Expression of Lymphoid-Associated Cell Surface Antigens by Childhood Acute Myeloid Leukemia Cells Lacks Prognostic Significance

By Franklin O. Smith, Beatrice C. Lampkin, Connie Versteeg, David A. Flowers, Patricia A. Dinndorf, Jonathan D. Buckley, William G. Woods, G. Denman Hammond, and Irwin D. Bernstein

The prognostic significance of cell surface antigens associated with lymphoid and myeloid lineage differentiation on the blasts of children with acute myeloid leukemia (AML) was evaluated. Leukemic blasts from 176 patients enrolled on Children's Cancer Study Group Protocol 213 determined to have AML by standard morphologic and cytochemical criteria were immunophenotyped. Cell surface antigens associated with myeloid differentiation were found on blasts from 88.1% of patients (CD15, 44%; CD33, 65%; CD36, 53%; glycoprotein Ib, 9.3%). However, blasts from 30.7% of patients expressed surface antigens thought to be specific for lymphoid lineage differentiation (CD2, 9.4%; CD5, 2.7%; CD19, 34.5%; CD20, 0.8%). In addition, CD34, a glycoprotein found on immature cells of both myeloid and lymphoid lineages, was expressed on the blast cells of 48.2% of patients. With the exception of the lymphoid lineage nonspecific antigen CD4, no correlation was found between white blood cell count at diagnosis, age, and French-American-British morphology, and the expression of any of the lymphoid- or myeloid-associated cell surface antigens studied.

Nor was the expression of these antigens prognostically significant with respect to response to induction therapy, death during induction, survival, event-free survival, or survival/event-free survival following remission induction. Multivariate analysis showed that CD4 expression was not an independent predictor of outcome. Thus, this prospective study suggests that expression of lymphoid-associated cell surface antigens as well as myeloid-associated antigens by childhood AML blasts lacks prognostic significance.

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ACUTE MYELOID LEUKEMIA (AML) is a heterogeneous disease derived from hematopoietic precursor cells. Previous studies have found few clinical and laboratory parameters that are reproducibly predictive of outcome. In children, these include a high white blood cell (WBC) count at diagnosis and French-American-British (FAB) M4 and M5 morphology. One approach to identifying prognostic factors has been to characterize the surface antigens displayed on AML blasts. In some studies, the expression of cell surface antigens associated with normal myeloid differentiation on AML blasts, including CD11, CD13, CD14, CD15, and CD33, were found to be of prognostic significance. In addition, studies in adults with AML have suggested that the expression of the hematopoietic antigen CD34 was predictive of a poor outcome.

While blasts from most patients with AML display antigens associated with normal myeloid development, a portion also display antigens associated with lymphoid development. Studies of relatively small numbers of patients have suggested that the presence of these lymphoid-associated cell surface antigens on AML blasts are predictive of a poor response to therapy. Contrary to these results, a study of a large number of adult patients with the lymphoid-associated antigens CD2 and CD19 on AML blasts found these patients to have a more favorable prognosis. In this report, we have prospectively examined the prognostic significance of cell surface antigens associated with myeloid differentiation (CD15, CD33, CD36, glycoprotein Ib), lymphoid differentiation (CD2, CD3, CD5, CD8, CD19, CD20), those antigens present on cells of both myeloid and lymphoid lineages (CD4, CD7, CD9, CD10), as well as the CD34 antigen, present on blasts from children with AML. We found that the presence of these lymphoid- or myeloid-associated cell surface antigens were not of prognostic value in this clinical trial of intensive therapy for childhood AML.

MATERIALS AND METHODS

Patients. Childrens Cancer Study Group (CCSG) protocol 213 for children more than 1 month and less than 21 years of age with previously untreated AML was conducted from January 1986 to February 1989. Five hundred ninety eligible patients were enrolled in the study. Immunophenotyping studies were performed on all 176 (29.8%) diagnosis bone marrow samples that were sent to the CCSG AML Reference Laboratory in Seattle. Patient follow-up is ongoing with a median time of 1,154 days. Objectives of the study included (1) comparison of two methods of induction therapy, and (2) comparison of three arms of therapy after remission induction that included allogeneic bone marrow transplantation, intensive consolidation chemotherapy followed by no further therapy, or consolidation chemotherapy followed by maintenance therapy. The treatment regimen is summarized in Fig 1.

Diagnosis. A diagnosis of AML was made at the institution of origin based on standard morphologic and cytochemical criteria including Wright-Giemsa, α-naphthyl butyrate esterase, chloroacetate esterase, periodic acid Schiff, peroxidase, and Sudan Black stains. The diagnosis was confirmed by review in a central laboratory.

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were performed. The percentage blasts in the patient's marrow was
were stained with isotype identical antibodies of irrelevant speci-
were suspended in 10% dimethyl sulfoxide (Sigma, St Louis, MO)
mary of the specificity of these antibodies is presented in Table 1.
were separated by Ficoll-Hypaque density centrifugation (specific
enrolled on CCSG protocol 213 were collected in preservative-free
mulations of ascites fluid at dilutions of 1:250 to 1:1,000 or as high perfor-
ere HPLC purified antibody. A summary of the antigen expression if more than 25% of the
cells were not evaluated were assessed using Chi square tests.
were performed comparing patients whose blasts expressed 0, 1, 2, or 3
bility, T11D7 or H12C12 (IgM, antimouse Thy1.1, and Thy1.2,
respectively), 1A14 (IgG2a, antimouse Thy1.1), or 31.A (IgG1,
antimouse Thy1.1). Cells were counter-stained with propidium
antimouse Thyl.1). Cells were counter-stained with propidium
iodide 8 pg/mL (Sigma) to exclude nonviable cells from analysis.
counter-stained with propidium
scatter properties consistent with blast-size cells were analyzed for
patients whose blasts were immunophenotyped and those whose
clinical features and outcome parameters between the groups of
potential differences in the
cell surface antigens associated with lymphoid or myeloid
differentiation. This included the analysis of patients whose
blast cells expressed multiple antigens associated with T-cell
cell surface antigens in a series of pair-wise
lymphoid-associated cell surface antigens in a series of pair-wise
comparisons.

Indirect immunofluorescence studies. After thawing of the cells in
RPMI with 10% fetal calf serum and 100 U/mL deoxyribonuclease 1 Type II (Sigma), the cells were incubated with MoAb
diluted in phosphate-buffered saline (PBS) plus 2% human AB
serum (GIBCO Laboratories, Grand Island, NY) for 30 minutes
at 4°C, washed with cold PBS plus 2% human AB serum, then
incubated with a 1:40 dilution of affinity purified fluorescein-
conjugated goat, antimouse IgM plus IgG antibody for 30 minutes
at 4°C (Tago, Inc, Burlingame, CA). As a negative control, cells were
stained with isotype identical antibodies of irrelevant speci-

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<td>Platelets</td>
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Results of MoAbs

Table 1. Specificity of MoAbs

Cells. Samples of marrow from 176 of 590 evaluable patients
enrolled on CCSG protocol 213 were collected in preservative-free
heparin and sent to Seattle at initial diagnosis. These specimens were
separated by Ficoll-Hypaque density centrifugation (specific gravity 1.077 g/cm3) (Pharmacia, Inc, Piscataway, NJ). The cells
were suspended in 10% dimethyl sulfoxide (Sigma, St Louis, MO)
and frozen in liquid nitrogen until immunophenotyping studies
were performed. The percentage blasts in the patient's marrow was
estimated from the standard morphologic slide preparation.

Monoclonal antibodies (MoAbs). MoAbs were used in the form
of ascites fluid at dilutions of 1:250 to 1:1,000 or as high perfor-
mations of ascites fluid at dilutions of 1:250 to 1:1,000 or as high perfor-
mance liquid chromatography (HPLC) purified antibody. A summary
of the specificity of these antibodies is presented in Table 1.

MuMoAbs were used in the form
for 30 minutes at
The use of MoAbs allows the discrimination between normal and neoplastic
lymphoid and myeloid elements in a biological sample.

A comparison of the specificity of MoAbs to cell surface antigens of
lymphoid and myeloid elements is presented in Table 1.

Table 1. Specificity of MoAbs

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RESULTS

Study population. Preliminary results for all 590 patients
enrolled on CCSG 213 showed no difference in the remission
induction rate between the two induction regimens
(Ara-C plus daunomycin versus Ara-C, daunomycin, etoposide,
dexamethasone, and 6-thioguanine: 80.8% vs 76.3%,
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respectively, \( P = .29 \). EFS at 2 years postinduction was not different for patients undergoing bone marrow transplantation and those patients who received chemotherapy only: EFS = 50% versus 41%, respectively, \( P = .41 \). In addition, there was no difference in EFS at 1 year between patients receiving maintenance chemotherapy and those patients who did not receive maintenance therapy: EFS = 63% versus 64%, respectively, \( P = .26 \).

To exclude a selection bias among patients who had marrow samples available for analysis, we compared the clinical characteristics and outcome in patients whose leukemia cells were immunophenotyped and all evaluable study patients. There was no difference between the 176 patients tested and the full cohort of 590 eligible patients enrolled on the study with respect to induction rate \( (P = .21) \), death during induction \( (P = .51) \), completion of induction \( (P = .58) \), survival \( (P = .22) \), EFS \( (P = .31) \), FAB classification \( (P = .95) \), age at diagnosis \( (P = .77) \), initial WBC \( (P = .43) \), sex \( (P = .77) \) and percentage of blasts in marrow \( (P = .087) \).

Of the 176 patients whose blast cells were phenotyped, 130 (73.9%) achieved remission, whereas 14 (7.9%) died during induction. Thirty-two patients (18.2%) failed induction therapy. There was no difference between the distribution of patients who received chemotherapy alone or chemotherapy plus marrow transplantation in the immunophenotyped patients, and the total group of 590 patients enrolled on study \( (P = .54) \). In the group of 176 patients who had their cells analyzed, 93 received chemotherapy alone while 29 received chemotherapy plus bone marrow transplantation. Actuarial survival at 4 years from diagnosis for the group of immunophenotyped patients was 38.9 ± 3.0% compared with an actuarial survival of 38.3 ± 3.9% for all 590 patients enrolled on this protocol \( (P = .46) \).

Expression of leukocyte differentiation antigens by leukemia cells. All patients were determined to have AML based on morphologic and cytochemical criteria (see Materials and Methods). An assessment of antigen expression by leukemic cells was consistent with a diagnosis of AML as evidenced by the expression of cell surface antigens associated with myeloid differentiation \( (CD15, CD33, CD36, glycoprotein Ib) \) in the majority of samples. The blast cells from 155 of 176 (88.1%) patients displayed cell surface antigens that are expressed predominantly on normal cells of the myeloid lineage but not on cells of the lymphoid lineage. The overall frequency of expression of these antigens was: CD15, 44%; CD33, 65%; CD36, 53%; glycoprotein Ib, 9.3%.

We found that 54 of 176 (30.7%) patients had blasts that expressed cell surface antigens that are currently thought to be displayed by cells of the lymphoid and not myeloid lineage \( (CD2, CD3, CD5, CD8, CD19, CD20) \). In addition, 31.2% of patient samples had blasts that expressed antigens which are present on cells of both lymphoid and myeloid lineages \( (CD4, CD7, CD9, CD10) \). The overall frequency of lymphoid-associated cell surface antigens was: CD2, 9.4%; CD3, 0%; CD4, 34.5%; CD5, 2.7%; CD7, 16.8%; CD8, 0%; CD9, 79.8%; CD10, 0.6%; CD19, 34.5%; CD20, 0.8%.

Twenty-one of 176 (11.9%) patients had marrow blasts that did not express myeloid-associated antigens \( (CD15, CD33, CD36, glycoprotein Ib) \). Five of these samples did not express lymphoid-associated cell antigens. Review of these 21 marrow samples showed morphologic and cyto logic findings consistent with the original diagnosis of AML.

Blasts were also tested for the cell surface expression of CD34, a glycoprotein that is found on immature marrow cells of both lymphoid and myeloid lineages. CD34 was expressed on the blasts present in the marrow of 80 of 166 patients (48.2%).

Immunophenotype and clinical characteristics. We found no association between age at diagnosis, initial WBC count, or FAB classification, and the expression of antigens known to be associated with myeloid, but not lymphoid maturation \( (CD15, CD33, CD36, glycoprotein Ib) \). Similarly, the expression of antigens associated with lymphoid but not myeloid differentiation \( (CD2, CD3, CD5, CD8, CD19, CD20) \) did not show a significant correlation with patient age at diagnosis, initial WBC count, or FAB classification \( (M1-M7) \). However, there was an association between expression of the CD4 antigen and FAB M4/M5 morphology \( (P < .000001) \) and age less than 2 years at diagnosis \( (P = .018) \).

No significant correlations between patient characteristics and immunophenotype were found in the 21 patients whose blasts did not express myeloid-associated antigens. These patient’s blasts comprised a variety of FAB classes \( (M1, N = 6; M2, N = 9; M4, N = 4; M5, N = 2) \). The expression of CD34 by blasts did not correlate with age at diagnosis, initial WBC count, or FAB classification. However, the absence of CD34 expression was significantly associated with age less than 2 years at diagnosis \( (P = .012) \) and FAB M4/M5 morphology \( (P = .0052) \), but did not correlate with WBC at diagnosis.

Immunophenotype and outcome. We found no prognostic significance for the cell surface expression of those antigens associated only with myeloid cells \( (CD15, CD33, CD36, glycoprotein Ib) \). With the exception of CD4 expression, the outcome of those patients whose blasts expressed antigens associated nonspecifically \( (CD4, CD7, CD9, CD10) \) or specifically \( (CD2, CD3, CD5, CD8, CD19, CD20) \) with lymphoid cells, was not different from those patients whose blast cells did not express lymphoid-associated antigens (Fig 2). This lack of prognostic value was found in both the therapeutic subgroup of patients who received chemotherapy alone (Fig 3) as well as patients who received chemoradiation therapy followed by allogeneic bone marrow transplantation (Fig 4).

The expression of CD4 by marrow blasts predicted a poorer survival \( (P = .03) \) but not EFS \( (P = .07) \). However, if patients under 2 years of age at diagnosis and FAB M4 morphology were excluded from the analysis, CD4 expression was not of prognostic value. The presence or absence of CD34 on leukemic blasts did not distinguish patients with a different outcome \( (survival P = .12) \). Because the absence of CD34 expression was associated with age under 2 years and FAB M4/M5 morphology, we analyzed the
outcome of those patients with and without CD34 expression after excluding patients younger than 2 years and patients with M4/M5 morphology. This analysis showed no difference in outcome (survival $P = .39$).

The presence of any two lymphoid lineage specific antigens (CD2, CD3, CD5, CD8, CD19, or CD20) did not predict a different outcome (actuarial survival at 4 years from diagnosis $= 60.0\% \pm 21.9\% \pm 38.5\% \pm 4.4\%, P = .17$). Actuarial survival at 4 years from diagnosis for patients whose blasts expressed any two lymphoid-associated antigens (CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD19, or CD20) was $38.2\% \pm 7.8\%$ compared with a survival of $39.2\% \pm 5.3\%$ for patients without lymphoid-associated antigens ($P = .46$). Similarly, patients whose blast cells expressed any three lymphoid-associated antigens did not fare differently (actuarial survival $= 45.0\% \pm 12.2\%; P = .31$).

The outcome of the 21 patients whose blast cells did not express myeloid-associated cell surface antigens was not different from the patients whose blast cells expressed antigens associated with myeloid differentiation (survival: $P = .55$, EFS: $P = .54$).

**DISCUSSION**

In this study, we used antibodies to detect cell surface antigens that are commonly displayed on cells of the myeloid, but not lymphoid lineages (CD15, CD33, CD36, glycoprotein Ib), those antigens displayed by lymphoid, but not myeloid cells (CD2, CD3, CD5, CD8, CD19, CD20), and antigens expressed on cells of both myeloid and lymphoid lineages (CD4, CD7, CD9, CD10). Leukemic blasts from 88.1% of the patients in this study displayed one or more of the cell surface antigens associated with myeloid differentiation. We found no correlation between the expression of antigens displayed only on myeloid cells and clinical features. Nor did we obtain prognostic information based on the expression of these myeloid lineage specific antigens. Previous studies in adults and children with AML have suggested that expression of cell surface antigens associated with myeloid differentiation are prognostically significant (CD13, CD14, CD15, and CD33).\(^\text{5}-\text{11}\) Contrary to these results, we did not find CD15 or CD33 to be of prognostic value in this study of childhood AML. Similarly, Ward et al\(^\text{15}\) were unable to show prognostic significance for expression of CD13, CD14, CD15, or CD33 in their study of adults with AML. Discrepancies in various reports may be explained by small study populations, the combined retrospective analysis of patients from different treatment regimens, the effect of different therapies on patient outcome, nonspecificity of MoAbs used in immunophenotyping studies, differences in criteria for positive antigen expression, and
the inclusion of normal cells in the analysis of leukemic blasts. Finally, since AML may have different biologic characteristics in different age groups, factors that are found to be prognostic in one age group may not have value in the other group.

We sought to determine if there is an association between patient characteristics and outcome and the expression of antigens associated with lymphoid lineage differentiation on the blasts of children treated on the same study protocol. When the analysis was restricted to those antigens that are thought to be specific for lymphoid differentiation, (CD2, CD3, CD5, CD8, CD19, CD20), 30.7% of AML samples were found to express one or more of these cell surface antigens. It is unlikely that the presence of these lymphoid-associated antigens on the blast cell population was the result of inclusion of normal lymphoid cells. This conclusion is supported by the fact that no samples were positive for CD3. The frequency of expression of CD2 in our study was consistent with that published by Cross et al1 who showed that CD2 was present on the blasts in 10% of children with AML. However, our results differ from those reported by Ball et al33 in which CD2 and CD19 antigens were expressed on the blast cells of 21% and 33%, respectively, of adult patients with AML.

We found no difference in the age at diagnosis, initial WBC count, and FAB classification in patients whose leukemic blasts expressed lymphoid lineage-specific antigens. Patients whose AML blast cells expressed these antigens did not fare differently from those patients whose leukemic blasts lacked expression of these lymphoid lineage-specific antigens. The overall probability of EFS at 4 years from diagnosis was 38.4%, regardless of the expression of those antigens found exclusively on cells of the lymphoid lineage (P = .54) (Fig 2).

We also evaluated antigens normally expressed by both lymphoid and myeloid cells. These included CD4, present on T cells and monocytes; CD7, expressed on T cells and possibly on myeloid progenitors8; CD9, present on pre-B cells, monocytes, and platelets; and CD10 (CALLA), expressed on pre-B cells and granulocytes. With the exception of the lymphoid lineage nonspecific antigen CD4, no correlation was found between the presence of these lymphoid lineage nonspecific antigens and age, WBC count at diagnosis, FAB classification, and outcome.

Although CD4 expression was associated with poorer survival, CD4 expression was not an independent prognostic factor. CD4 expression was found predominantly in the subgroup of patients under 2 years of age at diagnosis and FAB M4 morphology. When a multivariate analysis controlling for this previously identified poor outcome group of patients was performed, CD4 expression had no prognostic value.

Cross et al31 reported that CD2 and CD7 expression by AML blasts predicted a poor response to remission induction therapy (P = .05) and represented a subtype of AML with distinct biologic properties. However, their results may have been influenced by relatively small numbers of patients (N = 10) as well as the analysis of patients on two separate treatment protocols. A larger study of two treatment regimens by the same group later failed to show prognostic value for lymphoid-associated antigens expressed by childhood AML blast cells (CD2, CD5, CD7, CD10, CD19, CD20, CD22).52 Contrary to these reports, Ball et al33 reported that adults with AML whose blast cells expressed CD2 or CD19 had a more favorable prognosis with higher complete remission rates (P = .04), longer time to failure (P = .02), and improved survival (P = .02). In our analysis of a large population of patients treated on a single treatment protocol, we found no prognostic value for the expression of CD2, CD7, CD19, or other antigens associated with lymphoid lineage differentiation on AML blasts.

In our study, CD34 was displayed on leukemic blasts from 48% of patients. This result is consistent with previously reported frequency of 45%.11 Borowitz et al11 reported that although adults whose AML blasts displayed CD34 were less likely to enter a complete remission, there was no difference in their overall survival. We did not observe any difference in the rate of remission induction or survival in children whose leukemic blasts expressed CD34.

A large number of statistical analyses were performed in this study. Given the number of analyses performed, it is possible that statistical significance for any given comparison could occur by chance alone. However, despite this risk, no statistical corrections were used in the analyses. As noted in the Results and Discussion above, statistical significance was shown only for an association between the cell surface expression of CD4 and CD34 and the clinical characteristics of age at diagnosis and FAB classification. No statistically significant associations were shown for antigen expression and outcome. Therefore, it is unlikely that the absence of statistical corrections in the analyses had any bearing on the results of this study.

Leukemic blasts that express cell surface markers of a lineage different from the one to which they are assigned have often been termed “biphenotypic” or acute mixed-lineage leukemias (AMML). The clinical and biologic significance of “biphenotypic” leukemia is a subject of considerable interest and controversy. While this study and the study reported by Pui et al52 clearly show that lymphoid-associated antigen expression on childhood AML blast cells has no prognostic value, the presence of myeloid-associated antigens on childhood acute lymphocytic leukemia (ALL) cells is much less clear. This discrepancy in “biphenotypic” ALL is demonstrated in three recent studies by Pui et al52,53 and Wiersma et al.54 Pui et al found no prognostic value for the presence of myeloid-associated antigens on ALL blasts. In contrast to this finding, Wiersma et al was able to show that myeloid antigen expression on ALL cells was an independent predictor of a poor response to chemotherapy.

This study suggest that childhood “biphenotypic” AML may not represent a biologically distinct form of leukemia because children with this disease have similar clinical features and respond to therapy in a similar manner to those patients whose AML blasts do not express lymphoid-associated antigens.
### Appendix. Participating Principal Investigators: Children's Cancer Study Group

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LYMPHOID ANTIGENS ON CHILDHOOD AML

Appendix. Participating Principal Investigators: Childrens Cancer Study Group (Cont’d)

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