Cancer and Leukemia Group B Leukemia Correlative Science Committee: Major Accomplishments and Future Directions

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Abstract The Cancer and Leukemia Group B (CALGB) Leukemia Correlative Science Committee (LCSC) has a remarkable history of outstanding productivity and has been at the cutting edge of correlative science for adult leukemia for almost 25 years. Its work, initially focused on the use of immunophenotyping for diagnosis and prognosis of acute lymphoblastic leukemia and acute myeloid leukemia, has, for the last 15 years, focused on the clinical use of cytogenetic and molecular genetic markers in acute myeloid leukemia and acute lymphoblastic leukemia as well as in chronic lymphocytic leukemia. Numerous CALGB LCSC studies have had a major effect on the way we currently diagnose, predict outcome, select appropriate treatment, document complete remission, and monitor residual disease in adults with acute leukemia. In part as a result of the work of the CALGB LCSC, we are increasingly moving toward molecularly targeted therapy in acute and chronic leukemias. In this report, we briefly review those contributions from the CALGB LCSC that have had, or are likely to have in the future, a major effect on how we currently manage leukemia and outline directions of ongoing and future research conducted by the CALGB LCSC.

The Cancer and Leukemia Group B (CALGB) Leukemia Correlative Science Committee (LCSC) has been at the cutting edge of correlative science for adult leukemia for almost 25 years. Studies from this Committee have significantly affected on how we diagnose, predict outcome, select treatment, document complete remission (CR), and monitor residual disease in hematologic malignancies, especially acute leukemia. Indeed, work from this Committee is allowing us to increasingly move toward molecularly targeted therapy in adult leukemia.

The Committee as we currently know it began as the Immunology and Cytogenetics Committee in 1982 under the leadership of Clara D. Bloomfield, M.D., who chaired the Committee until 1999. Since then, the Committee has been chaired by Michael A. Caligiuri, M.D. The Committee initially focused on the use of immunophenotyping for diagnosis and prognosis, primarily in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Early on, however, it started to explore the clinical importance of cytogenetics in ALL and AML, and already by 1987 had protocols exploring the clinical use of molecular markers in adult leukemia. In recent years, there has been an increasing emphasis on the clinical application of genetics in chronic lymphocytic leukemia (CLL) in a way similar to that so successfully used in AML and ALL. In this brief review, we discuss those contributions from the CALGB LCSC to using cytogenetics and molecular markers in understanding and treating adult leukemia that have had, or are likely to have in the future, a major effect on how we manage leukemia.

Clinical Utility of Cytogenetics in Adult Leukemia

In June 1984, CALGB opened protocol 8461, a prospective karyotyping study to accompany all front-line acute leukemia treatment studies. Its major goals were to define the pretreatment leukemic karyotypes in adult acute leukemia and investigate their importance for diagnosis and prognosis and thus selecting therapy. This was the first prospective karyotyping study undertaken by any cooperative group conducting clinical trials in the United States and became the model for other groups, such as the Children’s Cancer Group and Southwest Oncology Group, regarding how to approach quality control of their cytogenetics studies. The structure for performing karyotyping by CALGB was important in that, instead of setting up a central cytogenetics laboratory, it allowed each CALGB main member institution to identify a laboratory that could be approved as a CALGB cytogenetics laboratory if it met certain standards. Cytogenetic analysis was thus done at institutional laboratories, but the karyotypes were then sent to a central CALGB cytogenetic data management center for central review by a group of expert cancer cytogeneticists. Although an ambitious undertaking, this has contributed substantially toward improving the quality of leukemia karyotyping in this country. However, recent review of CALGB data shows that central karyotype review is still required. Currently, ~25% of AML and ALL karyotypes require revision at central review (1).
New cytogenetic abnormalities

Discovery of new recurring chromosome aberrations is not only important in the search for genes whose disruption and/or deregulation contribute to leukemogenesis but also allows recognition of clinical and prognostic subgroups in AML and ALL. CALGB was the first to identify isolated trisomy of chromosome 13 (+13) as a recurrent, primary chromosome abnormality in AML and to show that patients with sole +13 fared poorly (2). Another CALGB study reported several novel cytogenetic variants of t(8;21)(q22;q22) in AML, including the first description of a subtle insertion of material from 21q22 into band 8q22, confirmed by spectral karyotyping, which resulted in RUNX1/CBFA2T1 (AML1/ETO) gene fusion (3).

A study of adult ALL with t(9;22)(q34;q11.2) as a primary chromosome aberration not only showed an adverse prognostic effect of the nonrandom secondary aberrations [monosomy 7 or 7p and an extra copy of der(22)(t(9;22))] but also identified six novel recurrent secondary chromosome aberrations: two structural [(t(2;7)(p11;p13) and der(18)(8;18)(q11.2;p11.2)] and four numerical, occurring as the sole secondary change (+X, +2, +17, and +9; ref. 4). The prognostic significance of these aberrations and of der(16)(1;16) (q11~21;q11~21), another abnormality first described by CALGB as a nonrandom secondary rearrangement in t(9;22)-positive ALL (5), has not yet been determined.

CALGB was also the first to report isochromosome 12p [i(12p)], an aberration typical of germ-cell tumors and AML or myelodysplastic syndrome with concurrent or preceding germ-cell tumors, in AML without clinical evidence of germ-cell tumors suggesting that i(12p) does not always indicate neoplastic disease of germ-cell origin (6).

Prognostic significance of cytogenetics

The International Workshops on Chromosomes in Leukemia (IWCL) first convincingly suggested that cytogenetic abnormalities were important independent prognostic factors in adult ALL (7) and AML (8). However, the IWCL ALL study was retrospective and the IWCL AML study included relatively few patients treated with standard intensive chemotherapy. Thus, large prospective trials evaluating the role of pretreatment cytogenetics in the context of modern chemotherapy were needed to definitively ascertain the role of specific cytogenetic findings in predicting outcome of adults with AML and ALL. CALGB has been a leader in establishing the importance of pretreatment karyotype as an independent prognostic factor in adult acute leukemia. The major contributions of CALGB include early publication of large series examining the role of karyotype using contemporary induction regimens in ALL (9), AML (10), older patients with AML (11, 12), and core-binding factor (CBF) AML (13); the first large study of prognostic factors in adult AML with a normal karyotype (14); and several smaller studies exploring prognostic significance of less common cytogenetic abnormalities (15–18).

ALL. The IWCL ALL study included 172 adults and was the first to clearly show the independent prognostic significance of karyotype for predicting outcome (7). In 1999, CALGB published an analysis of the prognostic significance of cytogenetics in 256 consecutive adults with ALL other than Burkitt’s leukemia treated on front-line CALGB ALL trials who had adequate pretreatment karyotypes (9). This study showed that, even with the introduction of current intensive multiagent therapy, karyotype remained the most important prognostic factor for disease-free survival on multivariable analysis. This publication along with large French (19) and British (20) studies confirmed the IWCL data on prognostic significance of karyotype in adult ALL and resulted in the standard use of pretreatment karyotyping for adult ALL. The CALGB study clearly showed that modern chemotherapy alone failed to cure patients with the t(9;22) and rarely cured those with t(4;11). CALGB 8461 resulted in CALGB early recommending all such patients undergo transplantation in first CR and in 1992, a protocol, 9113, using stem cell transplantation (SCT) in these patients. Since 2002, patients with t(9;22) have been treated separately on CALGB 10001 with therapy including imatinib and SCT when feasible.

AML. Although the IWCL study first showed that karyotype was an independent prognostic factor in AML, this study also included children, and only 305 patients received induction therapy that included cytarabine and an anthracycline (8). Several relatively small studies using standard intensive therapy in adults were published subsequently that seemed to confirm that karyotype was the most important prognostic factor in AML (21, 22). However, it was clear that very large studies were required to resolve conflicting data regarding the prognostic implications of specific cytogenetic abnormalities in adult AML. In 2002, CALGB published data on the prognostic significance of prospectively obtained pretreatment karyotypes in 1,213 consecutive adults with de novo AML treated with the same intensive standard induction therapy (10). Patients who achieved CR were randomized or assigned to one of six postinduction therapies, three of which comprised three or four cycles of intermediate-dose (IDAC) or high-dose (HDAC) cytarabine. Extensive data for specific karyotypes were obtained on achievement of CR and, with a median follow-up of 8.3 years, on long-term remission duration and overall survival (OS). Specific karyotypes were classified into three risk groups for achievement of CR, cumulative incidence of relapse, and OS. Cytogenetic abnormalities were not necessarily classified in the same risk group for different outcomes. Thus, an abnormality might be classified as having intermediate risk for achievement of CR but adverse risk for OS. Multivariable analyses found these cytogenetic risk groups to be the most important prognostic factor for achievement of CR and cumulative incidence of relapse. For survival, age entered the model before cytogenetic risk group, although cytogenetic risk group remained highly significant (10). Along with two large Medical Research Council trials (23, 24), this CALGB report conclusively documented the role of pretreatment karyotype as one of the most important prognostic determinants in adult AML.

CBF AML. Patients with t(8;21) and inv(16) are often grouped together in clinical studies because they have a similar, relatively favorable prognosis and because t(8;21) and inv(16) are related at the molecular level through disruption of the subunits of CBF. However, although t(8;21) occurs in 7% and inv(16)/t(16;16) in 8% of de novo AML patients, few studies comprising >100 patients have evaluated the clinical characteristics of t(8;21) and inv(16) AML (25–28), and only one large study had compared the two cytogenetic groups directly (28). Hence, a 2005 CALGB study compared pretreatment features and prognostic factors of...
144 consecutive adults with t(8;21) and 168 with inv(16) (13). This second largest study, and the one with the longest follow-up, concluded that AML with t(8;21) constitutes a distinct clinical entity from AML with inv(16) and thus should be stratified and reported separately. After adjusting for covariates, t(8;21) patients had significantly shorter OS and survival after first relapse. Unexpectedly, race was an important prognostic factor for t(8;21) AML (see below). Importantly, inv(16) patients with secondary +22 were less likely to relapse than those with sole inv(16), a finding also reported by others (28). A novel finding was that among inv(16) patients ages <60 years women were significantly more likely to relapse than men after controlling for other variables (13). Because treatment outcome of CBF AML is still disappointing, a recent CALGB study investigated the prognostic significance of mutations in the KIT gene, potential targets of tyrosine kinase inhibitors, and using multivariable analysis showed their negative prognostic effect on CBF AML with t(8;21) and, for the first time, inv(16) (29).

**Significance of race.** The relationship between race and outcome in adult AML has not been extensively evaluated. In recent studies, CALGB found that there were significantly more African Americans among patients with t(8;21) (30) and t(6;11)(q27;q23) (18, 30) and significantly more Whites among patients with normal karyotypes (30). We found on multivariable analysis that non-Whites with t(8;21) significantly more often failed to achieve CR (odds ratio, 5.7; P = 0.006) and that among non-Whites those with secondary cytogenetic abnormalities other than del(9q) had shorter survival; interestingly, secondary aberrations did not affect outcome of Whites with t(8;21) (13). Molecular differences between Whites and African Americans are currently being evaluated within cytogenetic subgroups and could provide important clues to leukemogenesis.

**AML with normal cytogenetics.** Patients with a normal karyotype constitute the largest subgroup of de novo AML, comprising 40% to 45% of the cases (22). Although improved management of these patients is obviously critical to increasing survival of adult AML, no large study has explored prognostic factors or the effect of specific therapies in this group of patients. Thus, we evaluated these issues in 490 adults ages <60 years (14). Interestingly, we found that splenomegaly was significantly associated with a substantially lower CR rate and that the addition of etoposide to daunorubicin and cytarabine improved the CR rate in patients ages ≤45 years. It will be important to confirm the importance of etoposide in randomized trials. Importantly, the postremission strategies of either four cycles of IDAC or HDAC or one cycle of HDAC and etoposide followed by autologous peripheral blood SCT were associated with improved disease-free survival and reduced relapse compared with therapies including fewer cycles of cytarabine or no transplantation (14). Until this large cytogenetic group of de novo AML is further dissected molecularly (see below), it is important that patients do not receive the latter therapies.

**t(9;11)(p22;q23).** Translocations involving band 11q23 are among the more common chromosome abnormalities in adult de novo AML and involve multiple chromosome partners. The t(9;11)(p22;q23) is the most common 11q23 translocation, occurring in ~2% of patients (22). Due to the infrequency of most 11q23 translocations and the fact that most involve rearrangement of the MLL gene, most investigators had grouped adults with these translocations into the same adverse prognostic group. Following reports that children with t(9;11) had a better prognosis than those with other 11q23 translocations, CALGB compared outcome of adults with de novo AML with t(9;11) with outcome of patients with other 11q23 translocations. We showed for the first time that adults with t(9;11) had a significantly better CR duration, event-free survival, and OS than adults with other 11q23 translocations (31). This study was important because, together with the pediatric studies (32), it showed significant differences in outcome based on the 11q23 translocation partner and that t(9;11) patients should be considered separately from patients with other 11q23 translocations. A subsequent CALGB study showed that although 85% of adults with t(9;11) achieved CR only 40% survived 5 years (10). Thus, the CALGB LCSC is currently evaluating the effect of different therapies in t(9;11) AML and looking at clinical and molecular factors that might identify those patients who require new approaches.

**Utility of cytogenetics for selecting therapy**

Work from the CALGB LCSC has changed the way several cytogenetic subgroups of leukemia are managed. The most important contribution of the Committee has been the demonstration that response to different doses of postinduction cytarabine varies depending on the cytogenetic subtype of AML. At the 1994 American Society of Hematology plenary session (33), CALGB first reported that HDAC was of particular benefit for patients with t(8;21) or inv(16), of some benefit for cytogenetically normal patients, and of no clear benefit in other cytogenetic subgroups (33, 34). The primary, leukemia-associated cytogenetic abnormality is now widely recognized as important for selecting effective therapy, especially in the context of molecularly targeted therapy. The importance of the presence of the t(15;17)(q22;q21)/PML/RARA for responsiveness to all-trans-retinoic acid convinced even the most skeptical therapists that genetic information, not simply morphology, was required. However, the concept that the primary cytogenetic abnormality in AML might influence the responsiveness to the dose of a specific cytotoxic agent was a totally unexpected finding and changed how patients with t(8;21) or inv(16) are managed.

The initial CALGB report was based on a randomized trial of four cycles of postinduction cytarabine given in one of three schedules: HDAC (3 g/m<sup>2</sup> every 12 hours on days 1, 3, and 5), IDAC (400 mg/m<sup>2</sup>/continuous infusion/d for 5 days), or low-dose cytarabine (100 mg/m<sup>2</sup>/continuous infusion/d for 5 days). The study suggested not only that CBF AML patients who achieved CR and were treated with HDAC had longer remissions than patients with other abnormalities but also that as many as 65% of CBF AML patients were cured (34). The UK Medical Research Council AML 10 trial showed that, for patients with CBF AML, transplantation in first CR caused more harm than good owing to the toxicity of the procedure (35). Thus, these two studies together resulted in the use of chemotherapy with HDAC alone for management of CBF AML, and this remains the current standard of care. Consequently, CALGB initiated cytogenetic-based, risk-adapted, postremission intensification in February 1997 in its front-line AML trial 9621 for patients ages ≤60 years (36), and most large cooperative groups now also treat CBF AML patients...
separately from other cytogenetic types of AML and incorporate HDAC in some way.

The cytarabine dose required to cure adults with t(8;21) or inv(16) AML remains unknown. Two subsequent CALGB studies found that three or four cycles of HDAC were significantly better than one cycle (37, 38). CALGB recently confirmed the benefit of three or four cycles of IDAC or HDAC over single cycle of HDAC. It also found that ~70% of patients ages <60 years who achieved a CR and were so treated seemed to be cured (13).

Another CALGB LCSC study that should direct therapy for a specific cytogenetic subtype in AML involved patients with t(6;11)(q27;q23) (18). The only patients surviving beyond 21 months were those receiving allogeneic transplant. Our data suggest that t(6;11) patients, the vast majority of whom are ages <60 years, should receive allogeneic transplant in first CR.

Cytogenetics for documenting CR

Elimination of the Philadelphia chromosome has been an important end point in chronic myelogenous leukemia since the introduction of IFN therapy. However, the use of cytogenetics to document CR has not been routine in acute leukemia. In 2003, an International Working Group proposed a separate category of CR (cytogenetic CR) that includes reversion to a normal karyotype in AML patients with clonal abnormalities at diagnosis (39). This was suggested based primarily on preliminary data from M. D. Anderson Cancer Center in 71 patients (40) and from CALGB in 83 patients studied at various time points in CR (41). Subsequently, CALGB documented the importance of cytogenetic CR in predicting outcome in 118 patients with pretreatment abnormal karyotypes who were studied cytogenetically on the first date of documented morphologic CR (42). Persistence of even one metaphase with an abnormality present pretreatment resulted in significantly inferior cumulative incidence of relapse, disease-free survival, and OS. These results were confirmed by multivariable analyses. Although these data should be corroborated in a larger study, based on this CALGB study, therapy is increasingly being altered for patients who do not achieve cytogenetic CR.

Clinical and Biological Utility of Molecular Markers in Adult Leukemia

CALGB was among the first clinical cooperative trial groups to initiate molecular studies in adult leukemia. Banking of leukemic material was already routine by 1984. As a result, CALGB has made important contributions to both the use of molecular markers in the management of adult leukemia and understanding the biology of the disease and leukemogenesis. Some of these contributions are reviewed below.

Prognostic utility of molecular markers

AML

Normal cytogenetics. Among adults with de novo AML, 40% to 45% present with normal cytogenetics when rigorous criteria, requiring karyotyping ≥20 bone marrow metaphases, are used (10) as is done in all CALGB studies. Unfortunately, some studies consider cases as normal cytogenetically when <20 metaphases or only blood has been studied. Because blood may be cytogenetically normal when marrow is abnormal, which occurred in ~5% of AML patients in the CALGB database whose marrow and blood were studied, this can lead to misclassification and should be discouraged.

CALGB has shown that among cytogenetically normal adults with de novo AML <60 years 82% achieve CR when treated with the addition of etoposide to daunorubicin and cytarabine with or without PSC-833 (14). Among patients achieving CR, 40% to 45% are cured when treated with four cycles of IDAC or HDAC or one cycle of HDAC and etoposide followed by autologous peripheral blood SCT in first CR. The 5-year survival for cytogenetically normal patients treated on CALGB 9621 (36) was 41%, the best outcome obtained to date on any CALGB trial. Obviously, it is important to determine which patients are currently cured and how the remaining 60% should be managed to increase their cure rate. Over the last 8 years, the CALGB LCSC has been a leader in the molecular dissection of karyotypically normal de novo AML and showed that, at least for younger patients, varies substantially depending on the presence or absence of specific molecular genetic alterations.

Partial tandem duplication of the MLL gene. The MLL partial tandem duplication (PTD) was the first molecular marker found to have independent prognostic significance in cytogenetically normal AML. This novel molecular rearrangement was discovered using material from the CALGB Leukemia Tissue Bank (LTB) and represented a new genetic mechanism for leukemogenesis (43). In a collaboration involving the CALGB LCSC and investigators from the University of Helsinki and Roswell Park Cancer Institute, pretreatment samples from 98 AML patients with normal cytogenetics were studied for the MLL PTD. Eleven patients with the MLL PTD had a significantly shorter duration of CR (P = 0.01; median, 7.1 versus 23.2 months) but not OS (44). Subsequent studies of cytogenetically normal AML, including one by CALGB, have confirmed the MLL PTD as an independent adverse prognostic factor for remission duration (45).

Obviously, a major goal of discovery of prognostic molecular markers is to assist in developing improved therapy for subsets of AML patients with normal cytogenetics. Recently, Whitman et al. (46), using material from the CALGB LTB, have suggested that the MLL wild-type allele is silenced in AML blasts harboring the MLL PTD, most likely as a result of epigenetic modifications. Because repression of the MLL wild-type allele is associated with selective sensitivity to cell death, these data suggest that therapy that includes DNA methyltransferase and/or histone deacetylase inhibitors should be explored in patients with MLL PTD.

Mutations of the FLT3 gene. FLT3 mutations are among the most common genetic anomalies in AML patients with normal cytogenetics. These mutations include FLT3 internal tandem duplication (ITD) in as many as 38% of patients. CALGB first showed in cytogenetically normal AML patients that those with FLT3 ITD had an inferior prognosis for both remission duration and survival (47). We also showed that the worst outcome was conferred by FLT3 ITD in the absence of a FLT3 wild-type allele (FLT3ITD/wild-type; ref. 47). Others subsequently confirmed the importance of the FLT3 ITD in cytogenetically normal AML and that the FLT3 mutant/wild-type allele ratio was important (48). In multivariable analyses, FLT3 ITD and MLL PTD have both been independent predictors for remission duration. The poor prognosis of the relatively young adults with normal
cytogenetics and FLT3ITD/−, despite treatment with current dose-intensive regimens, suggests that new treatments, such as therapy with FLT3 tyrosine kinase inhibitors, are needed for these patients. Indeed, using samples from the CALGB LTB, we have shown biological differences between AML blasts with FLT3ITD/−, those with both the FLT3 ITD and the wild-type allele, and those with only the FLT3 wild-type allele and that the first group is most sensitive to AML (54), we hypothesized that amplification and was also detected in cytogenetically normal adults with AML, and preliminary data suggest that allogeneic SCT in first CR might overcome the adverse prognostic effect of high BAALC expression (45, 52).

Overexpression of the BAALC gene. In 2003, CALGB was the first to identify another molecular marker that predicted outcome in karyotypically normal de novo AML (50). In this case, overexpression of a novel gene, BAALC, in pretreatment blood adversely affected remission duration and OS in patients treated on CALGB 9621 (50). The BAALC gene encodes a protein with no homology to any known proteins or functional domains and was discovered using material from the CALGB LTB (51). Other groups have since confirmed the adverse prognostic significance of high BAALC expression in blood of cytogenetically normal adults with AML, and preliminary data suggest that allogeneic SCT in first CR might overcome the adverse prognostic effect of high BAALC expression (45, 52).

Overexpression of the ERG gene. In 2005, CALGB identified yet another molecular marker, ERG, whose overexpression predicts adverse outcome in karyotypically normal de novo AML (53). In part using material from the CALGB LTB, ERG was found to be frequently overexpressed in AML patients with prognostically unfavorable complex karyotypes that contained cryptic amplification of chromosome 21 (54). Because ERG overexpression was not always associated with genomic amplification and was also detected in cytogenetically normal AML (54), we hypothesized that ERG overexpression might adversely affect prognosis. Indeed, we found that high ERG expression in pretreatment blood adversely affected remission duration and OS in karyotypically normal de novo AML patients ages <60 years (53). In multivariable models, high ERG expression and FLT3 ITD independently predicted worse remission duration and OS. When analysis was restricted to the more favorable subset of patients expressing a FLT3 wild-type allele, high ERG expression and MLL PTD both affected remission duration (53). These very recent findings await corroboration but may be important for both understanding leukemogenesis and developing molecularly targeted therapies for cytogenetically normal AML.

Mutations of RAS in AML. RAS mutations occur in 12% to 27% of AML patients. A CALGB LCSC study showed that de novo AML patients with RAS mutations, compared with both AML patients without RAS mutation and healthy control subjects, have a higher frequency of working in high-risk occupations and having work-related chemical exposure (55). This suggests that activation of RAS may identify an etiologic subgroup of AML caused by occupation and chemical exposure and that disease etiology may be better understood if epidemiologic measures of exposure are integrated with molecular assays of the cancer-related genetic defects.

The CALGB LCSC has also addressed the clinical importance of RAS mutations in AML, showing that the presence of RAS mutations was a significant predictor for favorable survival (56). Other studies, most of which did not include the type of postremission treatment in the clinical outcome analysis, yielded conflicting results, with some suggesting that patients with RAS mutations have a worse (57) or similar (58, 59) clinical outcome than patients with wild-type RAS. A recent CALGB study of 125 AML patients has shown a significant interaction between cytarabine dose and RAS mutational status (60). On multivariable analysis, among patients receiving HDAC, those with RAS mutations had half of the relapse risk of those with wild-type RAS. RAS status had no prognostic significance in patients assigned to receive low-dose cytarabine (60). Although requiring confirmation, this study suggests that AML patients carrying RAS mutations benefit from higher cytarabine doses more than patients with wild-type RAS and should receive consolidation with HDAC.

ALL

BCR/ABL fusion gene. Translocation (9;22), first reported in ALL by Bloomfield et al. (61), is the most frequent primary abnormality in adult ALL. CALGB was among the first, if not the first, to show in a prospective study the prognostic significance of molecular detection of the BCR/ABL fusion gene in adult ALL (62). We found that cytogenetics underestimated the incidence of t(9;22) and that BCR/ABL was present in almost one third of all cases and 50% of cases with B-lineage cell surface markers (62). Moreover, in contrast to earlier reports, most BCR/ABL-positive adults (77%) had the P190 gene subtype, similar to pediatric ALL. As with cytogenetic detection of the t(9;22), Philadelphia chromosome-positive patients identified by molecular methods did poorly with intensive therapy (62).

HOX11 overexpression. Outcome of adult T-ALL is clearly heterogeneous and cytogenetic markers have been of limited help in dissecting this subset. In children, activation of oncogenic transcription factors defines distinct molecular subsets of T-ALL and has prognostic relevance. CALGB, in a collaboration with the Eastern Cooperative Oncology Group, was the first to show that overexpression of HOX11 confers a good outlook for adult T-ALL (63). We have suggested that such patients should not undergo SCT in first remission. A recent large German study has confirmed the independent prognostic significance of HOX11 overexpression in predicting relapse-free survival (64). These data may be important for both understanding leukemogenesis and stratifying therapy for adult T-ALL.

CLL

Molecular markers in chronic lymphocytic leukemia. The CALGB LCSC is playing a major role in defining the effect of specific genetic abnormalities relative to the new treatment paradigm in chronic lymphocytic leukemia, chemoimmunotherapy. We recently were the first to show that high-risk chronic lymphocytic leukemia patients characterized by unmutated IgV(H) mutational status (≥98%) or high-risk interphase cytogenetics, including del(17p) and del(11q), seem to have a shorter progression-free survival and OS when treated with fludarabine and rituximab (65). Larger prospective studies are required to determine the independent influence of IgV(H) mutational status and interphase cytogenetics on treatment outcome. These studies are likely to result in stratification of therapy based on molecular markers in chronic lymphocytic leukemia.

Gene expression profiling in acute leukemia

Gene expression profiling using DNA microarray technology is a powerful tool allowing analysis of expression of thousands
of genes in one experiment. The CALGB LCSC in collaboration with Golub et al. did the first molecular classification of cancer using gene expression profiling. We showed that it was possible to distinguish AML from ALL correctly based on gene expression profiles (66). Subsequent studies have revealed that several cytogenetically and molecularly defined AML subtypes, such as t(15;17)/PML/RARA, t(8;21)/RUNX1/CBFB/T1, and inv(16)/t(16;16)/CBFB/MYH11, display characteristic gene expression signatures (67, 68) that, not surprisingly, correlate with clinical outcome (68). Moreover, novel gene clusters, seemingly not corresponding to cytogenetic aberrations, have been identified and some have had prognostic significance (68).

However, karyotypically normal AML seems to have more heterogeneous gene expression profiles; patients with these karyotypes have been found in more than one gene expression cluster. In one study (67), a relatively small cohort of karyotypically normal patients segregated mainly into two clusters with significantly different survival. This suggested that gene expression profiling could potentially become useful in predicting clinical outcome, but it required verification, especially because cytogenetically normal AML patients in another study (68) segregated into several distinct clusters. CALGB has recently confirmed the prognostic significance of the gene expression signature identified by Bullinger et al. (67) using the Affymetrix U133plus2.0 GeneChips (Affymetrix, Santa Clara, CA; ref. 69). More studies are necessary to assess the role of microarray gene expression profiling in clinical practice and prognostication of cytogenetically normal AML.

### Leukemogenesis and CALGB LTB Studies

Several important advances in understanding the molecular biology of leukemogenesis have come from CALGB LTB studies. Selected contributions are summarized in Table 1 and some are expanded below.

#### Molecular dissection of complex karyotypes

Approximately 10% to 20% of AML patients have complex karyotypes with three or more abnormalities, most of which are unbalanced (70). The distribution of genomic imbalances is nonrandom, but the underlying molecular mechanisms and consequences are largely unknown. Following discovery of a novel, cryptic overrepresentation of 21q in several complex karyotype AML patients, which did not involve amplification of RUNX1 (71), CALGB did a high-resolution array-based comparative genomic hybridization study searching for chromosome 21 genes whose amplification likely contributes to leukemogenesis (54). Comparative genomic hybridization disclosed amplification of two regions harboring the APP gene and the ERG and ETS2 genes, respectively. Microarray gene expression analysis revealed that APP was the most overexpressed gene compared with a control group of cytogenetically normal AML, whereas ERG and ETS2 also ranked among the most highly expressed chromosome 21 genes. Overexpression of APP, a gene not known previously to be involved in AML, occurred even in some patients with cytogenetically normal AML (54). This study highlighted the value of molecularly dissecting leukemic cells with complex karyotypes and led to the CALGB analysis described above of ERG expression in karyotypically normal AML (53).

#### Molecular basis of isolated trisomy

Little is known about the biological significance of isolated trisomies in cancer. Material from the CALGB LTB helped show for the first time that sole trisomy 11 (+11) in AML is consistently associated with rearrangement of MLL (72) and that this rearrangement, a MLL PTD, represented a novel mechanism of leukemogenesis (43, 73). Our finding of the frequent presence of MLL PTD in AML patients with +11 represents the first identification of a specific gene rearrangement associated with recurrent trisomy in human cancer.

### Table 1. Selected studies contributing to our understanding of the molecular basis of leukemogenesis that used material from the CALGB Leukemia Tissue Bank

<table>
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<th>Year</th>
<th>Description</th>
<th>Refs.</th>
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<tr>
<td>1994</td>
<td>First identification of a specific gene rearrangement associated with recurrent trisomy in human cancer (i.e., PTD of the MLL gene associated with +11).</td>
<td>(43, 72)</td>
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<td>1998</td>
<td>Discovery of the molecular mechanism (Alu/Alu-mediated homologous recombination) by which the MLL PTD is generated.</td>
<td>(75)</td>
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<td>1998</td>
<td>The first molecular classification of cancer (acute leukemia) using GeneChip technology, showing class discovery and class prediction.</td>
<td>(66)</td>
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<td>2000</td>
<td>Assessed the molecular, morphologic, cytogenetic, immunophenotypic, and clinical features of adult APL patients with the type V PML/RARA isoform, but no distinguishing features were found.</td>
<td>(76)</td>
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<tr>
<td>2000</td>
<td>First demonstration of nonrandom and tumor type-specific patterns of global aberrant CpG island methylation in cancer.</td>
<td>(77)</td>
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<td>2001</td>
<td>Evidence that BCR/ABL suppresses CAAT/enhancer binding protein-α expression through inhibitory action of a RNA-binding protein, hnRNP E2, in blast crisis of chronic myelogenous leukemia.</td>
<td>(78)</td>
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<td>2001</td>
<td>Demonstration of fundamental biological differences in AML with isolated trisomy 8 and normal cytogenetics using GeneChip expression profiling.</td>
<td>(79)</td>
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<tr>
<td>2001</td>
<td>Demonstration that aberrant CpG island methylation in AML has a prevalence within chromosome 11 loci.</td>
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<td>2001</td>
<td>Identification of BAALC, a human member of a novel mammalian neuroectoderm gene lineage involved in acute leukemia and may be associated with a poor prognosis.</td>
<td>(51)</td>
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<td>2004</td>
<td>Molecular dissection of AML with complex karyotype and abnormal chromosome 21 reveals amplification of APP, ERG, and ETS2 genes.</td>
<td>(54)</td>
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</table>
Material from the CALGB LT5 was also used to show that, in cases with +11, only one chromosome contains the mutated MLL allele (74), whereas the wild-type alleles are silenced by epigenetic modifications (46). Pharmacologic reversal of MLL wild-type expression results in a lower threshold for apoptosis of the myeloid blast, which is further enhanced by targeted silencing of the MLL PTD expression with antisense. Thus, targeted therapy for patients with MLL PTD AML is now being developed.

Conclusion

The CALGB LCSC studies have at times led, and at other times substantially contributed to, progress in diagnosis, prognostication, and treatment of the acute and chronic leukemias. We believe that in the future the cure of these diseases will come from understanding their molecular heterogeneity, confirming the prognostic significance of each defect in large prospective analyses of uniformly treated patients and then collaborating with our colleagues in industry to target such defects with curative intent. These tasks can likely only be done by large national or international cooperative groups. Thus, state-of-the-art tissue procurement, availability of the most advanced molecular analyses, and statistical expertise will be critical to achieving this important goal. The CALGB LCSC will continue to participate in and at times lead these endeavors, collaborating with basic and clinical scientists throughout the world who wish to play an important role in this "team approach." We believe this will ultimately lead to the design of novel, more effective molecularly targeted therapies.

References


83. Balas CD, Murphey SM, Grunstein M, et al. Primary acute myeloid leukemia (AML) cells with the FLT3 internal tandem duplication (ITD) and absence of wild type (WT) FLT3, (ITD/WT) genotype are distinct from AML with either FLT3-WT or FLT3-ITD/WT genotypes [abstract]. Blood 2002;100:89a.


94. Balas CD, Murphey SM, Grunstein M, et al. Primary acute myeloid leukemia (AML) cells with the FLT3 internal tandem duplication (ITD) and absence of wild type (WT) FLT3, (ITD/WT) genotype are distinct from AML with either FLT3-WT or FLT3-ITD/WT genotypes [abstract]. Blood 2002;100:89a.