Reanalysis of Cancer Drugs: Old Drugs, New Tricks

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INTRODUCTION

Most cancer chemotherapy protocols in current use have been established empirically, and combination therapies have generally been designed based on single-agent activity and nonoverlapping toxicities. However, advances in our understanding of the molecular pathophysiology of human cancers indicate that certain molecular features of neoplastic cells can be associated with their sensitivity to chemotherapeutic agents and therefore can be used as the basis for rational drug selection and therapy design. In addition, the ability to discover markers that predict responsiveness to therapy is possible today as never before. Specimens (fixed tissues, immortalized cells, frozen specimens, body fluids, and so forth) obtained 5–10 years before the clinical outcome is known represent a major reservoir of information. Exploiting this resource can shorten the time necessary for retrospective trials and can then be used empirically when prospective trials are mounted. Furthermore, prognostic and predictive measures can be determined with microarrays (1).

Identifying potential responders is critical to successful drug treatment and is potentially a major use of biomarkers. Such biomarker-based strategies can be key to the development and approval of new therapies. For instance, Koo et al. (2, 3) have observed a striking correlation between activating ras mutations in human tumor cells and enhanced sensitivity to deoxycytidine analogs as well as to topoisomerase (topo) II inhibitors. Compared with tumor cells with wild-type ras alleles, human tumor cell lines harboring or transformed with activated ras oncogenes display significantly enhanced cytotoxic sensitivity to deoxycytidine analogs, including 1-beta-D-arabino-furanosylcytosine (cytarabine) and gemcitabine. Activated ras oncogenes similarly sensitize human tumor cells to topo II inhibitors and more prominently sensitize human tumor cells to epipodophyllotoxin-derived inhibitors such as etoposide and teniposide. Furthermore, these findings are supported by retrospective studies in patients with acute myeloid leukemia (AML) showing that the presence of ras oncogenes in leukemic cells is associated with increased complete remission rate, longer complete remission duration, and improved overall survival of the patients in response to treatment with cytarabine or the combination of cytarabine and a topo II inhibitor (4, 5). These results indicate that the ras oncogene plays a determinant role in rendering tumor cells sensitive to deoxycytidine analogs and topo II inhibitors and may provide a rationale for the design of potentially synergistic combination therapy (deoxycytidine analog + topo II inhibitor) for other cancers bearing frequent ras mutations. In Wilm’s tumor, the most common pediatric cancer of the urinary tract, previous studies have identified topo II alpha overexpression; the highly effective chemotherapeutic regimens are mainly constituted with topo II inhibitors (6–8). These results may provide a molecular basis for the success of topo II inhibitor-based treatment of Wilm’s tumors and indicate that topo II overexpression is a biomarker for the choice of topo II inhibitor treatment.

Strategies for selecting those most likely to respond to therapy have already proven beneficial to patients. For example, only patients with breast cancer expressing Her-2/neu respond to trastuzumab (Herceptin). Without identification of the susceptible subpopulation, Herceptin would not have been made available for treatment of this poor-prognosis breast cancer (9). The findings of Staedt (10) that gene expression profiles can be used prospectively to define lymphomas that respond to standard chemotherapy (Fig. 1) reaffirm that the thesis proposed herein has promise and that other examples of this utility will derive from the proposed analyses.

This article proposes to explore the possibilities of analyzing (reanalysis) chemotherapeutic drugs and protocols in practice through conventional and newer molecular and/or imaging technologies to discover surrogate end point or other biomarkers predicting and measuring effectiveness of the therapies. These analyses should first be performed through clinical trials using approved drugs and established protocols. The studies can be prospectively designed or developed from retrospective studies, where appropriate patient material is available. The purpose of these studies will be severalfold: (a) first, to identify potential surrogate end point and other biomarkers markers that will be informative of patient outcome and response; and (b) second, to identify when a specific protocol should be used (e.g., as indicated by biomarkers and/or gene expression profiles). As candidate biomarker(s) are identified, they should be verified or validated against currently used standards or biomarkers. Efficacy in diagnosis, prognosis, or molecular classification for treatment must be demonstrated.

Whereas the thrust of this paper is on evaluation of responses to approved cytotoxic therapies, other types of drugs and protocols should also be evaluated. It would be particularly beneficial to validate biomarkers of response to the newer
molecular therapies that do not produce dramatic tumor shrinkage. Interestingly, modulation of expression of the molecular target might not always be a good predictor of response. For example, both epidermal growth factor receptor (EGFR)- and Her-2-overexpressing tumors are sensitive to the EGFR-selective tyrosine kinase inhibitor gefitinib (Iressa), and sensitivity to this agent is correlated with its ability to down-regulate Akt. However, EGFR-overexpressing MDA-468 cells, which lack PTEN function, are resistant to Iressa, and Iressa is unable to down-regulate Akt activity in these cells. This resistance to Iressa is attributable to EGFR-independent constitutive Akt activation caused by PTEN loss. Reconstitution of PTEN function through tetracycline-inducible expression restores Iressa sensitivitvity and reestablishes EGFR-stimulated Akt signaling (11).

Gene or protein expression patterns such as these could be identified in other settings and with other drugs. Just as for the analysis of cytotoxic drugs, patients might be evaluated after a short treatment period with molecularly targeted agents; those who did not show the expression pattern could be spared further, possibly futile treatment.

In the following sections, techniques and material sources for assessing and developing new biomarkers are delineated. Sample clinical settings for the conduct of research to identify and validate prognostic and predictive (drug effect and clinical outcome) markers are discussed. A separate section on AML provides data from completed and ongoing clinical trials as a

Fig. 1 Examples of molecularly and clinically distinct subgroups of lymphoma (A) and leukemia (B). A shows the levels of expression of 57 genes that distinguish 3 subgroups of diffuse large B-cell lymphoma (84): germinal center B-cell-like lymphoma (orange); type 3 lymphoma (purple); and activated B-cell-like lymphoma (blue). The Kaplan-Meier curve shows that overall survival differs among the subgroups after chemotherapy. B shows 39 genes that are differentially expressed in 2 subgroups of B-cell chronic lymphocytic leukemia (85): one with unmutated (wild-type) immunoglobulin genes (purple); and one with somatically mutated immunoglobulin genes (blue). The Kaplan-Meier curve shows that the two subgroups differ with respect to the time to initial treatment after diagnosis. (Copyright © 2003 Massachusetts Medical Society. All rights reserved. Reprinted with permission.)
DEVELOPMENT OF BIOMARKERS

POTENTIAL TECHNOLOGIES FOR DEVELOPMENT OF BIOMARKERS

Technologies that could be used to develop biological markers to predict drug response or clinical outcomes for a cancer chemotherapeutic agent include the following: immunologically based assays for a given protein, peptide, or glycoprotein; PCR assays for quantitation of the expression of a specific gene product; genomic analysis; gene expression profiling to characterize the transcription level of multiple genes; proteomics; DNA methylation patterns; detection of circulating cancer cells; and functional imaging. Each of these is described below.

Table 1 lists examples of data resources on potential biological markers that may be identified with these technologies.

**Immunological and PCR-Based Assays**

Conventional methods for detection of tumor markers in body fluids involving isolation of the marker (usually a protein or glycoprotein), developing a specific antibody to the marker, and developing an immunologically based assay using the antibody, such as an ELISA, will continue to be useful in evaluating biomarkers. ELISA is the standard assay for detection of well-known serum markers such as carcinoembryonic antigen, human chorionic gonadotrophin, α-fetoprotein, CA-125, and prostate-specific antigen.

More recently, the development of the PCR technique has allowed determination of transcription levels of the above serum markers as well as of additional markers such as onecogenes in circulating cancer cells, serum, urine, and fecal samples.

**Genomic Analysis**

Comparative genomic hybridization and spectral karyotyping are two techniques used to measure tumor-associated chromosomal alterations. In comparative genomic hybridization, tumor DNA and normal reference DNA with different fluorescent labels are hybridized simultaneously to normal chromosome spreads. Regions of gain or loss of DNA sequences, such as deletions, duplications, or amplifications, are seen as changes in the ratio of the intensities of the two fluorophores along the target chromosomes. Spectral karyotyping allows visualization of all 23 pairs of human chromosomes by labeling each pair with a different fluorescent color.
Gene Expression Profiling

The Human Genome Project has revealed detailed information about the base sequence of human genes. This has allowed the development of DNA microarrays that enable the simultaneous detection of thousands of human genes displayed on a matrix as small as a slide coverslip. Two different kinds of target DNA sequences are widely used: (a) cDNA copies derived from a mRNA coding for a specific gene transcript; and (b) oligonucleotide sequences representing highly specific segments of the target genes. Data derived in this way can be used to systematically search for single genes whose over- or under-expression may be related to prognosis, disease progression, or drug response. Alternatively, an entire set of expressed genes can be used to subclassify tumor types, tumor stage, and/or tumor aggressiveness (17).

In some tumor types, a minimal number of genes can be used to classify cancers. For example, a “lymphochip” representing a subset of genes expressed in B-lymphocytes can be used to characterize distinct types of diffuse large B-cell lymphomas that vary in aggressiveness and predict patient survival (18). Similarly, a gene expression profile has been identified that predicts survival of patients with breast cancer (19).

Proteomics

Proteomics can be defined as the study of the protein expression profile of a tissue or specific cell type to define a set of functions. The term “proteome,” coined in 1994 to provide an analogy to the concept of the genome, is used to define the complete set of proteins that is expressed by a cell, tissue, or whole organism. In the context of cancer, the term is used to describe a protein expression profile that reflects the phenotype of a malignant cell that differentiates it from normal cells of the tissue in which the tumor developed.

Several techniques are available to determine protein expression profiles. These include the classic technique of two-dimensional gel electrophoresis; selective adsorption techniques to fractionate proteins based on size, hydrophobicity, ligand binding, and so forth; and mass spectrometer analysis (e.g., matrix-assisted laser desorption ionization time-of-flight and liquid chromatography tandem mass spectrometry). These techniques generate large data sets that require detailed bioinformatics analysis. Theoretically, 100,000 or more proteins may be expressed in a cell, if one considers all of the posttranslational modifications that can occur in the protein products of ≈30,000 human genes.

Although characterization of the proteome that defines a cancer appears to be an overwhelming task, the determination of protein expression patterns based on pattern recognition of serum proteins has been used to diagnose ovarian cancer, even though the specific protein(s) present in each peak of the profile may not be known (20). It is hoped that algorithms based on assessments of protein patterns for other cancers can also be established. This could provide a way to use proteomics for cancer diagnosis, prognosis, and drug responsiveness.

DNA Methylation Patterns

The methylation status of DNA in a cell type provides an indication of genome function. The sites of DNA methylation generally occur on CpG-rich base sequences, the so-called CpG islands. CpG islands are found in promoter regions of about half of all cellular genes, and intense methylation of these sequences is associated with gene silencing (21). Aberrant methylation of CpG islands in cancer tissue has been associated with inactivation of tumor suppressor genes (22). Aberrantly methylated DNA can be detected in body fluids such as serum, sputum, or breast ductal lavage from patients with lung (23, 24), liver (25), or breast (26) cancers.

Detection of Circulating Cancer Cells

Epithelial cells are not normally found in blood, and their detection usually indicates the presence of a pathological state, frequently cancer. Circulating epithelial cells can be isolated from plasma, even against a huge background of blood cells, because they bear specific cell surface markers, called cytokera- rins, not present on hematological cell types. Changes in circulating carcinoma cells have been found to correlate with disease status and drug response in patients with prostate (27) or breast (28) cancer, suggesting their use as biomarkers for drug response and disease progression.

Detection of Circulating Differentiated Endothelial Cells

Viable and apoptotic circulating endothelial cell levels in peripheral blood may serve as a biomarker for tumor angiogenesis, and their detection and quantitation may reflect the effects of antiangiogenic therapy (29). Circulating endothelial cell levels have been shown to rise significantly in the peripheral blood of untreated lymphoma and breast cancer patients, returning to healthy control levels in patients who achieve complete remission after chemotherapy (30).

Detection of Circulating Endothelial Progenitor Cells

In addition to fully differentiated endothelial cells, immature circulating endothelial progenitor cells derived from bone marrow also exhibit potential as a surrogate marker for tumor angiogenesis (31, 32). Although prognostic and therapeutic value has not been demonstrated, accumulating evidence in both mouse tumor models (33, 34) and cancer patients (30) suggests that malignant transformation is associated with increased numbers of circulating endothelial progenitor cells.

Imaging Technologies

Imaging technologies use energy-tissue interactions to noninvasively visualize internal tissues (35–37). Imaging modalities most commonly used in clinical settings include magnetic resonance imaging, X-ray computed tomography, positron emission tomography (PET), single photon emission computed tomography, and contrast and Doppler ultrasound. In the past, in vivo imaging methods revealed gross anatomy; diseases or treatment effects were mostly detected as anatomical structural abnormalities (structural or conventional imaging). Higher resolution detection and the use of imaging agents have enabled resolution on a finer scale and the evaluation of physiological parameters in live tissue (functional imaging). For drug development and evaluation, the main advantages of high-resolution imaging over other traditional biomarkers (such as those requiring tissue sampling, excision, and fluid analysis) are the direct
visualization of disease processes and of tumor heterogeneity, the ability to quantitate changes over time, and the noninvasive nature of the tests. Optical technologies under development using fluorescence and bioluminescence may prove particularly useful in early drug development.

More recently, imaging of specific drug and drug target molecules (molecular imaging) has evolved as a subset of functional imaging. Molecular imaging can be used to directly follow the path of an administered drug (e.g., the distribution and target binding of a labeled drug) or to monitor the drug’s target (e.g., detection of receptor expression and modulation of downstream targets). Nuclear techniques, particularly quantitative PET imaging, are being used clinically for such evaluations. For example, many drugs can be labeled with the positron emitters $^{11}$C or $^{18}$F, and tissue distribution of these labeled drugs can be followed by PET imaging. Another PET technique is evaluation of target binding inhibition of a PET radioligand by unlabeled drug. Thus, molecular imaging techniques are particularly promising for drug distribution and pharmacokinetics and, when used for this purpose, may be able to explain responders and nonresponders to a given drug.

Imaging (both anatomical and functional) can also play a key role in measuring response to therapy. The functional measurement of key biological parameters of neoplastic disease is possible: for example, angiogenesis and vascular responsiveness (using magnetic resonance imaging and ultrasound); cellular proliferation and apoptosis (e.g., 2-$^{18}$F-2-deoxy-D-glucose-PET [FDG-PET]); cell proliferation and apoptosis (e.g., FLT and $^{99m}$Tc-annexin imaging); and resistance to chemotherapy ($^{99m}$Tc-sestamibi imaging). Innovations in imaging devices allowing simultaneous combined modality measurements (e.g., PET/computed tomography or PET/magnetic resonance imaging) have great promise for increasing the utility of imaging in measuring the response to therapy.

Two examples of functional/molecular imaging probes applicable to cancer drug development are FDG-PET and $^{99m}$Tc-annexin. FDG-PET already is well established for use in the diagnosis and management of melanoma and lymphoma, as well as cancers of the lung, colon, breast, thyroid, esophagus, and head and neck. The apoptosis imaging probe $^{99m}$Tc-annexin V has recently shown promise in predicting tumor response to apoptosis-inducing chemotherapeutics in a Phase I study (38).

**Material Sources for New Technology Assays**

The techniques described above often have specific requirements regarding the material source. Particularly relevant material sources and their applications are described in the following paragraphs.

**Fresh Tissues.** This is the ideal source of material for all analyses described in this article and is required for certain types of analyses, particularly microarray-based gene expression profiling. With the introduction of laser capture microdissection (39), it is feasible to isolate single cell types and separate normal tissues from tumor tissues to carry out functional genomic and/or proteomic analyses. Primary cell culture can also be established for cell-based assays and surrogate biomarker analyses.

**Fixed Tissues.** Fixed tissues are commonly used for immunohistochemistry to detect biomarker expression. Tissue arrays can be developed for biomarker comparisons between patients or between pre- and posttreatment within patients, where appropriate samples are available. PCR-amplifiable-quality DNA and mass spectrometer-quality protein may be obtained from fixed tissues, but this material is not proven to be easily applicable for RNA-based analyses.

**Body Fluids (Serum, Plasma, Urine, Pleural Effusion, Ascites) and Feces.** These less invasive material sources can be used in analyses of DNA, proteins, and cancer cells from a variety of tissues [e.g., isolated from bronchial or peritoneal washes (20, 40)].

**Circulating Cancer Cells/Endothelial Cells.** For example, leukemic cells can be isolated directly from body fluids. Methods to isolate circulating carcinoma cells from plasma are also available for a variety of tumors (27, 28). Measurement of circulating cancer and endothelial cells is described above.

**STRATEGIES FOR NEW BIOMARKER EVALUATION IN CANCER CLINICAL TRIAL AND TREATMENT SETTINGS**

The measurement of a candidate biomarker pre- and posttreatment provides the best evidence for adequate evaluation of the biomarker and for establishing correlation with clinical outcomes. One promising clinical setting where such measurements is possible is cancers in which serum biomarkers or anatomical images are now used routinely to monitor patient response to therapy. A second setting is neoadjuvant therapy, where tissue samples or images are available pretreatment and after treatment, when definitive surgical (or radiological) ablation of the cancer occurs. A third is late-stage disease, in which pre- and posttreatment pathology or imaging is often available. Strategies for evaluation of biomarkers in these settings are outlined in the following paragraphs.

**Settings Where Tissue Biopsy or Pathology Is Not Available: Serum Biomarkers and Imaging Modalities**

Certain serum biomarkers are now an important aspect of diagnosis, prognosis, and posttreatment or postsurgical follow-up, even in solid tumors (e.g., CA-125 in ovarian cancer). For example, in testicular cancer, $\alpha$-fetoprotein, human chorionic gonadotrophin, and lactate dehydrogenase may detect tumors too small to be seen on physical examination and are monitored monthly for 1 year after surgical treatment (41, 42). These standard biomarkers, together with computed tomography parameters, can be used to investigate whether the presence or expression of specific new biomarkers is predictive of, or correlated with, clinical response to the standard therapy as well as disease progression. Although therapy is successful in many patients, a particular interest in testicular cancer is to identify those subgroups of patients less likely to respond to therapy and/or to survive. In support of the approach, a recent study of three additional biomarkers (Ki-67, apoptosis, and p53) used in combination with standard serum biomarkers could identify those with better survival among nonseminomatous testicular cancer patients (43).
Neoadjuvant Treatment

Because validating samples are available both pre- and posttreatment, patients undergoing neoadjuvant treatment provide a setting in which to evaluate whether the expression of specific biomarkers is predictive of or correlated with responses to chemotherapy. For instance, breast cancer often requires a multimodality approach to treatment. Over the past 30 years, the use of neoadjuvant chemotherapy has been increasing. Preoperative chemotherapy may decrease the spread of metastatic disease and may enable breast conservation therapy in patients initially deemed ineligible because of the large size of their tumors (44, 45). However, the success of this approach is still under evaluation (44, 46); a recent Phase II study found that neoadjuvant docetaxel treatment was associated with a 68% clinical response rate in stage II–III primary breast cancer patients (47). Although it is clear that chemotherapy given in the adjuvant setting after surgery does prolong patients’ overall and disease-free survival, it has not yet been proven that neoadjuvant chemotherapy increases survival. A potential trial design would include characterization and correlation of biomarker expression and response in biopsies and/or surgical specimens before and after neoadjuvant treatment with taxanes, as well as correlation with clinical response measured by pathology, imaging, clinical signs of progression, and survival. This approach is exemplified by one recent study of the gene signature associated with chemosensitivity in 24 patients; gene expression profiling of biopsy samples before neoadjuvant docetaxel identified a differential pattern of expression of 92 genes (of a total of 12,625 genes studied) that correlated with response (48). The gene expression signature exhibited a 93% and 83% positive and negative predictive value, respectively.

Advanced Disease: Response to Chemotherapy

Lung cancer is the leading cause of cancer-related mortality (49), and the basis of chemotherapeutic efficacy in lung cancer is poorly understood. There is a clear opportunity in the course of standard therapy for late-stage lung cancer to evaluate whether the presence or expression of specific biomarkers in biopsy and/or surgical specimens is predictive of or correlated with clinical response. Despite poor overall survival, a high percentage of patients with small cell lung cancer respond to therapy with increased median survival. Because of the short course of the disease and the availability of pre- and posttreatment imaging data (together with pretreatment and occasionally posttreatment pathology specimens), small cell lung cancer provides a unique setting for trials aimed at understanding individual patient response to standard therapy. The anticipated trial would compare biomarker presence and expression before and after therapy and would correlate biomarker presence and response with clinical responses measured by pathology, imaging, clinical progression, and survival. A similar approach can be used in non-small cell lung cancer, which responds poorly to standard therapy but represents about 80% of lung cancer cases. It is already known that the presence of the ErbB-2 or K-ras mutations confers negative prognostic significance; the prognostic potential of other markers (such as CYFRA 21-1, nitric oxide synthase, carcinoembryonic antigen, and CA-125) is also being explored. For example, a recent study used carcinoembryonic antigen and CYFRA 21-1 to identify a high-risk sub-

group of stage I patients, in whom aggressive adjuvant chemotherapy may be appropriate (50).

CASE STUDY: TREATMENT OF AML WITH CYTARABINE/TOPO II INHIBITOR COMBINATION THERAPY

In this section, the characterization of prognostic and treatment effect biomarkers in AML is reviewed, and strategies for further research are discussed. A potential trial design is presented in Fig. 2. A complex disease in which sensitivity to standard antileukemic chemotherapy is highly variable, AML is well suited to the discovery and study of biomarkers in all aspects of cancer drug development and therapy (patient selection and prognosis, as well as predicting therapeutic effectiveness). Such effort is expected to significantly impact patient management, especially with regard to stratification of patients within and among risk groups (e.g., to determine patient suitability for aggressive postremission therapy). Considerable ongoing effort has sought to characterize prognostic and predictive factors in AML, ranging from cytogenetic abnormalities to specific gene mutations. In some cases, the development and testing of new therapies have been stimulated by these findings. The promise of this general approach has already been shown in other leukemias; the Philadelphia chromosome, a t(9;22) translocation found in 95% of chronic myeloid leukemia cases and in some acute lymphoblastic leukemia patients, is of diagnostic and prognostic utility. Study of this translocation led to discovery of the BCR-ABL fusion protein as the critical initiating event in chronic myeloid leukemia, which in turn enabled the development of molecular tests for this biomarker that can sensitively detect minimal residual disease, even when standard hematological parameters appear normal. The subsequent development of imatinib mesylate (Gleevec), a small molecule inhibitor of the BCR-ABL tyrosine kinase, has transformed treatment paradigms in patients with chronic myeloid leukemia (51).

AML is a heterogenous disease that is composed of eight different morphological subtypes (including acute myeloblastic leukemia, acute promyelocytic leukemia, acute monocytic leukemia, acute myelomonocytic leukemia, erythroleukemia, and acute megakaryoblastic leukemia). There is also significant heterogeneity in the genetic defects that lead to AML. Cytogenetic abnormalities, regardless of other risk factors including age, are one of the most significant determinants of outcome and can guide risk-adapted treatment strategies (52, 53). For example, chromosome 11q23 abnormalities leading to MLL gene rearrangements confer highly unfavorable prognosis (54). The translocation (8;21)(q22;q22) and inversion or translocation of chromosome 16, which are commonly found in de novo AML, are associated with disruption of the core binding factor (CBF) transcription factor. These abnormalities define the CBF AML subtype, which is associated with the most favorable risk, including response to therapy (55). A Cancer and Leukemia Group B trial found higher overall survival with high-versus low-dose cytarabine therapy in CBF AML; complete response rates were about 90%, and overall survival was >50% at 5 years in these patients (56). These patients also have a high response rate and excellent outcome with repeated cycles of high-dose
cytarabine postremission therapy (57). However, allogeneic
bone marrow transplant was of less value to CBF AML patients
due to their reduced relapse risk (58). Reverse transcription-
PCR detection of the CBF fusion transcripts has been used, with
some success, to monitor residual disease and to predict relapse
in CBF AML patients (59).

Whereas cytogenetic abnormalities have significant value
as biomarkers, they are absent in a large proportion (approximately 45%) of younger patients (<60 years of age) with de
novo AML. Thus, there is high interest in identifying molecular
markers that can be used to stratify AML patients by risk,
regardless of the presence or type of chromosome markers. It is
estimated that nearly half of AML patients have a specific
alteration in a molecular marker that can be detected using PCR
(60). In addition to those that detect specific chromosomal
translocations (e.g., MLL and CBFβ/MYH11), abnormal expression
of several other genes can be found at diagnosis and has potential in disease monitoring and prognosis [e.g., Wilms’
tumor gene WT1, p53, cyclin E, p27, MDR1, ras, and tumor
necrosis factor α, as well as novel genes such as BAALC (brain
and acute leukemia, cytoplasmic; Refs. 4, 5, and 61–71)]. Mu-
tations that lead to constitutive activation of the FLT3 gene,
which encodes a tyrosine kinase growth factor receptor, are
found in 30–40% of AML patients from both favorable and
intermediate risk groups. A recent study assessed the prognostic
significance of FLT3 internal tandem duplication (FLT3/ITD) in
AML trials conducted by the United Kingdom Medical Re-
search Council. Regardless of cytogenetic risk group, FLT3/ITD
was significantly associated with reduced disease-free and over-
all survival (72). In a retrospective analysis, FLT3/ITD had
greater prognostic value in patients with normal versus t(15;17)
karyotype (73). A separate retrospective trial found that the poor
prognosis associated with FLT3/ITD could be partially over-
come by reinforced courses of standard, non-FLT3 targeted
chemotherapy (cytarabine with a topo II inhibitor), suggesting a
possible benefit of aggressive therapy in patients in whom the
mutation is identified early (74).

Ongoing Phase II clinical trials assessing the potential
therapeutic value of inhibitors of FLT3 and other kinases (e.g.,
PKC412, CEP-701, and SU5416) have shown limited efficacy
in refractory AML (75–78). Interestingly, new evidence sug-
gests divergent sensitivity among FLT3 mutants (e.g., FLT3/
ITD, FLT3 Asp835Tyr, and novel mutations within the activa-
tion loop) to the FLT3 inhibitors SU5416, AG1296, and
PKC412 (79). In addition, FLT3 overexpression in the absence
of FLT3/ITD mutation was recently reported to be associated
with reduced survival (80). Together, these findings suggest that
careful delineation of the FLT3 mutation (and/or alternative
mechanism of FLT3 overexpression) is an important consider-
atation in the design and conduct of clinical trials testing the
efficacy of FLT3 inhibitors in AML.

Prospective trials may characterize molecular markers (and
patterns) and/or cytogenetic abnormalities from leukemic cells,
associate these with clinical features of the disease, and establish
correlation with clinical responses in both induction therapy and postremission therapy settings. The findings of Koo et al. (2, 3) on activated ras cited above are an example of this use of biomarkers in AML. Whereas a standard cytarabine/topo II inhibitor protocol should be used for the induction therapy setting, a randomized multiarm design may be considered for the postremission setting. To achieve this goal, the biomarker study could be undertaken as a companion trial to one or several efficacy studies for a novel therapy. In support of this approach, an ongoing NCI-sponsored study will correlate specific chromosomal abnormalities with clinical parameters, molecular alterations, treatment response rates, response duration, survival, and cure in 6400 patients with previously untreated AML, acute lymphoblastic leukemia, or myelodysplastic syndromes, who are to be treated with various induction and postinduction regimens (81).

Prospective trials in AML are well suited to the application of new genomic, proteomic, and gene expression array analyses to identify prognostic and predictive markers. Indeed, ongoing efforts are seeking to identify the gene expression patterns and genetic lesions associated with chemosensitivity in AML patients (reviewed in Ref. 54). For example, the expression pattern of 28 genes (of 23,040 genes characterized in 76 AML patients) assessed using microarray analysis could discriminate nonresponders from responders to induction chemotherapy (82). Additional research in this area is expected to facilitate the further characterization of drug response in AML and is encouraged. A complementary goal is to identify potential molecular targets in AML; microarray analyses were recently applied to identify a novel gene, ZFP91, that was elevated in 27 of 29 AML cases studied (83).

Data sets on solid cancers equivalent to those in AML have been slower to develop, partially due to intrinsic differences between solid tumors and leukemias but also due in part to less frequent access and inadequate sampling of the solid cancers. The access in solid tumors to baseline biopsies and the occasional availability of posttreatment tissue specimens, along with the almost certain availability of noninvasive imaging and serum assays, provide every opportunity to develop biomarkers in the three treatment settings delineated in this article.

SUMMARY AND RECOMMENDATIONS

This article presents a clinical research strategy to evaluate and understand the basis for therapeutic efficacy and safety and the variation of individual responses to approved oncologic drugs. New genomic, functional genomic, proteomic, and imaging technologies will be integrated into standard of care and investigative treatment protocols to accomplish this evaluation. These new methodologies may also facilitate the development and evaluation of new oncologic drugs, including new cytotoxic and noncytotoxic molecularly targeted drugs. Execution of this research plan will initially involve reanalysis of approved drugs. Recommendations for accomplishing this reanalysis are as follows.

(a) There is high value of making measurements (molecular profiling, imaging, and so forth) at both baseline (pretherapy) and posttherapy. Pretherapy measurements give insight into future stratification based on validating clinical outcomes; posttherapy evaluation provides data relevant to planning treatment schedules, changing therapies, and so forth.

(b) The best arena for this clinical research will be neoadjuvant settings where validating tissue biopsies will be available both pre- and posttherapy.

(c) Where tissue biopsy is not feasible and for multiple measurements and longer term follow-up, noninvasive imaging, both molecular (functional) and clinical (anatomical), will be particularly valuable.

(d) Based on available tissue archives with adequate specimen availability and rigorous demographic data, some retrospective studies will be possible. However, for the most part, efficient, small prospective studies will be needed and are anticipated to be very productive.

(e) Initially, these studies will involve cytotoxic standard of care therapies where proportional outcomes are known, and rapid validation of new technologies with clinical responses can be obtained most efficiently. This research would be followed by evaluation of experimental therapy regimens, often drug combinations and often with newer molecular targeted agents, radiotherapy, and other modalities of treatment.

(f) A collaborative initiative among pharmaceutical companies, biotechnology firms, the NCI (and other government), and academic scientists is needed to foster these goals. The primary functions of this collaboration will be to facilitate access to both novel technologies as they become available (e.g., genomic, proteomic, functional imaging technologies, and so forth) and clinical trial samples (e.g., serum, biopsy tissue, DNA imaging data, and so forth). It is anticipated that the NCI will take an active role in fostering this important collaborative effort.

(g) Existing NCI technology, translational programs, and clinical networks could be leveraged for the proposed collaboration. For example, the NCI’s Cancer Bioinformatics Informatics Grid project (85) is building a biomedical informatics network to facilitate access to data, data management tools, and analytical software. It is expected that the proposed bioinformatics system to collect, store, and disseminate outcome, demographic, and other pertinent clinical data would be implemented in accord with the Cancer Bioinformatics Informatics Grid framework. The initiative could also draw from the Specialized Programs of Research Excellence program’s experience fostering multidisciplinary teams to address biomarker identification and validation.11 Access to patients might be best achieved via the NCI’s established collaborative physician networks (e.g., Oncology Cooperative Groups).

(h) Other efforts to facilitate translational research and biomarker development may also inform the proposed collaboration. Private endeavors include MdBIO, a Maryland nonprofit corporation supporting the growth of bioscience.13 The NIH roadmap is a series of initiatives intended to “speed the movement of research discoveries from the bench to the bed side.”14 Comparable efforts are being supported by the European Organization for the Treatment of Cancer.13 The United Kingdom’s National Cancer Research Institute16 is a formal partnership among the government, charity, and private organizations funding cancer research in the United Kingdom (Medical Research Council, Cancer Research United Kingdom, Leukemia Research Fund, Department of Health). The National Cancer Research
Institute has strategic oversight over the National Cancer Research Network, which provides infrastructure support for clinical cancer research. The National Cancer Research Network’s sister organization, the National Translational Cancer Research Network, supports the advancement of novel anticancer therapeutics and diagnostics from the laboratory into early clinical testing. Comparable with the goals of the proposed collaboration, initiatives of these groups include the following: (a) to establish research networks throughout the United Kingdom (34 created to date; a searchable database of clinical trials has also been established); (b) to facilitate routine collection and analysis of biological samples associated with high-quality clinical data via the National Cancer Tissue Resource; (c) to develop an informatics platform to enable access and analysis of data across disciplines [proteomics, genomics, imaging, all National Cancer Research Institute-funded clinical trials, and so forth (this initiative is in collaboration with NCI’s Cancer Bioinformatics Informatics Grid project)]; (d) to perform systematic reviews to address gaps in the evidence-base; and (e) to establish links with industry.

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Reanalysis of Cancer Drugs: Old Drugs, New Tricks

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