CdA was administered at a dose of 0.3 mg/kg/d if body weight was below 10 kg. Courses were repeated every 4 weeks. The study ceased after two courses of the therapy and subsequent treatment was planned on an individual basis. Because pancytopaenia (haematopoietic dysfunction/bone marrow failure) was a common feature, neutropaenia and thrombocytopaenia were not a contraindication to therapy. Sulphamethoxazole/trimethoprim was used as *Pneumocystis carinii* prophylaxis, and anti-emetics and G-CSF were administered as necessary.

2.4. Patient monitoring and follow-up

Patient monitoring was based on medical charts and was performed on site at the participating hospital by a clinical research associate. A disease activity score evaluating LCH severity was calculated retrospectively: (i) at diagnosis; (ii) prior to the first course of 2-CdA + Ara-C; (iii) 28 d after the second course of 2-CdA + Ara-C. Briefly, the score takes into account multiple clinical parameters related to the severity of the disease, such as pain, tumour burden, skin involvement, liver and spleen size, liver enzyme levels and transfusion frequency [19]. Partial response was defined as any decrease in the activity score, and a complete response by a score below 2. The adverse effects (haematological, neurological, infectious, renal, gastrointestinal) of the regimens were analysed retrospectively.

2.5. Statistical methods

Stata version eight software was used for all statistical analyses. The cut-off date for analysis was 1st September 2004 and median duration of follow-up after

the first day of 2-CdA and Ara-C was 2.8 years (range 0.03–6.4 years), calculated using Schemper's measure [20]. Among survivors, median follow-up was 3.8 years (range 2–6.4 years). Significance of the reduction in LCH activity scores following 2-CdA and Ara-C treatment was determined by the Wilcoxon matched pairs test.

2.6. Patient characteristics

Patient characteristics prior to treatment by 2-CdA and Ara-C are shown in Table 1. Four male and 6 female patients were enrolled, with a median age at diagnosis of 6 months (range 0.16–1.5 years). The median delay between disease diagnosis and 2-CdA/Ara-C administration was 1 year (range 0.1–1.7 years). At diagnosis, all patients had had skin, liver and spleen involvement and 'haematopoietic dysfunction', with an initial activity score ranging from 6 to 15 (median 10.5). Seven patients presented with a haemophagocytic syndrome, associated in 6 cases with a viral infection (CMV $n = 2$, EBV $n = 2$, HHV8 $n = 1$ parvovirus $n = 1$), three had bone lesions and two presented with gut involvement. Initial therapy combined vinblastine (at least 4 doses of 6 mg/m^2/d) and corticosteroids (40 mg/m^2/d) in all cases. No other drug was administered in 6 patients, but three children also received etoposide (VP16) (6 pulses at 150 mg/m^2/d) and 1 received methotrexate (three courses at 500 mg/m^2). In 5 cases there are been a poor response to initial chemotherapy as defined by an increase in the 'LCH activity score' compared with the score at diagnosis; the other 5 patients had had a complete or partial response to initial therapy, but subsequently relapsed and their disease was not responsive to re-treatment with the same initial chemotherapy. Salvage therapies included VP16 and Ara-C in 5 cases, retinoic acid in 3 cases, anti-thymocyte globulin (ATG), 2-CdA or high-dose methotrexate in 1 case. Splenectomy was performed in 2 cases and a third patient underwent splenic irradiation. At the time of 2-CdA/Ara-C treatment all the patients were refractory to these treatments, as demonstrated by an increased 'LCH activity score' ranging from 11 to 19 (median 14) when treatment with 2-CdA and Ara-C was commenced (Table 2).

Table 1
Patient characteristics prior to treatment with 2chlorodeoxyadenosine (2-CdA) and cytosine arabinoside (Ara-C)

Patient number	Age at diagnosis (years)	Sex	Organs involved at diagnosis	Score at diagnosis	Initial therapy	Score at 6 weeks	Initial occurrence (IO) or relapse (R)	Second-line treatments before inclusion in the pilot study
1	0.4	F	Haemato., skin, liver, spleen, haemoph. syndr. (CMV)	10	VBL, Ster.	14	IO	ATG, VP16, Ster. 2-CdA (one course)
2	0.65	M	Haemato., skin, liver, spleen	8	VBL, Ster., VP16	3	R	VP16, Ara-C
3	0.53	F	Haemato., haemoph. syndr. (parvovirus), skin, liver, spleen, bone	8	VBL, Ster.	2	R	VP16, Ara-C
4	0.52	F	Haemato., haemoph. syndr. (CMV), skin, liver, spleen	7	VBL, Ster.	2	R	VBL, Ster.
5	1.4	F	Haemato., haemoph. syndr. (HHV8), skin, liver, spleen	13	ATG, VBL, Ster.	3	R	VP16, Ara-C
6	0.46	F	Haemato., skin, liver, spleen, gut	11	VBL, Ster., VP16	17	IO	VP16, ret. ac. RT spleen
7	0.86	F	Haemato., haemoph. syndr., skin, liver, spleen	13	VBL, Ster., VP16	12	IO	VP16, Ara-C, 2-CdA (five courses), ret. ac., splenectomy
8	0.16	M	Haemato.,skin, liver, spleen, bone	13	VBL, Ster.	5	R	VP16, Ara-C, 2-CdA, ret. ac., Anti-TNF-α
9	1.5	M	Haemato., haemoph.syndr. (EBV), skin, liver, spleen, bone	6	VBL, Ster.	8	IO	VBL + VP16, VP16 + Ara-C, Ara-C s.c., 2-CdA alone
10	1.1	M	Fever, skin, liver, haemoph. syndr. (EBV), spleen.	15	VBL, Ster., MTX	18	IO	Splenectomy

Haemato., haematopoeitic dysfunction; haemoph. syndr., haemophagocytic syndrome; CMV, cytomegalovirus; EBV, Epstein Barr virus; HHV8, Herpes virus type 8; VBL, vinblastine; Ster, corticosteroids; ATG, anti-thymocyte globulin; ret. ac., retinoic acid; MTX, methotrexate; Ara-C, cytosine arabinoside; s.c., subcutaneous; VP16, etoposide (Vepesid); RT spleen, splenic irradiation.

Table 2
2-chlorodeoxyadenosine (2-CdA) and cytosine arabinoside (Ara-C) treatment outcomes

Patient number	Number of courses	Associated therapy	Score before treatment	Score after two courses	Drugs received between the end of the first two courses of 2-CdA + Ara-C and a complete response	Duration (years) until LCH activity score <2	Maintenance therapy or subsequent therapy	Duration of FU (years)	Outcome	Complete blood count[b] at the last visit
1	2	–	12	5	2-CdA	0.45	2-CdA, VBL, Ster.	6.4	NAD	Hb: 13.7 WBC: 7620 Neutro: 4390 Plat: 239,000
2	2	–	13	13	2-CdA and ret. ac.	1	2-CdA, ret. ac., VBL	3	NAD	Hb: 13.4 WBC: 3200 Neutro: 512 Plat: 260,000
3	4	–	11	5	Two courses of 2-CdA and Ara-C	0.3	2-CdA, VBL, Ster.	5.4	NAD	Hb: 12.8 WBC: 5720 Neutro: 2250 Plat: 342,000
4	4	–	15	3	Two courses of 2-CdA and Ara-C	0.3		2.9	NAD	Hb: 12.9 WBC: 7490 Neutro: 4350 Plat: 356,000
5	2	–	12	3	2-CdA	0.6	2-CdA (12 courses)	4.7	NAD	Hb: 12.3 WBC: 7560 Neutro: 2400 Plat: 166,000
6	1	–	19		NE			0.03	Dead	
7	1	–	14		NE			0.1	Dead	
8	4	–	15	8	Two courses of 2-CdA and Ara-C	0.7	VBL, Ster.	2.5	NAD	Hb: 13.7 WBC: 6380 Neutro: 2810 Plat: 103,000
9	1	–	15		NE		ATG, COPAD, ret. ac., allo BMT	0.9	Dead	
10	3[a]	Anti-TNF-α	17	10	One course of 2-CdA and Ara-C[a]	0.4	2-CdA	2	NAD	Hb: 12.8 WBC: 9000 Neutro:5310 Plat: 114,000

[a] At two-thirds of dose.
[b] units: Hb in g/dl, WBC/mm^3, neutrophils/mm^3 and platelets/mm^3 VBL, vinblastine; Ster., corticosteroids; ret. ac., retinoic acid; allo BMT, allogeneic bone marrow transplant; COPAD, cyclophosphamide, vincristine, prednisolone, adriamycin; ATG, anti-thymocyte globulin; CR, complete response; NE, not evaluable; FU, follow-up

3. Results

3.1. Compliance with protocol

The results are shown in Table 2. Seven patients received at least two courses of 2-CdA and Ara-C, and in 1 of these cases anti-tumour necrosis factor-α (anti-TNF-α) antibody (infliximab: 4 injections) therapy was also administered concurrently with the first course. In 3 cases, a total of four courses of 2-CdA and Ara-C were administered, and in 1 case three courses. The 3 remaining patients received only one course of 2-CdA and Ara-C; of this latter group, 2 patients died of sepsis shortly after the first course, while a third was removed from the study and treated with a different chemotherapy regime, retinoic acid and allogeneic bone marrow transplantation.

3.2. Responses to the first two courses of 2-CdA and Ara-C treatment and subsequent therapies

Of the 10 patients treated, 7 could be evaluated for response because they had received at least two courses of therapy. In these patients, there was a highly significant reduction in the LCH activity score at 28 d after completion of the two courses (Wilcoxon matched pairs test: $P = 0.013$). A partial response was observed in 6 of these patients and 1 patient demonstrated 'no response' (Fig. 1), but at the last analysis, all 7 patients were alive with no active disease, the seventh patient having responded 'late', without receiving any other intensive therapy.

Complete response, defined by a disease activity score below 2, was eventually achieved following completion of the therapy after a median of 0.45 years (range 0.3–1 years).

The therapy provided after completion of the first two courses of 2-CdA and Ara-C and before documentation of complete response is detailed in Table 2. Four patients received additional courses of 2-CdA and Ara-C for a total number of four courses in 3 cases and of three courses in 1 case and 3 patients received courses of 2-CdA monotherapy, at a dose of 5 mg/m^2 for 3 d every 3 weeks, along with retinoic acid in 1 case. In 1 case, no additional therapy was given. Once a complete response was obtained, various 'maintenance' therapies were provided. One patient received no further therapy, in 5 cases 2-CdA alone was continued with a varied number of courses or in combination with vinblastine and corticosteroids (2 cases) or with retinoic acid followed by vinblastine (1 case). The last patient subsequently received vinblastine and corticosteroids as maintenance therapy.

Overall 3-year survival rate is 70%, but all patients who received at least two courses of 2-CdA and Ara-C therapy survived and at the last analysis exhibited no active disease. Their blood parameters were almost normal at the most recent clinic visit (Table 2).

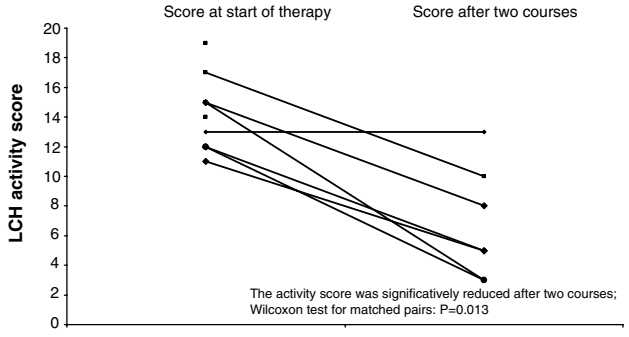

Fig. 1. Langerhans cell histiocytosis (LCH) activity score prior to and after two courses of 2-chlorodeoxyadenosine (2-CdA) and cytosine arabinoside (Ara-C).

3.3. Toxicities, late events and survival

The principal acute adverse effect (Table 3) was haematological, with all patients experiencing profound neutropaenia complicated by fever (>38.5 °C). Transient neuropathic pain was present as immediate toxicity in 3 children, requiring sedation by clonazepam, and 1 case of seizure was observed following the cessation of the second course of treatment. In 7 patients the absolute granulocyte count rose to 500/mm^3 after a median time of 24 d (range: 10–67 d). The median number of red blood cell and platelet transfusions per patient was 9.5 (range 2–14) and 17 (range 2–21), respectively. Four patients were hospitalised in positive air pressure rooms and 3 patients contracted microbial infections (*Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, aspergillosis). In 2 cases, auto-immune haemolytic anaemia and chronic immune purpura occurred 2 years after the end of the therapy.

Two patients died of severe sepsis, one at day 14 and the other at day 38 after the beginning of the first course of 2-CdA and Ara-C treatment; 1 from *Pseudomonas*

Table 3
Adverse effects of the therapy

Patient number	Number of courses	Days after onset until ANC >500/mm^3	Total number of RBC transfusions	Total number of platelet transfusions	Fever (>38.5 °C)	Positive air pressure	Toxicity/late events
1	2	25	10	18	+		Auto-immune haemolytic anaemia
2	2	10	7	10	+	+	Neuropathic pain
3	4	13	2	2	+	–	Chronic ITP (parvovirus), seizure
4	4	25	12	16	+	+	*Staphylococcus epidermidis* sepsis
5	2	23	10	19	+	+	Neuropathic pain
6	1	NE	NE	NE	+	–	*Pseudomonas aeruginosa* sepsis
7	1	NE	NE	NE	+	+	Neuropathic pain, pulmonary aspergillosis
8	4	67	14	19	+	–	–
9	1	26	5	7	+	–	–
10	3	21	9	21	+	–	–

RBC, red blood cell; ANC, absolute neutrophil count; NE, not evaluable.

aeruginosa sepsis and the other from pulmonary aspergillosis. Both patients had neutropaenia below 500/mm^3 at the time of the sepsis. It should be noted that both these deaths occurred in patients who had been treated heavily with other chemotherapy regimes between their initial therapy (vinblastine and corticosteroids) and the start of 2-CdA and Ara-C chemotherapy.

4. Discussion

This study was focused on refractory patients with 'haematopoietic dysfunction' because their short-term outcome is known to be extremely poor [1–4]. In our database, refractory 'haematopoietic dysfunction' accounts for more than two-thirds of deaths among patients with LCH. Improving the outcome of these patients could bring immense clinical benefit. Salvage therapies in LCH are numerous and varied. Some preliminary reports, such as for cyclosporin-A and interferon-alpha administration [21,22], seemed promising, but results have not been confirmed in larger studies. Treatment with anti-TNF-α, thalidomide and 2-deoxycoformycin has also been reported, but only as case reports [23–25]. Longer follow-up durations from larger studies are required to determine the precise contribution of these agents. Some patients have undergone haematopoietic stem cell transplantation, but the general status of the patients who survive for long enough to 'reach' transplant and, in many cases, the delay in finding an appropriate unrelated donor, limit this approach [26].

2-CdA has been used to treat LCH for nearly 10 years. A review of clinical publications reporting 2-CdA monotherapy yields 69 cases in 19 separate articles [6–12,27–38]. Most of these publications reported the therapy in adults; but 33 children were also studied, and most had 'single system' LCH. Good results were reported for 2-CdA monotherapy in these patients, but the efficacy of 2-CdA as a single therapeutic agent seems to be disappointing in patients with 'haematopoietic dysfunction'. Nine such cases have been reported [7–12]. Precise information regarding patients' haematological status prior to 2-CdA therapy was not provided, but complete response was reported in only 2 of the 9 cases. In our register, 2-CdA monotherapy has been used in 5 patients with haematopoietic dysfunction and failure of initial therapy; however, none responded. Thus, 2-CdA as a single agent is probably not very effective in patients with 'haematopoietic dysfunction' and refractory LCH. The successful use of an ra-C-containing combination for treating newly diagnosed disseminated LCH with organ dysfunction [13], and the additive effect between these two drugs, suggested that combination 2-CdA and Ara-C chemotherapy [39] may be an effective treatment for refractory haematopoietic LCH. This combination therapy has been reported in two previous cases, and its use was successful in one instance [16,17].

In the view of the varied clinical manifestations of LCH, we developed a comprehensive LCH activity scoring system, and used it to determine the initial severity of the disease and to assess the efficiency of 2-CdA and Ara-C therapy more objectively [19].

Two courses of 2-CdA and Ara-C treatment seemed to be the 'turning point' of the disease course in our patients (Table 2). Complete response was never obtained in the first month after completion of the two courses, but occurred late afterwards (between 2 and 6 months). In 1 case, a complete response was observed without any additional therapy. In 4 patients, additional courses of 2-CdA and Ara-C resulted in a complete response, whilst in the 3 other patients, less intensive treatment was administrated during this interval. In the absence of any published information regarding the time needed to obtain a complete response in patients with refractory LCH and 'haematopoietic dysfunction', we interpret this result as a delayed response of 2-CdA and Ara-C, rather than the results of the additional therapy.

This encouraging outcome is associated with considerable toxicity. Red blood cell and platelet transfusions were always mandatory, as was intensive supportive care. Two patients died of sepsis shortly after the first course of treatment. All 10 patients developed fever with severe neutropaenia and received multiple antimicrobials including anti-fungal agents. These complications are partly due to the underlying disease, as suggested by the high 'disease activity score', but are worsened, temporarily, by the treatment. Late complications, such as persistent thrombocytopaenia (50–150,000/mm^3), chronic parvovirus infection and haemolytic anaemia, have also been observed, and could be related to persistent immunosuppression or immunodysregulation after this regimen [40]. 2-CdA is known to be a powerful immunosuppressant, reducing CD4 lymphocyte counts for up to 2 years [27], but it is uncertain whether this causes any specific adverse consequences.

The encouraging results of our pilot study clearly warrant further investigation of 2-CdA and Ara-C combined therapy, in a properly structured phase II clinical study. Additional trials are needed to assess other questions arising from this study, such as (i) the appropriate time point to begin salvage therapy and (ii) whether or not 'maintenance' therapy is needed afterwards.

Conflict of interest statement

None declared.

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References

1. Gadner H, Grois N, Arico M, *et al*. A randomized trial of treatment for multisystem langerhans'cell histiocytosis. *J Pediatr* 2001, **138**, 728–734.
2. Minkov M, Grois N, Heitger A, *et al*. Response to initial treatment of multisystem Langerhans cell histiocytosis: an important prognostic indicator. *Med Pediatr Oncol* 2002, **39**, 581–585.
3. The French Langerhans' Cell Histiocytosis Study Group. A multicentre retrospective survey of Langerhans' cell histiocytosis: 348 cases observed between 1983 and 1993. *Arch Dis Child* 1996, **75**, 17–24.
4. Minkov M, Grois N, Heitger A, *et al*. Treatment of multisystem Langerhans cell histiocytosis. Results of the DAL-HX 83 and DAL-HX 90 studies. DAL-HX Study Group. *Klin Padiatr* 2000, **212**, 139–144.
5. Saven A, Piro LD. 2-Chlorodeoxyadenosine: a potent antimetabolite with major activity in the treatment of indolent lymphoproliferative disorders. *Hematol Cell Ther* 1996, **38**(suppl. 2), 93–101.
6. Saven A, Burian C. Cladribine activity in adult langerhans-cell histiocytosis. *Blood* 1999, **93**, 4125–4130.
7. Weitzman S, Wayne AS, Arceci R, *et al*. Nucleoside analogues in the therapy of Langerhans cell histiocytosis: a survey of members of the histiocyte society and review of the literature. *Med Pediatr Oncol* 1999, **33**, 476–481.
8. Dimopoulos MA, Theodorakis M, Kostis E, *et al*. Treatment of Langerhans cell histiocytosis with 2-chlorodeoxyadenosine. *Leuk Lymphoma* 1997, **25**, 187–189.
9. Frost JD, Wiersma SR. Progressive Langerhans cell histiocytosis in an infant with Klinefelter syndrome successfully treated with allogeneic bone marrow transplantation. *J Pediatr Hematol Oncol* 1996, **18**, 396–400.
10. Grau J, Ribera JM, Tormo M, *et al*. Results of treatment with 2-chlorodeoxyadenosine in refractory or relapsed Langerhans cell histiocytosis. Study of 9 patients. *Med Clin (Barc)* 2001, **116**, 339–342.
11. Stine KC, Saylors RL, Williams LL, *et al*. 2-Chlorodeoxyadenosine (2-CDA) for the treatment of refractory or recurrent Langerhans cell histiocytosis (LCH) in pediatric patients. *Med Pediatr Oncol* 1997, **29**, 288–292.
12. Stine KC, Saylors RL, Saccente S, *et al*. Efficacy of continuous infusion 2-CDA (cladribine) in pediatric patients with Langerhans cell histiocytosis. *Pediatr Blood Cancer* 2004, **43**, 81–84.
13. Egeler RM, de Kraker J, Voute PA. Cytosine-arabinoside, vincristine, and prednisolone in the treatment of children with disseminated Langerhans cell histiocytosis with organ dysfunction: experience at a single institution. *Med Pediatr Oncol* 1993, **21**, 265–270.
14. Crews KR, Gandhi V, Srivastava DK, *et al*. Interim comparison of a continuous infusion versus a short daily infusion of cytarabine given in combination with cladribine for pediatric acute myeloid leukemia. *J Clin Oncol* 2002, **20**, 4217–4224.
15. Kornblau SM, Gandhi V, Andreef HM, *et al*. Clinical and laboratory studies of 2-chlorodeoxyadenosine cytosine arabinoside for relapsed or refractory acute myelogenous leukemia in adults. *Leukemia* 1996, **10**, 1563–1569.
16. Choi SW, Bangaru BS, Wu CD, *et al*. Gastrointestinal involvement in disseminated Langerhans cell histiocytosis (LCH) with durable complete response to 2-chlorodeoxyadenosine and high-dose cytarabine. *J Pediatr Hematol Oncol* 2003, **25**, 503–506.
17. Nagarajan R, Neglia J, Ramsay N, *et al*. Successful treatment of refractory Langerhans cell histiocytosis with unrelated cord blood transplantation. *J Pediatr Hematol Oncol* 2001, **23**, 629–632.
18. Histiocyte Society Writing Group Histiocytosis syndrome in children. *Lancet* 1987, **15**, 208–209.
19. Donadieu J, Piguet C, Bernard F, *et al*. A new clinical score for disease activity in Langerhans cell histiocytosis. *Pediatr Blood Cancer* 2004, **43**, 1–7.
20. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996, **17**, 343–346.
21. Arico M, Colella R, Conter V, *et al*. Cyclosporine therapy for refractory Langerhans cell histiocytosis. *Med Pediatr Oncol* 1995, **25**, 12–16.
22. Jakobson AM, Kreuger A, Hagberg H, *et al*. Treatment of Langerhans cell histiocytosis with alpha-interferon. *Lancet* 1987, **2**, 1520–1521.
23. Bertolini F, Mingrone W, Alietti A, *et al*. Thalidomide in multiple myeloma, myelodysplastic syndromes and histiocytosis. Analysis of clinical results and of surrogate angiogenesis markers. *Ann Oncol* 2001, **12**, 987–990.
24. Henter JI, Karlen J, Calming U, *et al*. Successful treatment of Langerhans'-cell histiocytosis with etanercept. *N Engl J Med* 2001, **345**, 1577–1578.
25. McCowage GB, Frush DP, Kurtzberg J. Successful treatment of two children with Langerhans' cell histiocytosis with 2′-deoxycoformycin. *J Pediatr Hematol Oncol* 1996, **18**, 154–158.
26. Akkari V, Donadieu J, Piguet C, *et al*. Hematopoietic stem cell transplantation in patients with severe Langerhans cell histiocytosis and hematological dysfunction: experience of the French Langerhans Cell Study Group. *Bone Marrow Transpl* 2003, **31**, 1097–1103.
27. Betticher DC, Fey MF, von Rohr A, *et al*. High incidence of infections after 2-chlorodeoxyadenosine (2-CDA) therapy in patients with malignant lymphomas and chronic and acute leukaemias. *Ann Oncol* 1994, **5**, 57–64.
28. Chang SE, Koh GJ, Choi JH, *et al*. Widespread skin-limited adult Langerhans cell histiocytosis: long-term follow-up with good response to interferon alpha. *Clin Exp Dermatol* 2002, **27**, 135–137.
29. Conias S, Strutton G, Stephenson G. Adult cutaneous Langerhans cell histiocytosis. *Australas J Dermatol* 1998, **39**, 106–108.
30. Dallafior S, Pugin P, Cerny T, *et al*. Successful treatment of a case of cutaneous Langerhans cell granulomatosis with 2-chlorodeoxyadenosine and thalidomide. *Hautarzt* 1995, **46**, 553–560.
31. Goh NS, McDonald CE, MacGregor DP, *et al*. Successful treatment of Langerhans cell histiocytosis with 2-chlorodeoxyadenosine. *Respirology* 2003, **8**, 91–94.
32. Ottaviano F, Finlay JL. Diabetes insipidus and Langerhans cell histiocytosis: a case report of reversibility with 2-chlorodeoxyadenosine. *J Pediatr Hematol Oncol* 2003, **25**, 575–577.
33. Pardanani A, Phyliky RL, Li CY, *et al*. 2-Chlorodeoxyadenosine therapy for disseminated Langerhans cell histiocytosis. *Mayo Clin Proc* 2003, **78**, 301–306.
34. Robak T, Kordek R, Robak E, *et al*. Langerhans cell histiocytosis in a patient with systemic lupus erythematosus: a clonal disease responding to treatment with cladribine, and cyclophosphamide. *Leuk Lymphoma* 2002, **43**, 2041–2046.

35. Rodriguez-Galindo C, Kelly P, Jeng M, *et al*. Treatment of children with Langerhans cell histiocytosis with 2-chlorodeoxyadenosine. *Am J Hematol* 2002, **69**, 179–184.
36. Saven A, Figueroa ML, Piro LD, *et al*. 2-Chlorodeoxyadenosine to treat refractory histiocytosis X. *N Engl J Med* 1993, **329**, 734–735.
37. Saven A, Foon KA, Piro LD. 2-Chlorodeoxyadenosine-induced complete remissions in Langerhans-cell histiocytosis. *Ann Int Med* 1994, **121**, 430–432.
38. Watts J, Files B. Langerhans cell histiocytosis: central nervous system involvement treated successfully with 2-chlorodeoxyadenosine. *Pediatr Hematol Oncol* 2001, **18**, 199–204.
39. Gandhi V, Estey E, Keating MJ, *et al*. Chlorodeoxyadenosine and arabinosylcytosine in patients with acute myelogenous leukemia: pharmacokinetic, pharmacodynamic, and molecular interactions. *Blood* 1996, **87**, 256–264.
40. Chasty RC, Myint H, Oscier DG, *et al*. Autoimmune haemolysis in patients with B-CLL treated with chlorodeoxyadenosine (CDA). *Leuk Lymphoma* 1998, **29**, 391–398.