ALK-positive anaplastic large cell lymphoma limited to the skin: clinical, histopathological and molecular analysis of 6 pediatric cases. A report from the ALC99 study

Ilske Oschlies,1 Jasmin Lisfeld,2 Laurence Lamant,3 Atsuko Nakazawa,4 Emanuele S. G. d’Amore,5 Ulrika Hansson,6 Konnie Hebeda,7 Ingrid Simonitsch-Klupp,8 Jadwiga Maldyk,9 Leonhard Müllauer,10 Marianne Tinguely,11 Markus Stucker,12 Marie-Cecile DeLeley,12 Reiner Siebert,13 Alfred Reiter,1 Laurent Brugières,14 Wolfram Klapper,1 and Wilhelm Woessmann2

1Department of Pathology, Hematopathology Section and Lymph Node Registry, Christian-Albrechts-University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; 2NHL-BFM Study Center, Department of Pediatric Hematology and Oncology, Justus-Liebig University, Giessen, Germany; 3Laboratoire Anatomie Pathologique, Centre Hospitalier Universitaire Purpan, Toulouse, France; 4Department of Pathology, National Center for Child Health and Development, Tokyo, Japan; 5O di Anatomia Patologica, Ospedale San Bortolo, Vicenza, Italy; 6Avdehingen foer patologi, Sahlgrenska Universitetssjukhuset, Gothenburg, Sweden; 7Radbound University Nijmegen Medical Centre, Department of Pathology, Nijmegen, The Netherlands; 8Institute of Pathology, Medical University Vienna, Vienna, Austria; 9Department of Pathology, Childrens Hospital, Warsaw, Poland; 10Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland; 11Department of Dermatology and Allergology, Ruhr University Bochum, St. Joseph, Germany; 12Department of Biostatistics, Institut Gustave Roussy, Villejuif, France; 13Institute of Human Genetics, Christian-Albrechts-University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany, and 14Department of Pediatric Oncology, Institut Gustave Roussy, Villejuif, France

ABSTRACT

Anaplastic large cell lymphomas are peripheral T-cell lymphomas that are characterized by a proliferation of large anaplastic blasts expressing CD30. In children, systemic anaplastic large cell lymphomas often present at advanced clinical stage and harbor translocations involving the anaplastic lymphoma kinase (ALK) gene leading to the expression of chimeric anaplastic lymphoma kinase (ALK)-fusion proteins. Primary cutaneous anaplastic large cell lymphoma is regarded as an ALK-negative variant confined to the skin and is part of the spectrum of primary cutaneous CD30-positive T-cell lymphoproliferative disorders. Thirty-three of 487 pediatric patients registered within the Anaplastic Large Cell Lymphoma-99 trial (1999 to 2006) presented with a skin limited CD30-positive lymphoproliferative disorder. In 23 of the 33 patients, material for international histopathological review was available, and the cases were studied for histopathological, immunophenotypical and clinical features as well as for breaks within the ALK gene. Five of 23 cases and one additional case (identified after closure of the trial) expressed ALK-protein. Complete staging excluded any other organ involvement in all children. Expression of ALK proteins was demonstrated by immunohistochemistry in all cases and the presence of breaks of the ALK gene was genetically confirmed in 5 evaluable cases. The histopathological and clinical picture of these skin-restricted ALK-positive lymphomas was indistinguishable from that of cutaneous anaplastic large cell lymphoma. Five children presented with a single skin lesion that was completely resected in 4 and incompletely resected in one. Three of these patients received no further therapy, 2 additional local radiotherapy, and one chemotherapy. All children remain in complete remission with a median follow up of seven years (range 1-8 years). We present 6 pediatric cases of ALK-positive primary cutaneous anaplastic large cell lymphomas. After thorough exclusion of systemic involvement, therapy confined to local measures seems to be sufficient to induce cure.

©2013 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2012.065664

Introduction

Anaplastic lymphoma kinase-positive (ALK-positive) anaplastic large cell lymphoma (ALCL) is characterized by a neoplastic proliferation of large pleomorphic (anaplastic) CD30-positive (CD30+ T cells with typical translocations involving the ALK gene and subsequent expression of chimeric ALK protein. This lymphoma accounts for approximately 15% of childhood non-Hodgkin’s lymphomas, but is rare in adulthood. ALK-positive (ALK+) ALCL is usually a systemic disease that frequently involves extranodal sites. In children, 18-25% of systemic ALCLs develop skin manifestations during the course of the disease and this is a poor prognostic factor. Systemic ALK-negative (ALK-) ALCL is included in the updated WHO classification as a separate preliminary entity. Systemic ALK-negative ALCL accounts for less than 5% of pediatric systemic ALCLs. However, both ALK-positive and ALK-negative ALCLs are considered potentially disseminated diseases. Primary cutaneous ALCL (cALCL) is regarded by the WHO as a separate disease entity and belongs to the spectrum of
primary cutaneous CD30-positive lymphoproliferations (CD30+LPD), a group that also includes lymphomatoid papulosis (LyP). CD30+LPDs share with systemic ALCL the presence of neoplastic CD30+ large T cells, but lack ALK translocations and protein expression. cALCLs remain confined to the skin, virtually never disseminate beyond local lymph nodes, and show an excellent prognosis after surgical resection without systemic therapy. Most cases of cALCL present as solitary skin lesions, but multiple skin nodules are also found. In contrast to systemic ALCL, cALCL is only rarely found in children and young adults. Recently published recommendations for the diagnosis of CD30+LPD state that immunohistochemical detection of ALK expression should be considered highly suspicious of a cutaneous manifestation of underlying systemic ALCL. In contrast, IRF4 translocations have been reported in cALCL and in ALK–ALCLs but not in ALK+ALCL. In the international multicenter trial ALCL99, children included with localized skin disease were not to receive systemic chemotherapy based on the assumption that their disease would be CD30+LPD. We describe a series of 6 pediatric ALCLs that clinically and histologically resembled cALCL but expressed ALK fusion proteins. These localized cutaneous ALK+ALCL followed the typical benign clinical course of a CD30+LPD.

**Design and Methods**

**Identification of cases and histopathological review**

In the ALCL99 multicenter study, 487 children and young adults with the diagnosis of ALCL were registered from 1999 to 2006, including 33 patients with a CD30+ lymphoproliferative disorder limited to the skin. Patients with isolated skin lesions diagnosed by complete staging procedures were to be followed after resection by ‘watchful waiting’ without further systemic therapy regardless of the ALK status. For 23 of these, skin limited lymphoma material was available for an international histopathological review. One additional case reported here was identified after completion of registration in 2006. The histological review of the cases was performed by members of the international pediatric lymphoma pathology panel (OJ, LL, AN, EdA, UH, KH, ISK, JM, LM, MT) using hematoxylin and eosin (H&E) stained slides as well as slides stained immunohistochemically in various laboratories (see below). The registered clinical data from the study center were reviewed and additional details were obtained by contacting the attending pediatric oncologist. The study was part of the scientific projects accompanying the ALCL99 study, for which informed consent was obtained. The study was carried out according to the local ethical guidelines and in accordance with the ethical guidelines of the studies in which the patients were treated.

**Immunohistochemistry and fluorescence in situ hybridization**

All immunohistochemical stainings were performed on whole tissue sections. The stainings were scored semiquantitatively as negative, weak (<30% positive tumor cells or all tumor cells weakly positive), positive (>30% positive tumor cells) or not interpretable. The minimal staining panel for each lymphoma included CD20, CD3, CD30, and ALK. Additional stainings for granzyme B, perforin, TIA1, EMA, CD2, and CD5 were available for individual cases. Due to the retrospective nature of the study, the staining procedures and antibody sources for these markers varied between the participating countries but had been previously established within the group as part of the ALCL99 study.

Fluorescence in situ hybridization (FISH) for chromosomal breaks in the ALK gene or at the IRF4/DUSP22 locus was performed as previously described.

**Results**

**Identification of the 6 ALK-positive cases limited to the skin**

Among the 23 cases with ALCL or CD30+ lymphoproliferations confined to the skin registered into the ALCL99 study and available for international histopathological review, 5 patients with expression of ALK protein were identified. During the preparation of the manuscript, another case of ALK+ALCL limited to the skin was identified by the NHL-BFM study center and included in this series.

**Table 1. Histopathological and immunohistochemical features of 6 pediatric cases of ALK-positive primary cutaneous anaplastic large cell lymphoma.**

<table>
<thead>
<tr>
<th>Case n.</th>
<th>Epidermal change</th>
<th>Epidermotropism of tumor cells</th>
<th>Dermal involvement*</th>
<th>Admixed inflammatory cells</th>
<th>CD30 (pattern)</th>
<th>ALK</th>
<th>ALK staining pattern</th>
<th>ALK small cell component</th>
<th>EMA</th>
<th>CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hyperplastic, ulceration</td>
<td>+</td>
<td>+</td>
<td>ni</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>n+cyt</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>ni</td>
<td>ni</td>
<td>+</td>
<td>neutrophils</td>
<td>++</td>
<td>++</td>
<td>n+cyt</td>
<td>– ni</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>normal</td>
<td>–</td>
<td>+</td>
<td>lymphohistiocytic</td>
<td>+</td>
<td>+</td>
<td>n+cyt</td>
<td>+ ++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>normal</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>n+cyt</td>
<td>+ ++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>hyperplastic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>cyt</td>
<td>– ++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>hyperplastic</td>
<td>–</td>
<td>+</td>
<td>lymphohistiocytic</td>
<td>++</td>
<td>++</td>
<td>n+cyt</td>
<td>+ ++</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Scoring of histopathological features: + present; - absent; ni: not interpretable. Scoring of immunohistochemistry: - negative staining; + weak staining or <30% of cells moderate to strong staining; ++ moderate to strong staining in >30% cells; n+cyt: nuclear and cytoplasmic staining; cyt: cytoplasmic staining only. *In all evaluable cases dermal involvement was superficial and deep.
Histological and immunohistochemical features

The main histological and immunohistochemical features of the 6 cases are summarized in Table 1. In most cases, a superficial and deep cutaneous infiltration extending into the subcutis was observed (3 of 4 cases in which all skin layers were included in the biopsy specimen). The lesions were rather poorly demarcated. In one case, an isolated subcutaneous nodule without dermal involvement was seen. In 5 cases, the epidermis was included in the specimen and was either normal in appearance (n=2), showed hyperplastic changes (n=2), or hyperplastic changes with additional focal superficial erosion (n=1). A large number of CD30+ neoplastic blasts forming cohesive sheets were detectable in 5 of 6 cases. However, one case displayed only scattered blasts. In 3 of 6 lesions, the growth pattern of the blasts was perivascular. Reactive inflammatory bystander cells were composed of a moderate number of neutrophils (1 of 6) or lymphohistiocytic cells (2 of 6). No inflammatory bystander cells were detectable in 3 of 6 lymphomas. Figure 1 shows one representative example of an ALK+ ALCL confined to the skin.

ALK expression was immunohistochemically detectable in all cases with nuclear and cytoplasmic staining in 5 of 6 cases. Interestingly, in 4 of 6 lymphomas, a small cell component was detectable, as indicated by predominately nuclear staining of small lymphoma cells (Figure 2). All lymphomas expressed at least one cytotoxic protein, such as granzyme B, TIA1 or perforin with the characteristic granular staining pattern (data not shown).

Fluorescence in situ hybridization

Material for fluorescence in situ hybridization was available for 4 lymphomas. Breaks in the ALK gene were detectable in all 4 analyzed cases (Figure 1). In the additional patient with multicellular skin disease NPM-ALK transcripts were detected in the bone marrow and blood by polymerase chain reaction (data not shown) so that the ALK-translocation was confirmed molecularly in 5 of 6 patients. In contrast, breaks affecting the IRF4/DUSP22 locus in 6p25 recurrently involved in cALCL were not detectable in the 3 cases studied.

Clinical characteristics, therapy and outcome

Table 2 summarizes the clinical characteristics of the patients reported in this series. Median age was 10.8 years.
(range 7.5-13.8 years). Three patients were male and 3 female. None of the children had a clinically documented history of lymphomatoid papulosis (LyP) or mycosis fungoides. The lymphomas presented clinically as papulonodular skin lesions (5 of 6) and/or subcutaneous nodules (3 of 6). One patient displayed multiple skin lesions (case 4) which were described as multiple pink nodules on the trunk, arms and neck. The isolated lesions in the other 5 patients involved the thigh (n=3), neck (n=1) or knee (n=1). Figure 1 shows the clinical presentation of one case with a solitary lesion on the thigh (case 6). None of the children suffered from B symptoms. All patients underwent a complete initial staging procedure to exclude systemic disease according to the ALCL99 protocol, including imaging of the abdomen and thorax, full blood cell count and bone marrow cytology. Lumbar puncture was performed in 5 of the 6 patients. In one patient, minimal disseminated disease (MDD) was detectable, measured by polymerase chain reaction for NPM-ALK transcripts in the bone marrow and blood (case 4, Table 2 and data not shown). The single skin lesion was surgically completely resected in 4 of the 5 patients. One patient received addi-

### Table 2. Clinical features of 6 pediatric cases of ALK-positive primary cutaneous anaplastic large cell lymphoma.

<table>
<thead>
<tr>
<th>Case n.</th>
<th>Age (years)</th>
<th>Maculopapular lesions</th>
<th>Subcutaneous nodules</th>
<th>Multiple skin lesions</th>
<th>Location</th>
<th>B symptoms</th>
<th>Staging*</th>
<th>Complete resection</th>
<th>Therapy Chemo/radiation</th>
<th>Relapse</th>
<th>Follow up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ventral thigh, approx. 2 cm in diameter</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>neck, approx. 3 cm in diameter</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>thigh, small red lesion</td>
<td>n.e.</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>anterior wall of thorax, neck, back: pink nodules</td>
<td>–</td>
<td>+ (MDD+ BM and pB)</td>
<td>–</td>
<td>chemo2</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>11.9</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>right knee</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>radiation</td>
<td>–</td>
<td>5.2</td>
</tr>
<tr>
<td>6</td>
<td>13.8</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>left thigh</td>
<td>–</td>
<td>+ (no CSF)</td>
<td>+</td>
<td>radiation</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

1MDD: minimal disseminated disease assessed by RTPCR for t(2;5) (NPM;ALK) in the bone marrow (BM) and peripheral blood (pB) was positive; chemotherapy according to ALCL99 was pre-phase, 3xA, 3xB, complete remission after A1. CSF: cerebrospinal fluid; n.e.: not evaluated; staging*+: complete clinical staging was performed and remained negative.

**Figure 2.** An example of ALK-positive ALCL (case 1, see Table 1) with epidermotropism of lymphoma cells and a subepithelial small cell tumor component. (A and B) Hematoxylin & Eosin staining. (C) ALK1.
Table 3. Literature review of reported ALK-positive cutaneous anaplastic large cell lymphomas and findings in this series.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Localization</th>
<th>ALK expression pattern</th>
<th>Therapy of first lesion</th>
<th>Local recurrence</th>
<th>Distant cutaneous recurrence</th>
<th>Number of recurrences reported</th>
<th>Treatment of recurrent lesions</th>
<th>Systemic dissemination</th>
<th>Outcome</th>
<th>Observation period in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al.</td>
<td>33</td>
<td>m</td>
<td>multiple: trunk, head, leg</td>
<td>nuclear and cytoplasmic</td>
<td>6 cycles of chemotherapy</td>
<td>no</td>
<td>yes</td>
<td>2</td>
<td>excision, chemotherapy</td>
<td>systemic relapse</td>
<td>2 years after diagnosis</td>
</tr>
<tr>
<td>Kadin et al.</td>
<td>57</td>
<td>m</td>
<td>single lesion leg</td>
<td>cytoplasmic</td>
<td>surgical</td>
<td>no excision</td>
<td>yes</td>
<td>6</td>
<td>surgical excision and radiotherapy</td>
<td>no</td>
<td>CCR</td>
</tr>
<tr>
<td>Sasaki et al. and Hosoi et al.</td>
<td>57</td>
<td>f</td>
<td>single lesion forehead</td>
<td>cytoplasmic</td>
<td>spontaneous regression without treatment</td>
<td>no</td>
<td>yes</td>
<td>several</td>
<td>total excision and radiotherapy</td>
<td>systemic relapse 2.5 years after diagnosis,</td>
<td>DOD</td>
</tr>
<tr>
<td>Beylot-Barry et al.</td>
<td>57</td>
<td>f</td>
<td>multiple lesions: trunk</td>
<td>cytoplasmic</td>
<td>6 cycles CHOP</td>
<td>no</td>
<td>no</td>
<td>0</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Su et al.</td>
<td>57</td>
<td>f</td>
<td>multiple lesions: leg</td>
<td>cytoplasmic</td>
<td>5 cases with nuclear and cytoplasmic</td>
<td>3 cases: surgical excision, 2 cases: excision and local radiotherapy, 1 case chemotherapy</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

This series mean age: 6 years, range (7-13) n=6


Discussion

We report here 6 cases of ALK+ ALCL limited to the skin. These lymphomas mimicked primary cutaneous CD30+ LPD in their histopathology, clinical presentation and response to therapy. CD30+ LPD comprise a spectrum of diseases confined to the skin, including LyP and cALCL, which show overlapping histological features. Both diseases are characterized by a neoplastic infiltrate of anaplastic CD30+ T cells with a variable admixture of reactive inflammatory cells. Single nodular skin lesion or, less frequently, multiple nodules that do not undergo spontaneous regression are the typical presentation of cALCL. Distinguishing a primary cutaneous CD30+LPD, such as LyP and cALCL, from secondary involvement of the skin by systemic ALCL is clinically relevant. Treatment of systemic ALCL consists of risk-adapted polychemotherapy. Secondary skin involvement is regarded as a clinical risk factor, often utilized to stratify patients to a more aggressive treatment regimen. In contrast, primary cutaneous CD30+LPD, which is limited to the skin and rarely disseminates, usually either resolves spontaneously or is treated locally, e.g. by surgical excision.

All of our cases fulfilled the clinical and histological criteria of a primary cutaneous anaplastic large cell lymphoma with predominantly solitary skin lesions, no history of LyP, no extracutaneous dissemination and response to local therapy, but all cases were ALK+. Given the higher incidence of cALCL in adults, most published series analyzing ALK expression have included predominately adult patients. There have been only single case reports and small series of pediatric cALCL, and in these ALK staining was inconsistently performed. We assume that our cases is not population-based as cutaneous CD30+LPD are diagnosed and treated either by dermatologists or pediatric oncologists. Nevertheless, our data suggest that ALK+ cALCL might be more frequent than anticipated within the pediatric population, and recommend that all CD30+LPD of the skin in children should be carefully analyzed for ALK expression.

Lamant et al. recently reported 5 children with systemic ALK+ ALCL that presented as skin lesions at the site of preceding insect bites, often with involvement of the draining local lymph node. Thus, the skin might not only present a preferred microenvironment for ALK+ ALCL but might even be the primary site of lymphomagenesis. At the moment, no reliable histopathological features are known to distinguish secondary skin involvement by a systemic ALCL from primary cutaneous CD30+LPD. EMA has been reported to be positive in most systemic ALK+ and ALK ALCLs but negative in cALCL. ALK protein expression, as well as the underlying ALK-gene translocation, are considered indicative of systemic ALK+ ALCL and are seen in nearly all pediatric systemic ALCL cases. In contrast, cALCL is considered ALK- both at the molecular and the protein level. Our cases were ALK and EMA...
positive on the one hand but localized and limited to the skin on the other. They, therefore, presented as and thus could be named as primary cutaneous ALK-positive ALCI.

One could discuss whether the child with multiple skin lesions and positive MDD should have been classified as child with systemic type ALCI. Nevertheless, for the moment, staging is determined by clinical imaging as well as by the evaluation of bone marrow cytology, and all these investigations were negative in this child, indicating isolated skin disease. In practical terms, the child was treated with systemic chemotherapy despite the isolated skin involvement, and we would support this treatment decision, especially since positive MDD has been shown to be an adverse prognostic factor in systemic ALCI. To the best of our knowledge, skin-confined variants of ALK+ ALCI have previously been published in 5 cases only. Table 3 shows a summary of the literature and the cases presented here. However, the published cases differ from our series in two main points. First, all previously published cases were adult patients (Table 2). Second, 2 of 5 previously published cases developed systemic disease years after the initial primary skin disease; a feature that was rare in our cohort (Table 2). Just recently, at the joint workshop of the Society for Hematopathology and the European Association for Hematopathology (SH/EAHP) on cutaneous lymphomas held in Los Angeles in October 2011, 5 new cases of ALK+ ALCIs confined to the skin were presented as case reports. Four of these occurred in adults with variable clinical scenarios, ALK-staining-patterns and histomorphological features, and only one ALK+ ALCI confined to the skin was described in a child with a very unusual mycosis fungoides as clinical and histological presentation. Therefore, more attention to ALK-staining in cutaneous T-cell lymphoproliferations seems justified.

Interesting histological features of the lymphomas reported here were the presence of a small component in 4 of the 6 cases, and a perivascular growth pattern in 3. The presence of a small cell component and a perivascular growth pattern have recently been reported to be associated with a poorer outcome in systemic ALK+ ALCI. However, there was no relapse among the 5 patients with exclusive local therapy reported in our series. This emphasizes again that ALK-positive ALCI limited to the skin may represent a specific subgroup of ALK+ ALCI for which prognostic parameters established in systemic ALK+ ALCI do not apply.

In summary, our cases illustrate that ALK+ ALCI can present as a localized skin-limited disease. Localized treatment with careful follow up seems justified after thorough exclusion of systemic disease in this rare variant. Understanding the biology of ALK+ ALCIs that are confined to the skin might influence therapy strategies for ALK+ ALCI also in other locations.

**Funding**

This work was supported by the José-Carreñas-Foundation (DJC/LS R08/09). RS and WK are supported by the Kinderkrebs Initiative Buchholz, Holm, Wilmersdorf, Germany. The ALCL99 study was supported by the Forschungsförderhilfe Pepper and the Association Cent pour Sang la Vie, France. None of the authors reported any other potential conflicts of interest.

**Acknowledgments**

The authors are indebted to all the children and parents who participated in this study, to Nathalie Bouvet, Institut Gustave-Roussy Villejuif, France, for database management, and Olivia Batic, Dimitry Abramov and Reina Zühlke-Jenisch for their technical assistance.

**Authorship and Disclosures**

Information on authorship, contributions, and financial and other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

---

**References**


