CD2 Antigen Expression on Leukemic Cells as a Predictor of Event-Free Survival After Chemotherapy for T-Lineage Acute Lymphoblastic Leukemia: A Children’s Cancer Group Study

By Fatih M. Uckun, Peter G. Steinherz, Harland Sather, Michael Trigg, Diane Arthur, David Tubergen, Paul Gaynon, and Gregory Reaman

We examined the prognostic impact of CD2 antigen expression for 851 patients with T-lineage acute lymphoblastic leukemia (ALL), who were enrolled in frontline Childrens Cancer Group treatment studies between 1983 and 1994. There was a statistically significant correlation between the CD2 antigen positive leukemic cell content of bone marrow and probability of remaining in bone marrow remission, as well as overall event-free survival (EFS) ($P = 0.0003$ and $P = 0.002$, log-rank tests for linear trend). When compared with patients with the highest CD2 expression level (>75% positivity), the life table relative event rate (RER) was 1.22 for patients with intermediate range CD2 expression level (30% to 75% positivity) and 1.81 for “CD2-negative” patients (<30% positivity). At 6 years postdiagnosis, the EFS estimates for the three CD2 expression groups (low positivity to high positivity) were 52.8%, 65.5%, and 71.9%, respectively. CD2 expression remained a significant predictor of EFS after adjustment for the effects of other covariates by multivariate regression, with a RER of 1.47 for CD2-negative patients ($P = .04$). Analysis of T-lineage ALL patients shows a significant separation in EFS after adjustment for the National Cancer Institute (NCI) age and white blood cell (WBC) criteria for standard and high-risk ALL ($P = .002$, RER = 1.67). The determination of CD2 expression on leukemic cells helped identify patients with the better and poorer prognoses in both of these risk group subsets. For standard risk T-lineage ALL, CD2-negative patients had a worse outcome ($P = .0007$, RER = 2.92) with an estimated 5-year EFS of 55.9% as compared with 78.3% for the CD2-positive patients. Thus, CD2 negativity in standard risk T-lineage ALL identified a group of patients who had a worse outcome than high-risk T-lineage ALL patients who were CD2 positive. The percentage of CD2 antigen positive leukemic cells from T-lineage ALL patients is a powerful predictor of EFS after chemotherapy. This prognostic relationship is the first instance in which a biological marker in T-lineage ALL has been unequivocally linked to treatment outcome.

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A CUTE LYMPHOBLASTIC leukemia (ALL) is a biologically diverse group of diseases.1,10 Leukemic cells from about 15% of ALL patients have a T-lineage immunophenotype as determined by expression of surface antigens found on normal T-lymphocyte precursors corresponding to discrete stages of maturation within the T-lymphocyte precursor pathway.1,2,6,12 While the remainder have a B-lineage immunophenotype as determined by surface expression of B-lineage lymphoid differentiation antigens.8,10 T-lineage immunophenotype is often associated with unfavorable presenting features (eg, high leukocyte counts, organomegaly) and poor prognosis.10,13-16 The identification of prognostically distinct patient subgroups within T-lineage ALL may lead to tailored and risk-adjusted therapy with improved therapeutic index. Furthermore, it may also allow appropriate stratification of T-lineage ALL patients in risk-adjusted clinical trials.3,10 Even though several prognostically important biological markers have been identified for patients with B-lineage immunophenotype, previous investigations have failed to discern clinically meaningful biological markers of leukemic T-lineage lymphoblasts.1 Consequently, all T-lineage ALL patients within a given program of therapy are treated alike, while B-lineage ALL patients may receive quite different treatments based on the biological features of their leukemic cells.3,10

With the introduction of intensive chemotherapy, a progressive and significant improvement has been achieved in the clinical outcome of T-lineage ALL patients.4,4 We recently reported an estimated 5-year event-free survival (EFS) probability of 75.2% for patients with T-lineage immunophenotype who were enrolled on contemporary Children’s Cancer Group programs of intensive chemotherapy, although 71.1% of the patients presented with poor-risk features.17 Obviously, there are prognostic influences at work that have so far escaped detection. Thus, it would be advantageous to know if any of the recognized immunophenotypic variables on leukemic T-lineage lymphoblasts have value in assessing prognosis. We now describe the effect of CD2 antigen expression on the treatment outcome of T-lineage ALL patients.

MATERIALS AND METHODS

Study patients From 1983 through 1994, patients with ALL who were less than 21 years of age were enrolled in the CCG-100 and CCG-1800 series of risk-adjusted treatment studies. The diagnosis was based on morphologic, biochemical, and immunologic features of leukemic cells, including lymphoblast morphology on Wright-Giemsa stained smears of bone marrow, positive nuclear staining for terminal deoxynucleotidyl transferase (TdT), negative staining
for myeloperoxidase, and expression of two or more lymphoid differen-
tiation antigens on the plasma membrane (see below). There were
4,398 patients with complete data for both clinical and immunophe-
notyping assessments. Standard definitions of remission, relapse, and
induction failure were previously reported. Results were also
presented according to the recently published NCI risk classification
criteria. These criteria classify a patient as being in the standard
risk category if age at diagnosis is 1 to 9 years and the white blood
(WBC) count is less than 50,000/mL. High-risk patients include
those who are diagnosed at an age of 10 years or greater and/or who
had an initial WBC equal to or greater than 50,000/mL. Infants
defined as less than 1 year of age at diagnosis are also classified as
high-risk.

**Immunophenotyping.** Mononuclear cell fractions containing
\( \geq 90\% \) leukemic cells were isolated from pretreatment bone marrow
aspirate samples by centrifugation of the cell suspensions on Ficoll-
Hypaque gradients. Immunophenotyping was performed centrally in
the CCG ALL Biology Reference Laboratory-linked immunopheno-
typing facilities in Minneapolis, MN, Washington, DC, and Seattle,
WA by indirect immunofluorescence and flow cytometry using
monoclonal antibodies reactive with the following lymphoid differ-
entiation antigens CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD9,
CD10, CD19, CD20, CD21, CD22, CD24, CD40, and CD72, as
previously described. All results were reviewed centrally in the CCG
ALL Biology Reference Laboratory in Minneapolis. Throughout this study, OKT11, 9.6 and 35.1 monoclonal antibodies
were used for evaluating leukemia cells for CD2 antigen expression.
Between 1983 and 1988, 52% of the T-lineage ALL cases were
immunophenotyped in Minneapolis, 22% in Washington, and 26%
in Seattle. Between 1989 and 1995, 56% of the T-lineage ALL cases
were immunophenotyped in Minneapolis, 35% in Washington, and
9% in Seattle. Among the 4,398 children enrolled in CCG treatment
studies for newly diagnosed ALL between 1983 and 1994, 730
(16.6%) had T-lineage ALL. Leukemic cells from the T-lineage
ALL patients expressed several surface differentiation antigens that
identify discrete stages of human T-cell ontogeny. Using expression
on \( \geq 30\% \) of leukemic cells as the arbitrary criterion for positivity
for the bulk population of leukemic cells, 84.5% of cases were
designated as CD2-positive, 33.9% as CD3-positive, 83.5% as CD5-
positive, 97.7% as CD7-positive, 19.5% as CD10/CALLA-positive,
and 45.8% as CD34-positive. The percentage of leukemic cells ex-
pressing each antigen showed a marked interpatient variation ranging
from 0% to 99% for CD2 (median, 83%; mean \( \pm SD, 70\% \pm 31\% \)),
0% to 98% for CD3 (median, 12%; mean \( \pm SD, 31\% \pm 28\% \)), 0%
to 99% for CD5 (median, 83%; mean \( \pm SD, 69\% \pm 31\% \)), 0% to
99% for CD7 (median, 90%; mean \( \pm SD, 81\% \pm 20\% \)), and 0%
to 97% for CD10/CALLA (median, 3%; mean \( \pm SD, 17\% \pm 27\% \)).

**Clinical treatment protocols.** In the first series of studies, pa-
tients were enrolled on six major protocols (CCG-104, -105, -106,
-107, -123, and -139), most of which relied on randomized designs
to compare therapeutic approaches of specific interest within a given
study population. The intensity of chemotherapy and the use of other
treatment modalities, such as radiation therapy, varied according to
the risk category to which a particular protocol was directed. Some
treatments, generally those based on intensification strategies, were
found to be significantly better than others. In the second series of
studies, patients were enrolled on five protocols (CCG-1881,
-1882, -1883, -1891, and -1901) that incorporated the superior regi-
ments from the CCG-100 series as controls for the newer treatment
approaches. Thus, since 1989, poor-risk patients have received the
best intensive therapies from the CCG-100 series or newer treatments
characterized by still greater intensification. Patients were assigned
to the study protocols according to their presenting risk features,
as described in other CCG reports. Comparisons between intensive
regimens almost always involved patients with unfavorable presenting
features (eg, WBC \( \geq 50,000/mL \), age \( \geq 10 \) years).

The T-lineage immunophenotype was not per se considered an ad-
verse risk factor. Each protocol was approved by the National Cancer
Institute, as well as the institutional review boards of the CCG-
affiliated institutions that participated in this study. Informed consent
was obtained from parents, patients, or both, as deemed appropriate,
according to the Department of Health and Human Services guidelines.

**Statistical methods.** The association of leukemic cell immuno-
phenotypes with the patients’ clinical, demographic, and laboratory
features, as well as their NCI risk category, was examined with
chi-square tests for homogeneity of proportions. Life-table estimates
were calculated by the Kaplan-Meier procedure. The primary end-
point was event-free survival (EFS) from the date of study entry.
An event is defined as induction failure (no response to therapy or
death during induction), leukemic relapse at any site, death during
remission, or the development of a second malignant neoplasm,
whichever occurs first. Patients who had not experienced an event
when last contacted were censored at that time in the EFS analysis.
Follow-up for event-free survivors ranged from 1 to 143 months
(median, 44 months). Other life-table end-points included site-spe-
cific relapses occurring as isolated initial events (ie, marrow relapse,
central nervous system [CNS] relapse, or testicular relapse). Site-
specific relapse analysis dealt only with the subset of patients who
achieved an initial remission and examined the duration of hematol-
ogy, CNS, or testicular remission from the time of induction. Pa-
tients who relapsed initially due to causes other than the one being
examined were censored at the time of that event.

Most comparisons of life-table estimates were based on the log-
rank statistic. Stratified log-rank tests were used in some in-
stances to adjust for the possible modifying effect of other factors.
Multivariate regression analyses relied on the Cox proportional haz-
ards model. Estimates of the life-table relative hazard rate (RHR)
for a particular event were calculated by the O/E method for log-rank
analysis and by the regression coefficient method in the proportional
hazards analysis. \( P \) values in the log-rank life-table comparisons
refer to survival patterns across the entire period of follow-up, with
the exception of occasional instances (limited to the text) where
comparisons of the Kaplan-Meier estimates are given for specific
time points. The log-rank test for trend or a regression test for trend
was used to test the significance of an ordering effect across different
expression levels of CD2 antigen in the bulk population of leukemic
cells as a candidate prognostic factor. The percentage of CD2-
positive blasts was used as a marker for the overall CD2 expression
level in the bulk population of leukemic cells. It should be noted,
however, that this percentage does not always reflect the actual
number of CD2 molecules on the surface of the individual leukemic
cells. The standard deviation of the life-table estimate was calculated
using Greenwood’s formula and was used in constructing confidence
limits. For purposes of comparisons, a statistically significant differ-
ance was defined as \( P < .05 \), and a borderline significant difference
as \( P > .05 \), but \( < .10 \).

**RESULTS**

Predictive value of CD2 antigen expression for isolated
bone marrow relapses and event-free survival after chemo-
Table 1. Presenting Features of the CD2- and CD2+ T-Lineage ALL Patients

<table>
<thead>
<tr>
<th>Variable Category</th>
<th>CD2- T-Lineage ALL (n = 101)</th>
<th>CD2+ T-lineage ALL (n = 550)</th>
<th>Chi-Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) &lt;1</td>
<td>5 (5.0)</td>
<td>2 (0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr) 1-9</td>
<td>53 (52.5)</td>
<td>366 (66.2)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Age (yr) ≥10</td>
<td>43 (42.6)</td>
<td>184 (33.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/L) 1-19</td>
<td>32 (31.7)</td>
<td>171 (31.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/L) 20-49</td>
<td>25 (24.8)</td>
<td>62 (11.3)</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/L) ≥50</td>
<td>44 (43.6)</td>
<td>317 (57.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Male</td>
<td>68 (67.3)</td>
<td>402 (73.1)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Sex Female</td>
<td>33 (32.7)</td>
<td>148 (26.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race White</td>
<td>77 (77.0)</td>
<td>409 (75.0)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Race Black</td>
<td>10 (10.0)</td>
<td>48 (8.8)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Race Other</td>
<td>13 (13.0)</td>
<td>88 (16.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down Syndrome Yes</td>
<td>1 (1.0)</td>
<td>7 (1.3)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Down Syndrome No</td>
<td>100 (99.0)</td>
<td>541 (98.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged liver No</td>
<td>88 (87.1)</td>
<td>493 (90.5)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Enlarged liver Yes</td>
<td>13 (12.9)</td>
<td>52 (9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged spleen No</td>
<td>76 (75.2)</td>
<td>448 (81.5)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Enlarged spleen Yes</td>
<td>25 (24.8)</td>
<td>102 (18.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged nodes No</td>
<td>76 (75.2)</td>
<td>383 (69.6)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Enlarged nodes Yes</td>
<td>25 (24.8)</td>
<td>167 (30.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of med mass No</td>
<td>63 (62.4)</td>
<td>358 (65.1)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Presence of med mass Yes</td>
<td>38 (37.6)</td>
<td>192 (34.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hgb (g/dL) &lt;1.7</td>
<td>35 (34.7)</td>
<td>155 (28.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hgb (g/dL) 1.7-9.9</td>
<td>40 (39.6)</td>
<td>184 (33.8)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Hgb (g/dL) ≥10</td>
<td>26 (25.7)</td>
<td>205 (37.7)</td>
<td></td>
<td></td>
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<tr>
<td>Platelets (×10⁹/L)≤149</td>
<td>41 (40.6)</td>
<td>186 (34.4)</td>
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<td></td>
</tr>
<tr>
<td>Platelets (×10⁹/L)59-149</td>
<td>26 (25.7)</td>
<td>214 (39.0)</td>
<td></td>
<td>.039</td>
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<tr>
<td>Platelets (×10⁹/L)≥150</td>
<td>34 (33.7)</td>
<td>146 (26.6)</td>
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<tr>
<td>FAB L1</td>
<td>67 (69.1)</td>
<td>411 (78.0)</td>
<td></td>
<td></td>
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<tr>
<td>FAB L1/L2 + L2/L1</td>
<td>21 (21.6)</td>
<td>83 (15.7)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>FAB L2</td>
<td>9 (9.3)</td>
<td>33 (6.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction M1 + M2</td>
<td>95 (95.0)</td>
<td>519 (97.9)</td>
<td></td>
<td>.088</td>
</tr>
<tr>
<td>Induction M3 + Death</td>
<td>5 (5.0)</td>
<td>11 (2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI risk group Good risk</td>
<td>32 (31.7)</td>
<td>162 (29.5)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>NCI risk group Poor risk</td>
<td>69 (68.3)</td>
<td>388 (70.5)</td>
<td></td>
<td></td>
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<tr>
<td>CNS disease at diagnosis Yes</td>
<td>6 (6.9)</td>
<td>32 (5.9)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>CNS disease at diagnosis No</td>
<td>95 (94.1)</td>
<td>512 (94.1)</td>
<td></td>
<td></td>
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<td>Modal chromosome number (n = 155) Normal 46, diploid</td>
<td>1 (6.7)</td>
<td>60 (42.9)</td>
<td></td>
<td></td>
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<tr>
<td>Modal chromosome number (n = 155) &lt;46</td>
<td>1 (6.7)</td>
<td>3 (2.1)</td>
<td></td>
<td></td>
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<tr>
<td>Modal chromosome number (n = 155) 46, Pseudodiploid</td>
<td>8 (83.3)</td>
<td>64 (46.7)</td>
<td></td>
<td>.006</td>
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<tr>
<td>Modal chromosome number (n = 155) ≥47-50</td>
<td>5 (53.3)</td>
<td>11 (7.9)</td>
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<tr>
<td>14q11 breakpoint (n = 155) Yes</td>
<td>3 (20.0)</td>
<td>28 (20.0)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>14q11 breakpoint (n = 155) No</td>
<td>12 (80.0)</td>
<td>112 (80.0)</td>
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</tbody>
</table>

Abbreviation: NS, not significant; FAB, French-American-British.

therapy. A total of 651 T-lineage ALL patients with data on CD2 expression on the surface of their leukemic cells were examined to determine whether the CD2-negative and CD2-positive groups differed according to various clinical and laboratory features, as well as response to induction chemotherapy (Table 1). CD2-negative patients less often were in the favorable 1 to 9 years age range at diagnosis (P < .0001) and less frequently had high leukocyte counts (>50,000/μL) at presentation. Otherwise, the two groups had very similar presenting features. Central cytogenetic review and classification was performed at presentation in a small subset of 155 T-lineage ALL patients enrolled in the more recent study series (ie, CCG-1800 series). The CD2-negative patients less often had a normal diploid karyotype and more often had hyperdiploidy with 47 to 50 chromosomes (P = .006). Both groups had a very low incidence of hyperdiploidy with >50 chromosomes and an identically high incidence (ie, 20%) of 14q11 rearrangement. Achievement of initial remission generally occurs in a very high proportion of ALL patients. Therefore, even in poor prognostic categories, it is usually very difficult to show statistically significant differences for remission achieve-
Table 2. Univariate and Multivariate Prognostic Factors for T-Lineage ALL

<table>
<thead>
<tr>
<th>Factor</th>
<th>Categories</th>
<th>RHR</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Univariate</td>
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<td></td>
<td></td>
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<tr>
<td>Marked splenomegaly</td>
<td>No</td>
<td>1.00</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td>Marked hepatomegaly</td>
<td>No</td>
<td>1.00</td>
<td>.0003</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.17</td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>&lt;30%</td>
<td>1.75</td>
<td>.0003</td>
</tr>
<tr>
<td></td>
<td>=30%</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/L)</td>
<td>&lt;20</td>
<td>1.00</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>20-49</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>=50</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>1-9</td>
<td>1.00</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
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</tr>
<tr>
<td></td>
<td>≥10</td>
<td>1.15</td>
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<td>1.00</td>
<td>.0005</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.92</td>
<td></td>
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<tr>
<td>Age (yr)</td>
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<td>1.00</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>4.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥10</td>
<td>1.23</td>
<td></td>
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<tr>
<td>Marked hepatomegaly</td>
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<td>1.00</td>
<td>.03</td>
</tr>
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<td></td>
<td>Yes</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>&lt;30%</td>
<td>1.49</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>=30%</td>
<td>1.00</td>
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</tr>
</tbody>
</table>

Despite this inherent difficulty, CD2-negative patients appeared to have poorer remission induction success with a failure rate of 5.0% compared with 2.1% in the CD2-positive group, reaching a borderline significant level (P = .088) (Table 2). These results are consistent with and extend the POG study published by Borowitz et al who reported that E rosette-negative T-lineage ALL patients were less likely to enter complete remission than E rosette-positive T-lineage ALL patients.

As shown in Fig 1A, CD2-negative cases relapsed sooner and much more frequently in the bone marrow than CD2-positive cases (RHR = 2.38, P = .0002), although the risk of relapse in CNS (P = .75) or testes (P = .83) was not affected by CD2 expression. Figure 1B compares the EFS outcome of CD2-negative and CD2-positive patients. The CD2-negative group had a significantly poorer outcome (RHR = 1.68, P = .002) with a 6-year EFS of 52.8% ± 5.6% compared with 69.8% ± 2.2% in the CD2-positive group (P = .003, KMp test). By comparison, the expression levels of the CD3, CD5, CD10, and CD34 antigens did not influence the overall EFS outcome of T-lineage ALL patients (data not shown). Because of the very small fraction of CD7 antigen negative T-lineage ALL cases (only 14 of 612 cases analyzed for CD7 expression were negative), we could not examine the prognostic value of CD7 expression with statistical precision in this analysis.

To further assess the predictive value of CD2 expression on marrow relapse and event-free survival, T-lineage ALL patients were divided into three groups according to the percentage of CD2-positive leukemic cells. Notably, CD2 showed a pronounced prognostic effect on marrow relapse and EFS, across its three categories of distribution (P = .0003 and P = .002, log-rank tests for linear trend). When compared with patients with the highest CD2 expression level (>75% positivity) in the bulk population of leukemic cells, the life-table relative event rate (RER) was 1.22 for patients with intermediate range CD2 expression level (30% to 75% positivity) and 1.81 for patients with the lowest CD2 expression level (<30% positivity). Figure 2 depicts the Kaplan-Meier estimates of EFS probabilities for patients with different levels of CD2 expression on their leukemic cells. At 6 years postdiagnosis, the EFS estimates for the three CD2 expression groups were (41.9% to 63.8%), (57.2% to 73.8%), and (66.8% to 77.1%).

Besides CD2 expression, splenomegaly (P < .0001), hepatomegaly (P = .0003), age (P = .02), and WBC count (P = .02) also had important univariate prognostic influence in T-lineage ALL patients (Table 2). Cox proportional hazards

Fig 1. Probability of relapse and EFS, according to CD2 antigen expression, of 651 children with newly diagnosed T-lineage ALL treated in risk-adjusted CCG-100 & CCG-1800 series therapy studies. The cumulative proportions of patients remaining free of bone marrow relapse (depicted in [A]) and surviving event-free (depicted in [B]) are shown according to the number of years after study entry.
regression analyses were conducted that simultaneously evaluated the prognostic effects of CD2 antigen expression, as well as these other potentially confounding prognostic factors. CD2 expression remained a significant predictor of EFS after adjustment for the effects of these covariates by multivariate regression, with a RER of 1.67 for the lowest CD2 expression level compared with the highest CD2 expression level (\( P = .04 \)). Thus, the prognostic effect of CD2 antigen expression remains relatively independent of any of the several patient features we considered (Table 2). Splenomegaly (\( P = .0005 \)), hepatomegaly (\( P = .03 \)), and age (\( P = .02 \)) also retained prognostic influence in the regression analysis, although WBC count was no longer significant once adjusted for the other factors.

Analysis of T-lineage ALL patients showed a significant separation in EFS according to the NCI age and WBC criteria for standard and high-risk ALL (\( P = .002 \) and RER = 1.67, stratified log-rank test). The determination of CD2 expression on leukemic cells helped identify patients with the better and poorer prognoses in both of these risk group subsets (Fig 3), although the separation seemed to be greater in the standard risk patients. For standard risk T-lineage ALL, CD2-negative patients had a worse outcome (\( P = .0007 \), RER = 2.92) with an estimated 5-year EFS of 55.9% as compared with 78.3% for the CD2-positive patients. For high-risk T-lineage ALL, CD2-negative patients had a borderline significant worse outcome (\( P = .07 \), RER = 1.44) with an estimated 5-year EFS of 52.7% as compared with 67.1% for the CD2-positive subset. Thus, CD2 negativity in standard risk T-lineage ALL identifies a group of patients who have a worse outcome than high-risk T-lineage ALL patients who are CD2-positive. The overall significance level for the absence of CD2 antigen adjusted for these two risk categories was \( P = .002 \) with a 1.70 times greater RER.

Patients with adequate bone marrow samples sent for central laboratory immunophenotyping, representing 61.3% of the total number of patients entered in CCG-100s or CCG-1800s studies, were compared for EFS outcome with the remaining CCG ALL patients entered in these studies. Results showed a similar EFS pattern with EFS differences between the groups never exceeding 2% at any time point, which suggests that no selection bias existed with regard to the prognosis of patients having central immunophenotyping performed.

T-lineage ALL patients with and without CD2 expression
data were examined for comparability of various patient characteristics (age, WBC count, organomegaly, CNS status, sex, FAB morphology, NCI prognostic group) and no significant differences were identified. Also, EFS outcome for those with and without CD2 assessment showed very similar outcome patterns \( (P = .48) \). Thus, there appears to be no selection bias among the T-lineage ALL patients regarding who had CD2 assessment performed.

**Effect of therapy on the prognostic significance of CD2 antigen expression in T-lineage ALL.** In the CCG-100 series of studies, conducted between 1983 and 1989, both the enhanced intensity of chemotherapy and the use of radiation therapy for CNS prophylaxis favorably influenced the EFS of T-lineage ALL patients, as compared with results obtained with unmodified regimens.\(^{20,30,32}\) In the second series, CCG-1800, conducted between 1989 and 1994, we incorporated the superior regimens from the CCG-100 series studies as controls for newer, more intensive programs of chemotherapy. The administration of more intensive therapy to poor risk categories, which include a substantial portion of the T-lineage ALL patients improved the 5-year EFS outcome of T-lineage ALL patients from 65.8% in the CCG-100 series to 78.1% in the CCG-1800 series.\(^{17}\) However, this overall improvement in EFS outcome over the two treatment eras did not abolish the prognostic significance of CD2 antigen expression. The RER for the CD2-negative subset was 1.55 in the CCG-100 series \( (P = .04) \) and 1.74 in the CCG-1800 series \( (P = .06) \).

**DISCUSSION**

In a large cohort of T-lineage ALL patients, we evaluated the biologic significance of CD2 antigen positivity of leukemic lymphoblasts. Absence of CD2 antigen on leukemic cells from T-lineage ALL patients was a very strong predictor of treatment failure within both the standard and poor risk categories according to the NCI Working Formulation. The determination of CD2 expression on leukemic cells helped identify patients with the better and poorer prognoses in both of these risk group subsets. Notably, CD2 negativity in standard risk T-lineage ALL identified a group of patients who had a worse outcome than high-risk T-lineage ALL patients who were CD2-positive. Multivariate analysis of potentially confounding covariates with the Cox proportional-hazards model established the expression level of the surface CD2 antigen on leukemic cells of T-lineage ALL patients as an independent predictor of treatment outcome after chemotherapy. This prognostic relationship is the first instance in which a biological marker in T-lineage ALL has been unequivocally linked to treatment outcome.

In our study, the expression of CD3, CD5, CD10, or CD34 antigens on leukemic cells did not affect the patients’ event-free survival. By comparison, in a recent POG study of 253 patients with T-lineage ALL, lack of CD10 expression was associated with poor treatment outcome.\(^{13}\) Furthermore, expression of CD5 antigen in patients with leukocyte counts less than 50,000/\( \mu \)L was associated with good prognosis, whereas CD3 antigen expression had no prognostic significance.\(^{13}\) In a single-institution study, which contradicted the aforementioned POG study, Pui et al\(^{14}\) found that the expression of the CD3 antigen in T-lineage ALL inferred an increased risk of treatment failure, whereas the expression of CD10 or CD5 antigens did not show a prognostic value. These apparent discrepancies and contradictions may be due to differences in the size of patient populations studied, as well as treatment differences.

The CD2 T-cell surface molecule\(^{39}\) is a glycoprotein monomer with a molecular mass of 50 kD. A detailed analysis of human T-cell antigen expression during the early stages of fetal thymic maturation showed that CD2 is acquired on thymocytes at 12 weeks of fetal gestation.\(^{40}\) CD2 can mediate both cell-to-cell adhesion and trigger mitogenic, as well as activation signals that are thought to play important roles in T-cell development.\(^{39,41}\) CD2 has been shown to interact with multiple ligands, including: (1) the LFA-3/CD58 molecule on thymic epithelial cells, (2) the complement regulatory protein CD59, and (3) the CD48 molecule.\(^{42}\) CD2 is physically associated with Src family protein tyrosine kinases Lck and Fyn.\(^{42}\) Association with CD2 receptor results in increased baseline activity of these tyrosine kinases and engagement of the CD2 receptor further triggers their activation leading to enhanced tyrosine phosphorylation of multiple phosphoprotein substrates and calcium mobilization.\(^{43-45}\)

Because apoptotic cell death of leukemic lymphocyte precursors is dependent on tyrosine kinase activation,\(^{45,46}\) it is possible that the intracellular CD2/Fyn, CD2/Lck, as well as the extracellular CD2/LFA-3 interactions decrease the apoptotic threshold of CD2-positive T-lineage ALL cells. Furthermore, the cascade of biochemical events triggered by CD2 engagement stimulates the proliferative activity of T-lineage ALL cells, which may increase the sensitivity to chemotherapeutic agents.\(^{47}\) Our preliminary studies suggest that primary leukemic cells from CD2-positive T-lineage ALL patients may be more susceptible to apoptosis than leukemic cells from CD2-negative patients (F.M. Uckun, unpublished observations, July 1996). A greater susceptibility of leukemic cells to drug or radiation-induced apoptosis may, in part, contribute to the observed EFS advantage of CD2-positive T-lineage ALL cases on risk-adjusted treatment protocols.

Our study provides direct evidence that the percentage of CD2 antigen positive leukemic cells from T-lineage ALL patients is a powerful predictor of EFS after chemotherapy. This prognostic relationship is the first instance in which a biological marker in T-lineage ALL has been unequivocally linked to treatment outcome. Patients with CD2-negative T-lineage ALL relapse at nearly 1.5 times the rate for patients with CD2-positive T-lineage ALL. More effective treatment of CD2-negative T-lineage ALL may require the introduction of novel agents that can either kill CD2-negative leukemic T lymphoblasts or enhance their sensitivity to contemporary chemotherapy. The problem of persistent leukemia in the high-risk subgroups of T-lineage ALL, including CD2-negative T-lineage ALL, will be addressed in the next generation of frontline CCG studies, which will employ apoptosis-inducing biotherapeutic agents, such as immunotoxins containing the plant-derived hemitoxin pokeweed antiviral protein (PAP)\(^{47}\) or immunonconjugates containing tyrosine kinase inhibitors,\(^{48,49}\) in an attempt to eradicate leukemic T-lineage lymphoblasts that are resistant to contemporary chemotherapy.
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CD2 EXPRESSION IN T-LINEAGE ALL

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CD2 antigen expression on leukemic cells as a predictor of event-free survival after chemotherapy for T-lineage acute lymphoblastic leukemia: a Children’s Cancer Group study

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