The Cooperative Group Bulletin Board

Cooperative Group Tissue Banks As Research Resources: The Cancer and Leukemia Group B Tissue Repositories[1]

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The CALGB[3] is a national cooperative cancer clinical trials group supported by the NCI. More than 250 academic medical centers, hospitals, and physician practices enroll patients on CALGB clinical trials in breast cancer, GI cancer, GU cancer, lung cancer, melanoma, leukemia, and lymphoma. For many years, a major goal of CALGB studies has been to understand the biological factors that determine the prognosis or predict response to therapy of various tumor types. The CALGB has made important contributions to identifying cytogenetic subgroups within the heterogeneous diagnosis of AML that have prognostic importance, as well as in describing relationships between c-erbB-2 expression in stage II breast cancer and treatment outcomes associated with the dose intensity of doxorubicin-based therapy. Conducting correlative science studies requires a coordinated system that includes the centralized collection of tumor cells and tissues, storage under controlled conditions, a comprehensive inventory, a process to distribute specimens to investigators and to receive the assay results from research laboratories, and policies to address responsible research, including safeguarding patient confidentiality. Ultimately, the results of the laboratory studies must be linked to and correlated with the clinical outcomes of patients treated on CALGB clinical trials so that statistically valid conclusions can be drawn regarding the relationship between tumor biology and treatment outcome. The CALGB has developed tissue repositories located at The OSU that collect viable leukemia cells, as well as formalin-fixed, paraffin-embedded solid tumor specimens for use by the investigator community. The purpose of this article is to briefly describe these repositories, give examples of the types of research they support, and inform investigators about how to submit proposals for access to specimens from these banks.

The CALGB LTB

The CALGB first received NCI funding to support a LTB in 1996, although the bank had been established four years before that time. Approximately 500 patients with various types of leukemia are enrolled annually on CALGB protocols and contribute specimens to the LTB. Highly skilled health care providers at each CALGB institution are familiar with obtaining informed consent, completing data questionnaires, and shipping specimens. The CALGB database contains information on the type of leukemia or myelodysplastic syndrome, clinical history, cytogenetics, treatment, and clinical outcome on most patients with specimens stored in the CALGB LTB, making it a unique and highly valuable research tool. The LTB is a repository currently comprising >80,000 samples (Fig. 1) obtained from >1,700 patients enrolled on CALGB leukemia treatment protocols and is housed at The OSU Comprehensive Cancer Center. Table 1 shows the distribution of cases by leukemia diagnosis.

After patient informed consent at CALGB member institutions, fresh bone marrow, peripheral blood, and buccal swab samples are obtained at diagnosis and shipped via overnight carrier to the central CALGB LTB procurement lab at OSU, where samples are processed immediately 6 days each week. A unique patient number is generated on receipt of the sample by the CALGB LTB, and all other patient identifiers are then removed. In many instances, follow-up samples obtained at the time of remission and relapse are also obtained. The bone marrow and blood samples are processed under sterile conditions using standard procedures and frozen in liquid nitrogen freezers equipped with an independent environmental monitoring system. Quality control testing shows that 90% viability is maintained in samples on proper thawing. The procurement lab is staffed by a full-time laboratory supervisor, a research assistant, two procurement technicians, and one data entry assistant. The laboratory receives samples Monday through Saturday, including many holidays, provided the carrier is in service. The CALGB LTB is equipped with seven large capacity (24,000 or 38,000 cryovials/freezer) liquid nitrogen freezers with automated fill systems for sample storage at −135°C, as well as −20°C and −80°C freezers for plasma and serum storage. Each freezer or refrigerator in the procurement lab is equipped with a temperature probe that is connected to a REES Environmental Monitoring System (Rees Scientific, Trenton, NJ). This system

Received 1/11/02; revised 1/24/02; accepted 1/27/02.
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[1] Supported in part by USPHS Grant CA31946 (to R. L. S.).
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[3] The abbreviations used are: CALGB, Cancer and Leukemia Group B; NCI, National Cancer Institute; LTB, Leukemia Tissue Bank; OSU, Ohio State University; ITD, internal tandem duplication; AML, acute myeloid leukemia; WT, wild-type; DFS, disease-free survival; OS, overall survival; PCO, Pathology Coordinating Office; CAF, cyclophosphamide, doxorubicin, and 5-fluorouracil; IHC, immunohistochemistry.

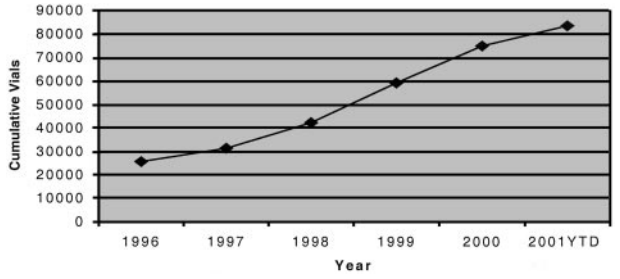

Fig. 1 Acquisition of specimens in the LTB, 1996–2001.

Table 1 Leukemia patients with specimens in the CALGB LTB

Diagnosis	No. of patients with specimens in the LTB (1996–2001)
ALL	337
AML	1066
APL	63
CLL	137
CML	62
MDS	67
TOTAL	1732

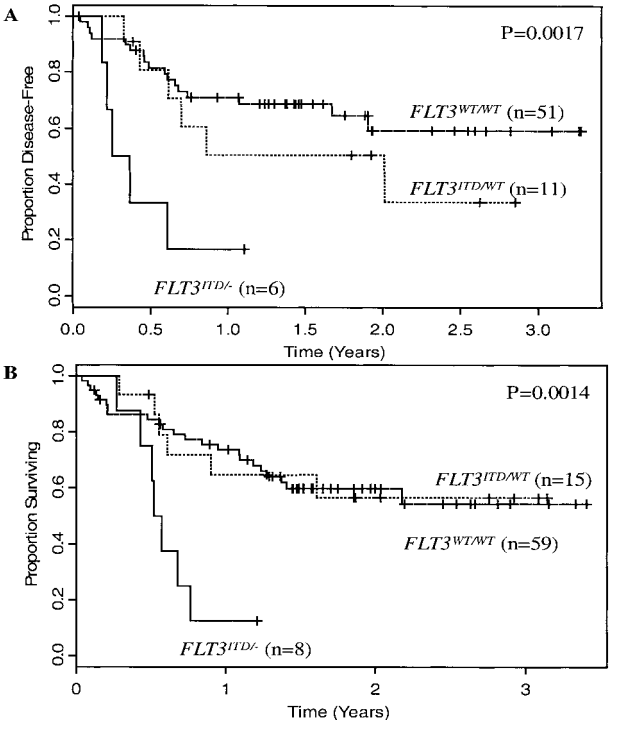

Fig. 2 DFS (*A*) and OS (*B*) of patients with AML according to FLT3 genotype (see Ref. 1 for additional details).

can notify laboratory personnel (via computer, telephone, and pager) of any fluctuation in temperature.

The work that can be accomplished using the CALGB LTB can be illustrated by a recent study characterizing the FLT3 ITD in AML and its correlation with clinical outcome (1). The *FLT3* gene is mutated by an ITD in 20–25% of adults with AML. However, its significance in predicting outcome was not consistent in earlier studies. In part, this was because of the heterogeneity of the AML patient samples used in earlier studies with respect to age, cytogenetics, and treatment regimens. Using material from the CALGB LTB, samples from 82 adults with primary AML, age < 60 years, and normal cytogenetics who received uniform therapy were studied. The *FLT3* ITD was found in 23 (28%) patients. When the 23 *FLT3* ITD+ cases were compared with the 59 cases with WT *FLT3*, DFS was inferior ($P = 0.03$), yet OS was not different ($P = 0.14$). However, 8 (35%) of 23 *FLT3* ITD+ cases also lacked a *FLT3* WT allele ($FLT3^{ITD/-}$) as determined by PCR and loss of heterozygosity. Thus, three genotypic groups were identified: normal $FLT3^{WT/WT}$, heterozygous $FLT3^{ITD/WT}$, and hemizygous $FLT3^{ITD/-}$. DFS and OS were significantly inferior for patients with $FLT3^{IDT/-}$ ($P = 0.0017$ and 0.0014, respectively; Fig. 2, *A* and *B*). Although DFS and OS for $FLT3^{WT/WT}$ and $FLT3^{ITD/WT}$ groups did not differ ($P = 0.32$ and 0.98, respectively), OS of the $FLT3^{ITD/-}$ group was worse than the $FLT3^{WT/WT}$ ($P = 0.0005$) and $FLT3^{ITD/WT}$ ($P = 0.008$) groups. These results have led to a proposal of a model in which $FLT3^{ITD/-}$ represents a dominant positive, gain-of-function mutation providing AML cells with a greater growth advantage compared with cells having the $FLT3^{WT/WT}$ or $FLT3^{ITD/WT}$ genotypes. This CALGB LTB study therefore led to the discovery of the $FLT3^{ITD/-}$ genotype as an adverse prognostic factor in *de novo* AML with normal cytogenetics. A poor prognosis of the relatively young $FLT3^{ITD/-}$ adults (median age, 37 years), despite treatment with current dose-intensive regimens, suggests that new treatment modalities, such as therapy with a FLT3 tyrosine kinase inhibitor, are needed for this group of patients.

Applications for research proposals to use the material in the CALGB LTB may be obtained by contacting the CALGB Central Office [Phone: (773) 702-9171; Fax: (312) 345-0117; E-mail: mkelly1@midway.uchicago.edu] or by visiting the CALGB Web site.[4] Any investigator is eligible to request material from the CALGB LTB. The Director of the CALGB LTB assists the investigator with developing and submitting the proposal, and the CALGB provides biostatistical support to determine the appropriate sample size to complete the study. Proposals are reviewed quarterly by the CALGB LTB Committee, comprising clinicians, scientists, pathologists, and biostatisticians. Because one of the unique and highly valued features of the CALGB LTB is its direct link to cytogenetic and clinical outcome data bases for the majority of cases in the bank, proposals with preliminary data demonstrating feasibility that also plan to link their investigation to the cytogenetics and/or clinical database receive the highest priority. Proposals that request material for projects that have insufficient preliminary data or will not require correlation to cytogenetics or clinical outcome are encouraged first to seek material from other banks that lack the cytogenetics/clinical database. The CALGB does not fund the proposals. Investigators must provide evidence of an adequate source of funding to complete the project outlined in the proposal.

[4] Internet address: http://www.calgb.org.

Table 2 Specimens submitted to the CALGB PCO, 2000–2001

Year	Protocols requiring sample submission	Tissue blocks received	Tissue slides received	Patients with samples submitted
Jan.–Dec., 2000	40	2044	3506	1473
Jan.–Dec., 2001[a]	48	2348	13324	2042

[a] Extrapolated to the end of 2001.

Furthermore, before receiving the material, the investigator must provide documentation of Institutional Review Board approval for research involving the material. On submitting their proposal, investigators must sign an agreement to abide by the CALGB policies and procedures governing data analysis and submission of the work for publication. These policies require that all laboratory clinical correlations be performed by the CALGB statistician assigned to the project and that all publications undergo review by the CALGB headquarters office before submission for publication. Any unused material must be returned to the LTB at the completion of the study.

Table 3 Inventory of specimens in the CALGB PCO (as of January 2002)

Disease	Blocks	Slides	Patients with specimens in PCO
Breast	9492	17784	6009
GI	3597	18931	2033
GU	287	866	175
Lung	387	350	257
Lymphoma	553	2923	386

The CALGB PCO

The PCO for the CALGB is a repository for formalin-fixed, paraffin embedded solid tumor specimens obtained from patients enrolled on CALGB protocols. It is located in the Department of Pathology at The OSU on the OSU Medical Campus at B054 Graves Hall, 333 West 10th Avenue, Columbus, Ohio 43210. The phone and fax numbers are (614) 688-5493 and (614) 292-5618, respectively. This information and more is found at the CALGB PCO Web site.[5] The PCO can also be reached by E-mail at path-calgb@medctr.osu.edu or via the CALGB Web site.[6]

The CALGB PCO is located in ~2200 square feet of space that consists of an office (300 square feet) and laboratory space (1900 square feet). The PCO receives paraffin blocks and pathology reports from the CALGB main member and at-large member institutions and their affiliated hospitals, as well as from other cooperative groups. Paraffin blocks are catalogued and either processed immediately for microtomy or stored vacuum packed at 4°C until needed. Tissue sections that are cut from the paraffin blocks are placed on microscope slides, vacuum packed, and stored at 4°C until they are shipped to an investigator. The PCO ensures that patient specimen blocks are handled with the greatest of care, taking precautions to prevent exhausting a block of all material and retaining a representative histological section from the surface of a cut block in the bank. Both the block and the histological section are available for return within 24 h to the submitting institution when requested. Institutions preferring to submit cut sections rather than blocks are required to adhere to PCO standard operating procedures and to the cutting schema of the scientific protocol to ensure both high quality and adequacy of samples for the planned scientific studies.

During calendar year 2001, the PCO received 2,348 tissue blocks and 13,324 slides from 2,042 cases in 48 active protocols. These numbers compare to 40 active protocols during the period from January through December 2000, with the receipt of 2,044 tissue blocks and 3,506 slides, from 1,473 cases (Table 2). Table 3 summarizes the current inventory of blocks and slides by disease.

Examples of the work supported by the CALGB PCO are studies of the relationship between expression of erbB-2 and treatment outcome after dose intensive doxorubicin-based adjuvant therapy for stage II breast cancer. In CALGB trial 8541, 1572 women with stage II breast cancer were randomly assigned to receive one of three different dosage regimens of adjuvant CAF. The overall study results demonstrated that the low dose regimen produced significantly inferior outcomes compared with the intermediate and high dose regimens, which did not differ from each other (2). Tissue blocks from the primary tumor specimens of 1013 women were collected and analyzed for erbB-2 expression by IHC and differential PCR. Histological analysis of sections from each block confirmed the presence of tumor in 994 cases. A proportional hazards model was used to relate various covariables to DFS and OS. The variables chosen were CAF dose, number of positive lymph nodes, tumor size, menopausal status, c-erbB-2 expression, and CAF dose interaction with c-erbB-2 expression. The latter results are depicted in Fig. 3, *A–D*. c-erbB-2 immunopositivity (as a continuous variable) was an independent prognostic factor for both DFS and OS ($P = 0.004$ and <0.001, respectively). More importantly, the interaction between c-erbB-2 expression and CAF dose was significant for both DFS ($P = 0.001$ by IHC) and OS ($P = <0.001$ by IHC). Patients who were assigned randomly to the dose-intensive treatment arm and whose tumors expressed high levels of c-erbB-2 (≥50% of cells positive) had a longer OS and DFS than similarly treated patients whose tumors expressed low levels of c-erbB-2 (OS = 78 *versus* 65% at 8 years; DFS = 69 *versus* 55% at 8 years; Refs. 3 and 4). These data, since confirmed by others (5–7), suggest that lymph node-positive breast cancer patients with c-erbB-2-positive tumors benefit from treatment with dose-intensive, doxorubicin-based adjuvant therapy; such therapy may not benefit patients with c-erbB-2

[5] Internet address: http://www.pathology.medctr.ohio-state.edu/calgb/.
[6] Internet address: http://www.calgb.org.

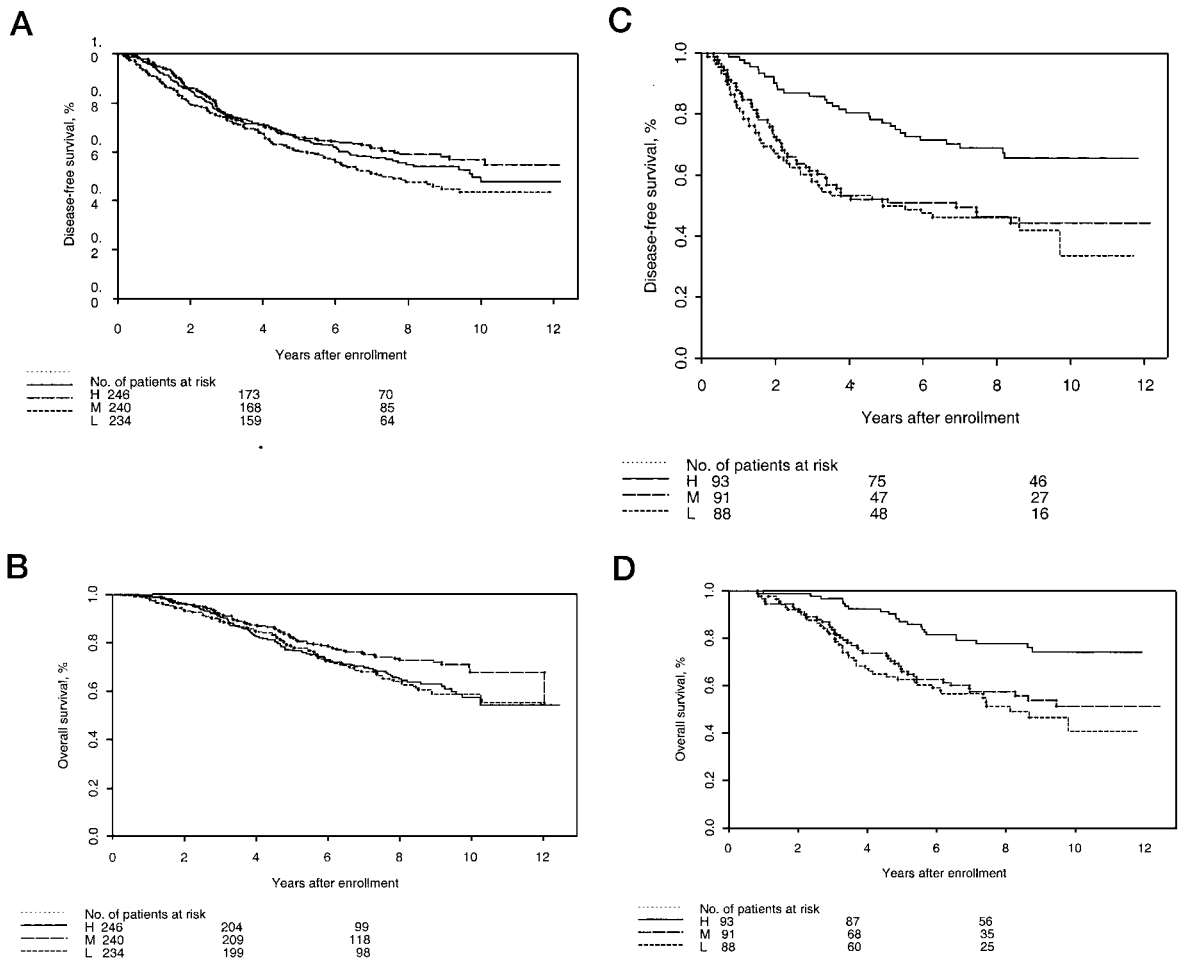

Fig. 3 Interaction of CAF dose (*H*, high; *M*, medium; *L*, low) with c-erbB-2 expression in patients with stage II breast cancer. *A*, DFS for low c-erbB-2 expression ($P = 0.058$); *B*, OS for low c-erbB-2 expression ($P = 0.048$); *C*, DFS for high c-erbB-2 expression ($P < 0.001$); *D*, OS for high c-erbB-2 expression ($P < 0.001$; see Ref. 4 for additional details).

negative tumors; the c-erbB-2 dose interaction might be specific for CAF; and the interaction might be modulated by other molecular abnormalities, such as p53 mutation (4). Ongoing studies are addressing the hypothesis that the beneficial effects of dose-intensive CAF therapy in erbB-2-positive patients are related to co-overexpression of topoisomerase II, the primary target of doxorubicin.

Proposals to use specimens stored at the PCO should be submitted in writing to Dr. Philip W. Kantoff, chair of CALGB Solid Tumor Correlative Science Committee (philip_kantoff@dfci.harvard.edu) or to Lynn Dressler, vice-chair of CALGB Solid Tumor Correlative Science Committee (dressler@med.unc.edu). Proposals should be five to seven pages in length and written in the format shown in Table 4.

Proposals will be reviewed by appropriate members of the CALGB Solid Tumor Correlative Science Committee according to the criteria summarized in Table 5.

Investigators who have proposals approved are expected to abide by the guidelines shown in Table 6 in conducting their research with specimens from the CALGB PCO.

CALGB Tissue Bank General Policies

The CALGB tissue banks were established after approval by the Solid Tumor Correlative Science Committee, Leukemia Correlative Science Committee, or Pathology Committee, as well as the Executive Committee of CALGB, and adhere to the policies and procedures of the CALGB. As a responsible guardian of specimens for the CALGB and the nation, each bank has safeguards in place to address medical legal, confidentiality, and privacy concerns of the patient, the submitting pathologist, or other physician and the institution submitting the specimen. These policies include the following:

(*a*) Samples can only be obtained from patients who are registered to a CALGB study (clinical trial study or a laboratory companion study).

(*b*) The tissue bank director agrees that these samples are under the guardianship of the CALGB and, as such, will procure, store, process, and distribute the samples according to CALGB policies. In addition, if the bank does not comply with CALGB policies, CALGB can and will move the bank and transfer samples to another approved CALGB location.

Table 4 Format for submission of proposals to obtain specimens from the PCO

(*a*) Title of project, investigator, and affiliation
(*b*) Specific aims
(*c*) Background and rationale
(*d*) Methods and technical feasibility
(*e*) Preliminary data
(*f*) Statistical considerations
(*g*) Relevance to CALGB/cooperative group setting
(*h*) CALGB resources required
(*i*) Funding available to complete the proposed study
(*j*) References

Table 5 Review criteria of the CALGB solid tumor correlative sciences committee for access to specimens in the CALGB PCO

(*a*) Will the study move the field forward; is it unique?
(*b*) Does the study require the resources of a cooperative group?
(*c*) Does the investigator have appropriate expertise/preliminary data?
(*d*) Can the work be done in a timely fashion?
(*e*) Will the results of the study impact patient care?
(*f*) Does the investigator have funding to conduct the work?

Table 6 Guidelines for investigators using specimens from the CALGB PCO

(*a*) Interact with CALGB staff as required to fully develop the research proposal;
(*b*) Present progress reports at CALGB meetings;
(*c*) Submit laboratory data to the CALGB Statistical Center for final analysis and clinical correlations;
(*d*) Use specimens only for approved projects and return unused specimens to the bank;
(*e*) Submit manuscripts and abstracts to the CALGB Central Office for review before submission for presentation/publication;
(*f*) Submit Institutional Review Board approval for the proposed research;
(*g*) Submit Conflict of Interest disclosure;
(*h*) Maintain confidentiality of research results unless required for protocol eligibility/randomization.

(c) Samples cannot be distributed to any investigator without prior approval of the scientific proposal from the appropriate CALGB scientific committee and, when necessary, the Executive Committee. Approval of the Cancer Therapy Evaluation Program of the NCI may also be required for some studies.

(*d*) If for any reason a patient/participant in a study decides that they do not want their sample to remain in the bank, their samples will be disposed of appropriately: either destroyed or, in the case of paraffin blocks, returned to the submitting institution.

(*e*) For tissue blocks/sections that have been submitted, quality control slides (H&E-stained sections and touch preps or imprints) will be prepared and remain on file at the CALGB PCO and will be available to the submitting institution for any medical legal need. In the case of whole blocks, any material remaining in the block will be returned to the submitting institution for any medical legal need.

(*f*) Only CALGB members can establish and maintain a bank for CALGB. If the member investigator moves to a non-CALGB institution, the bank of samples will be transferred to an appropriate CALGB investigator who will then take over the banking responsibilities of those samples.

(*g*) All samples collected, processed, banked, and distributed will be managed using the customized LabTrak database developed by the CALGB Statistical Center.

(*h*) To protect patient confidentiality and privacy, samples distributed to investigators do not contain any identifiable patient information, only the unique CALGB sample numbers are contained on the label of the sample to be stored and/or distributed. The sample numbers are generated by the CALGB Statistical Center. Only select approved CALGB personnel in the CALGB Statistical Center have the ability to match the sample number with clinical outcome information.

(*i*) Individual results/data from any correlative science study are not disclosed to the patient/participant or physician unless the results are necessary for eligibility/randomization on a CALGB clinical trial. In this way, clinical decisions/patient management will not be made based on results from CALGB correlative science studies. Aggregate data in the form of abstracts or manuscripts will be available to the patient or physician on request.

(*j*) Each CALGB tissue bank is designed according to the repository guardian model. In this model, as the CALGB guardian for the tissues, the repository provides the link between the patient, their sample, and the submitting institution's clinicians and/or pathologists, ensuring that appropriate policies and procedures are in place to address patient privacy, confidentiality, and medical legal concerns. The guardian also provides the link between the sample used in a laboratory study and the CALGB investigator. These links are monitored by the CALGB Statistical Center and Central Office.

(*k*) Collection of specimens for an individual study, not occurring at a main repository, will follow the same policy and procedures as established for repositories (*a–j* above). Remaining samples/aliquots not used in the study will be sent to the appropriate CALGB repository on completion of the study. Investigators collecting such specimens must agree to these policies and procedures in writing before sample collection. (This includes frozen specimens, extracted DNA, RNA, unstained sections, etc.).

(*l*) Oversight for appropriate compliance for repositories, main and individual, will be a function of the appropriate scientific committee of CALGB and the CALGB Group Chair.

The CALGB, as well as the other NCI-sponsored cooperative groups, maintain repositories of tissues collected from patients who are uniformly staged and treated and for whom long-term clinical outcomes are available. As such, these repositories are of enormous value in supporting correlative science studies that relate molecular markers to clinical outcomes for purposes of predicting the prognosis of patients and the likelihood of response to specific therapies. Although the cooperative group tissue banks are national resources, access to specimens must be carefully controlled so that these unique and well-characterized specimens are used in support of only the most meritorious research that has a high probability of producing definitive results. Investigators with an interest in obtaining CALGB specimens are encouraged to contact the representatives of the appropriate tissue repository described herein.

References

1. Whitman, S. P., Archer, K. J., Feng, L., Baldus, C., Becknell, B., Carlson, B. D., Carroll, A. J., Mrozek, K., Vardiman, J. W., George, S. L., Kolitz, J. E., Larson, R. A., Bloomfield, C. D., and Caligiuri, M. A. Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a Cancer and Leukemia Group B Study. Cancer Res., *61:* 7233–7239, 2001.

2. Wood, W. C., Budman, D. R., Korzun, A. H., Cooper, M. R., Younger, J., Hart, R. D., Moore, A., Ellerton, J. A., Norton, L., and Ferree, C. R. Dose and dose intensity of adjuvant chemotherapy for stage II, node positive breast carcinoma. N. Engl. J. Med., *330:* 1253–1259, 1994.

3. Muss, H. B., Thor, A. D., Berry, D. A., Kute, T., Liu, E. T., Koerner, F., Cirrincione, C. T., Budman, D. R., Wood, W. C., Barcos, M., and Henderson, I. c-erbB-2 expression and response to adjuvant therapy in women with node positive early breast cancer. N. Engl. J. Med., *330:* 1260–1266, 1994.

4. Thor, A. D., Berry, D. A., Budman, D. R., Muss, H. B., Kute, T., Henderson, I. C., Barcos, M., Cirrincione, C., Edgerton, S., Allred, C., Norton, L., and Liu, E. T. erbB-2, p53 and efficacy of adjuvant therapy in lymph node positive breast cancer. J. Natl. Cancer Inst. (Bethesda), *90:* 1346–1360, 1998.

5. Paik, S., Bryant, J. B., Park, C., Fisher, B., Tan-Chiu, E., Hyams, D., Fisher, E. R., Lippman, M. E., Wickerham, D. L., and Wolmark, N. erbB-2 and response to doxorubicin in patients with axillary lymph node positive, hormone receptor negative breast cancer. J. Natl. Cancer Inst. (Bethesda), *90:* 1361–1370, 1998.

6. Paik, S., Bryant, J., Tan-Chiu, E., Yothers, G., Park, C., Wickerham, D. L., and Wolmark, N. HER2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-15. J. Natl. Cancer Inst. (Bethesda), *92:* 1991–1998, 2000.

7. Ravdin, P., Green, S., Albain, K., Boucher, V., Ingle, J., Pritchard, K., Shepard, L., Davidson, N., Hayes, D., Clark, G., Martino, S., and Osborne, C. Initial report of the SWOG biological correlative study of c-erbB-2 expression as a predictor of outcome in a trial comparing adjuvant CAF T with tamoxifen alone. Proc. Am. Soc. Clin. Oncol. Annu. Meet., *17:* 97a, 1998.