Near-Tetraploidy in Childhood B-Cell Precursor Acute Lymphoblastic Leukemia Is a Highly Specific Feature of ETV6/RUNX1-Positive Leukemic Cases

Andishe Attarbaschi,1 Georg Mann,1 Margit König,2 Manuel Steiner,1 Michael N. Dworzak,1,2 Helmut Gadner,1,2 and Oskar A. Haas2 on behalf of the Austrian Berlin-Frankfurt-Münster (BFM) Cooperative Study Group

1St. Anna Children’s Hospital, Vienna, Austria
2Children’s Cancer Research Institute, St. Anna Children’s Hospital, Vienna, Austria

Near-tetraploidy (82–94 chromosomes) makes up fewer than 1% of childhood acute lymphoblastic leukemia (ALL) cases and has been reportedly associated with a possibly poorer prognosis compared with other ploidy groups. We analyzed 783 patients enrolled in the ALL-BFM-Austria 86, -90, -95, -99/2000 and Interfant-Austria 99 trials in order to assess its incidence, biological characteristics, and prognostic relevance. Twelve of 783 patients (1.5%) had a near-tetraploid ALL. Fluorescence in situ hybridization revealed that eight of the nine B-cell precursor (BCP) cases and none of the three T-cell ALL cases had an ETV6/RUNX1 rearrangement. After a median follow-up of 11.4 years, none of the patients has relapsed or died. Thus, near-tetraploidy appears to be a specific feature of ETV6/RUNX1 + BCP ALL cases that in turn may explain its excellent outcome.

INTRODUCTION

The ploidy and DNA content of leukemic blast cells are well-established prognostic markers of childhood acute lymphoblastic leukemia (ALL; Moorman et al., 2003; Harrison et al., 2004; Sutcliffe et al., 2005). High-hyperdiploidy (51–65 chromosomes) is usually associated with low-risk features and is reported to have a good prognosis, whereas hypodiploidy (<46 chromosomes) has an increased risk of relapse on various conventional treatment protocols (Moorman et al., 2003; Harrison et al., 2004). However, the prognostic meaning of near-tetraploidy (82–94 chromosomes) is rather unclear because this feature accounts for fewer than 1% of childhood ALL cases (Pui et al., 1990; Raimondi, 1993). According to the literature, near-tetraploid ALL appears to be associated with a T-cell phenotype, FAB L2 cytology, a higher median age at diagnosis and, possibly, a poorer prognosis, compared with other ploidy groups (Pui et al., 1990; Raimondi, 1993). The incidental detection of near-tetraploid ALL patients during systematic fluorescence in situ hybridization (FISH) screening for ETV6/RUNX1-positive (formerly known as TEL/AML1) cases led us to assess the prevalence, clinical characteristics, and prognostic role of near-tetraploidy in a series of unselected childhood ALL patients who were included in five successive multicentric ALL trials and had conventional karyotypes available (Attarbaschi et al., 2004).

PATIENTS AND METHODS

Between September 22, 1986, and June 1, 2005, 948 children and adolescents up to 18 years of age with ALL were enrolled in four consecutive multicenter trials of the Berlin-Frankfurt-Münster (BFM) group: ALL-BFM-A (Austria) 1986 (n = 142), ALL-BFM-A 1990 (n = 256), ALL-BFM-A 1995 (n = 230), and ALL-BFM-A 99/2000 (n = 312). From 1999 onward, infant cases were enrolled in trial Interfant-A 1999 (n = 8). ALL was diagnosed according to standard morphological, cytochemical, and immunophenotypic criteria. All cases were centrally reviewed and treated according to the respective BFM treatment protocols after obtaining written informed consent. Treatment stratification and protocols used in the ALL-BFM-A 86 and -90 trials were reported in detail previously (Schrappe et al., 2000).

Cytogenetic analyses of trypsin/Giemsa-banded bone marrow preparations were centrally performed according to standard procedures, and the...
results were classified according to the ISCN (1995). Near-tetraploidy was defined as a karyotype with 82–94 chromosomes. Cellular DNA content was determined by standard flow cytometry techniques. ETV6/RUNX1 FISH analysis was also centrally performed according to the manufacturer’s recommendation using the dual-color labeled LSI ETV6/RUNX1 ES probe set (Vysis, Downers Grove, IL). The hybridization signal patterns of at least 200 nuclei were assessed in each case. A sample was considered positive if 10% or more of the nuclei showed the respective abnormality pattern.

RESULTS AND DISCUSSION

Among the 783 of 948 (87%) patients with karyotypes available, we identified 12 (1.5%) who had near-tetraploid ALL, including nine with a B-cell precursor (BCP) and three with pre-T-cell ALL (Table 1). Modal chromosome number ranged from 82 to 94. The presence of tetraploid clones was studied and confirmed with DNA content measurements in 6 of the 12 patients. All 12 patients were good prednisone responders and in complete remission after induction therapy. After a median follow-up of 11.4 years (range 0.32–18.24 years), no patient has relapsed or died. One BCP ALL patient developed a secondary MLL-rearranged acute myeloid leukemia 4.5 years after ALL diagnosis. This patient was rescued with a high-dose regimen followed by bone marrow transplantation from an unrelated matched donor.

The most striking and novel finding of the present study, however, was that all but one of the BCP ALL cases with a near-tetraploid karyotype had an ETV6/RUNX1 rearrangement. Although such cases have been occasionally reported, the virtually exclusive association between these two features has not yet been shown (Raynaud et al., 1999; Attarbaschi et al., 2004). In contrast to the unexpected findings for the BCP ALL patients, all three patients with T-cell ALL initially presented with clinical and laboratory features frequently seen in this phenotypic ALL subset, such as male gender, higher age and leukocyte count at diagnosis, and, not surprisingly, lack of an ETV6/RUNX1 fusion gene (Raynaud et al., 1999; Attarbaschi et al., 2004). Furthermore, none of the four ETV6/ RUNX1-negative cases (BCP ALL, n = 1; T-cell ALL, n = 3) had a BCR/ABL, MLL/AF4 or TCF3/PBX1 fusion gene, as assessed by reverse transcription–polymerase chain reaction (RT-PCR).

With an incidence of 20%–25%, the t(12; 21)(p13;q22) with its genetic counterpart, the ETV6/RUNX1 fusion gene, is the most frequent

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<table>
<thead>
<tr>
<th>Patient Gender</th>
<th>Age (y)</th>
<th>VBM count (l)</th>
<th>Phenoype</th>
<th>Myeloid markers</th>
<th>Chromosome number</th>
<th>ETV6/ RUNX1</th>
<th>(Near-)diploid clone</th>
<th>(Near-)tetraploid clone</th>
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<tbody>
<tr>
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<td>m</td>
<td>12.1</td>
<td>pre-B ALL</td>
<td>CD33 +/CD33 +</td>
<td>94</td>
<td>+</td>
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<td>cALL</td>
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<td>++</td>
<td>85</td>
<td>2 2 2</td>
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**TABLE 1. Summary of Clinical and Biological Characteristics of the 12 Near-Tetraploid ALL Cases**

- **Patient Gender**: m, male; f, female
- **Age (y)**
- **VBM count (l)**
- **Phenotype**: pre-B ALL, cALL
- **Myeloid markers**: CD13 +/CD33 +
- **Chromosome number**: 94, 82, 88–91, 88–92
- **ETV6/ RUNX1**: +, ++, ++
- **(Near-)diploid clone**: 92, 90, 85, 85
- **(Near-)tetraploid clone**: 2 2 2, 2 2 2, 2 2 2, 2 2 2

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**RESULTS AND DISCUSSION**

Among the 783 of 948 (87%) patients with karyotypes available, we identified 12 (1.5%) who had near-tetraploid ALL, including nine with a B-cell precursor (BCP) and three with pre-T-cell ALL. (Table 1). Modal chromosome number ranged from 82 to 94. The presence of tetraploid clones was studied and confirmed with DNA content measurements in 6 of the 12 patients. All 12 patients were good prednisone responders and in complete remission after induction therapy. After a median follow-up of 11.4 years (range 0.32–18.24 years), no patient has relapsed or died. One BCP ALL patient developed a secondary MLL-rearranged acute myeloid leukemia 4.5 years after ALL diagnosis. This patient was rescued with a high-dose regimen followed by bone marrow transplantation from an unrelated matched donor.

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rearrangement in childhood BCP ALL (Rubnitz et al., 1999; Attarbaschi et al., 2004). According to event-free survival rates around 90%–100%, this aberration is mostly considered a favorable prognostic marker (Rubnitz et al., 1999; Attarbaschi et al., 2004). Thus, it was not surprising that the near-tetraploid BCP cases presented in this article also shared the typical biological and clinical features of ETV6/RUNXI- positive cases, such as favorable age and leukocyte count at diagnosis, coexpression of myeloid markers, and the common secondary genetic abnormalities, as well as the extremely good outcome. Furthermore, our observations indicated that near-tetraploidy in ETV6/RUNXI-positive cases did not adversely influence prognosis.

The ETV6/RUNXI-positive near-tetraploid cases offered the opportunity to study the timing of the acquisition of the individual secondary changes during clonal evolution (Table 1), especially in the four cases (patients 5, 6, 7, and 8) in which we could also identify a primary near-diploid clone. In the near-tetraploid clone of two cases (patients 7 and 8), the respective signal patterns were merely duplicated, whereas in the other two cases (patients 5 and 6), the ETV6 signal copy number was decreased. Intriguingly, we noted such a loss of ETV6 not only in four of the eight ETV6/RUNXI-positive cases (patients 2, 3, 5, and 6), but also in the only ETV6/RUNXI-negative patient who had a constitutional trisomy 21 (patient 9). In two cases (patients 6 and 9), these patterns imply that one ETV6 gene was only deleted after tetraploidization. FISH with a centromere-specific probe revealed that in both instances, an entire chromosome 12 homologue was lost. In two other cases without an evident diploid clone (patients 2 and 3), the near-tetraploid clone lacked all non-fused ETV6 alleles, indicating that this deletion had most likely taken place before tetraploidization in an unidentified (pseudo)diploid subclone. The remaining case (patient 5) can also be explained in a similar fashion, because the unequal signal patterns render it rather unlikely, although not impossible, that the near-tetraploid clone was a direct progeny of the concomitant near-diploid clone. It can thus be speculated that in some of the cases, tetraploidization was a relatively late chromosomal event.

Chromosomal missegregation can increase the frequency of binucleation, and, therefore, mono-nucleated tetraploid cells can arise through a process involving chromosome nondisjunction and furrow regression (Shi and King, 2005). Although even stochastic errors in chromosome segregation may be sufficient to induce tetraploidy, it remains unclear what renders ETV6/RUNXI-positive cells particularly vulnerable to this process (Shi and King, 2005). The mechanism giving rise to near-tetraploidy is most likely to be a doubling of a near-(pseudo)diploid clone with subsequent chromosome losses rather than a simultaneous gain of all additional chromosomes during a single abnormal cell division, as seen in high-hyperdiploid cases (Paulsson et al., 2005). This process is somewhat similar to the formation of hyperdiploid karyotypes in the rare ALL cases in which a coexisting near-haploid clone is duplicated (Onodera et al., 1992). Molecular genetic studies have shown that in most childhood BCP ALL cases, including the ETV6/RUNXI-positive and high-hyperdiploid leukemia subsets, the initiating chromosomal events had already occurred in utero (Wiemels et al., 1999; Panzer-Grumayer et al., 2002). Whether tetraploidization also is such a comparatively early evolutionary step that merely promotes the malignant potential of ETV6/RUNXI-positive blast cells or whether it is actually a rather late event or even the last trigger that sets off the clinical disease manifestation remains to be investigated. Moreover, further studies on larger series of patients with ALL are warranted to confirm the seemingly exclusive association of tetraploidy with an ETV6/RUNXI rearrangement.

REFERENCES


