Building better therapy for children with acute lymphoblastic leukemia

Childhood acute lymphoblastic leukemia is one of the most curable of all human cancers, but new approaches are urgently needed for children who relapse and to avoid severe side effects of curative therapy. Work from the laboratories of Rob Pieters and William Evans, including a paper in this issue of *Cancer Cell*, has led to the identification of genes whose expression correlates with drug crossresistance and long term outcome. The goal is now to integrate these and other findings using gene expression technology into the care of children with the most common pediatric malignancy.

The high cure rate of children with acute lymphoblastic leukemia (ALL) represents one of the most remarkable success stories in the war on cancer. Stepwise improvements in therapy that have taken place over the last four decades have increased survival rates from 15% to the 75%–80% observed today (Pui et al., 2004). Many factors have led to this high cure rate, including: (1) the use of combination chemotherapy, (2) presymptomatic treatment of the central nervous system, a sanctuary site, and, more recently, (3) the use of intensified therapeutic regimens. These major advances have been derived empirically through carefully controlled, randomized multiinstitutional clinical trials. More recently, clues to the underlying pathogenesis of ALL have come from the identification of somatically acquired genetic lesions in leukemic blasts, such as specific chromosomal translocations and gain or loss of whole chromosomes. It is quite remarkable that relatively little improvement can be attributed to this new information. If empiric therapy can lead to the 75%–80% cure rates seen today, what impact can we expect from more recent breakthroughs in laboratory science?

While the extremely high cure rates for these children are gratifying, major hurdles still exist. The therapy delivered today can be associated with significant short- and long-term side effects. Moreover, close to one in four children will suffer a recurrence, and their outcome is dismal. Identifying the biological mechanisms that mediate tumor response and resistance will undoubtedly lead to more effective and tumor-specific therapy.

A number of fundamental questions about cellular drug resistance have occupied investigators for years. Do drug-resistant tumor cells exist at diagnosis, or are they acquired during therapy? If present at diagnosis, are resistance mechanisms shared by the total population of tumor cells, or does the problem lie in a much smaller subset of blasts that may prove difficult to identify and study at initial diagnosis? Finally, will resistance mechanisms prove to be unique to individual agents or classes of drugs, or will dominant pathways that confer resistance to many agents emerge? The article by Lugthart and colleagues in this issue of *Cancer Cell* addresses these important questions (Lugthart et al., 2005).

The generation of resistant cell lines in vitro rarely mimics the de novo drug resistance seen in patients. However, these investigators have shown previously that the relative in vitro drug sensitivity of individual ALL patient samples correlates with outcome, suggesting that drug resistance exists at diagnosis and that there are certain features shared by the entire population that are associated with the eventual outgrowth of a truly resistant clone (Den Boer et al., 2003).

In a recent study (Holleman et al., 2004), the authors identified gene expression signatures that were associated with unique sensitivity to four commonly used drugs. There was little overlap between these signatures, indicating that diverse pathways mediate tumor response. This

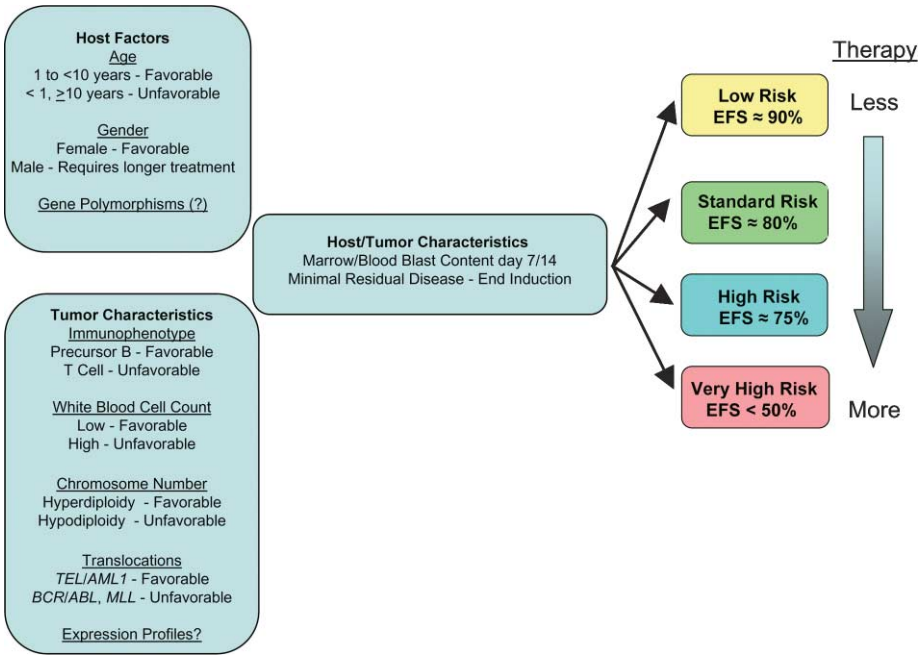

Figure 1. Risk group stratification in pediatric ALL

A combination of clinical and biological features of the tumor and host are currently used for risk group stratification. These features include a patient's age and initial white blood cell count (WBC), genetic features of leukemic blasts, and early treatment response. Therapy is based on the predicted risk of relapse, such that children in lower risk groups receive less intensive treatment than those in higher risk groups. The event-free survival (EFS) rates for patients in different risk groups are shown.

result is a bit sobering, since it might prove daunting to modulate many pathways in an attempt to improve the sensitivity of leukemia cells to conventional agents. In the current paper (Lugthart et al., 2005), the authors turn their attention to discovering pathways that are responsible for governing response to multiple drugs (the same four used in the earlier study). One might have expected that more distal effector pathways downstream of the genotoxic or cytotoxic "hit" supplied by the drugs might be identified in such an analysis. They identified two major patterns of in vitro response—one associated with poor response to all four agents, and the other, a more curious one, characterized by a discordant relationship between sensitivity to vincristine (VCR) and L-asparaginase (ASP).

The authors searched for robust gene expression signatures that defined the most resistant and sensitive samples (e.g., top and bottom quartiles) and again found a seemingly diverse group of genes whose expression appeared to correlate with sensitivity or resistance to multiple anticancer agents. As in their earlier study, few of these genes have been previously linked to drug resistance. The crossresistance profile was associated with older age, a well-known adverse prognostic variable. Samples characterized by either the presence of the t(12;21) (TEL-AML1) or hyperdiploidy were more likely to be cross-sensitive and have a favorable VCR-ASP discordant phenotype. There was little overlap between the sensitivity/resistance signatures described in this report and gene expression signatures discovered previously that predict genetic subgroups such as TEL-AML1 expression or hyperdiploidy (Yeoh et al., 2002; Moos et al., 2002). These results indicate again that the signatures are associated with intrinsic drug resistance/sensitivity pathways and are not simply surrogate markers for these genotypes.

The crossresistance gene signature did predict outcome in an independent set of patients, emphasizing the predictive value of this score. In contrast, the ASP-VCR gene expression score was predictive in the COALL/DCOG (German Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia/ Dutch Childhood Oncology Group), trials but not in patients treated on St. Jude protocols. This result underscores an important point that should not be overlooked—all predictive variables are highly dependent on the type of therapy delivered. So it is not surprising that some gene expression signatures associated with outcome might not be confirmed in trials using different treatment approaches.

This article, as well as prior results from the same group of investigators, begins to shed light on the biological basis of treatment failure. How, then, might these important results be applied to treatment today? First, the identification of pathways associated with drug resistance might define new targets for potential modulation. This is crucial if further improvements in therapy are to be gained, as the maximal benefit from dose intensification has already been reached in most circumstances. Blocking drug resistance pathways might be expected to improve outcome and could lead to the ability to decrease the dose of, or eliminate entirely, toxic chemotherapeutic agents. However, this will be challenging given the diversity of resistance genes identified in this report that do not easily point to attractive targets being pursued by industry currently. Moreover, the complexity of the response relationships, such as discordant patterns of responsiveness to vincristine and asparaginase, might prove problematic.

The second potential application of these results is their use in risk classification. Treatment is optimally tailored to each individual patient's risk of failure so that chances for cure can be maximized, while unnecessary toxicity can be avoided. A variety of clinical (age, gender) and laboratory (white blood cell count, blast surface immune phenotype, blast genotype) characteristics are now used to stratify patients into different risk groups (Figure 1) (Smith et al., 1996). Those predicted to have a good outcome typically receive less aggressive therapy, while those with a predicted higher risk of treatment failure receive more intensified therapy. In spite of these tools, however, many patients who fail treatment are those who were predicted to have a good outcome initially.

Can the gene expression signatures discovered by Lugthart et al. be used to augment current risk classification schemes? A few observations require consideration in this regard. First, the four drugs used to assess sensitivity in this report are used commonly during the first month of ALL therapy, a phase called induction. The overwhelming majority (>98%) of patients with ALL, regardless of associated prognostic features, enter remission at the end of induction, where there is no visible appearance of blasts in the bone marrow or blood. In addition, close to 50% of patients with adverse gene signatures as defined by Lugthart et al. are still cured of their disease, so altering therapy based on the crossresistance or VCR-ASP score alone seems unwarranted. However, these signatures might be used in conjunction with current risk criteria. One of the most important measures of drug sensitivity is the in vivo sensitivity defined by the kinetics of blast regression in the peripheral blood and/or bone marrow. Children whose blasts disappear by day 7 of treatment have a much better outcome compared to those with a slower rate of regression (Gaynon et al., 1997). Newer techniques designed to detect blasts well below the previous threshold defined by light microscopy, such as flow cytometry and molecular techniques focused on clonal markers (antigen receptor or translocations), now make it possible to detect one leukemic blast in a background of 10^4 or 10^5 normal cells (Szczepanski et al., 2001). Importantly, patients showing a slow early response can have their therapy augmented, and this has been shown to improve outcome (Nachman et al., 1998).

The in vivo sensitivity of the samples was not factored into the analysis by the authors, and it will be interesting to determine whether profiles that predict crossresistance also predict slow early response to therapy as measured by marrow blast clearance and the presence of minimal residual disease. Cario et al. (2005) used gene arrays to define a signature associated with residual disease at day 29 in a different cohort of patients, and there appears to be no overlap with the gene sets found by Lugthart et al. Nonetheless, one approach might be to use both the in vitro gene expression profiles and the kinetics of regression in vivo to risk-classify patients. Perhaps the 50% of patients with adverse crossresistance scores who are cured are those displaying rapid disease regression in vivo.

Over the past forty years, building successful therapy for children with ALL has relied on the empiric application of drugs whose exact biological effects were uncertain to say the least. Undoubtedly, physicians, patients, and family members will some day look back on this era as relatively primitive compared to what will unfold shortly in the age of molecular genetics. However, to

label the therapy as primitive would not do justice to the resounding success in the number of lives saved and the dedicated professionals who pioneered cures for these children. The fact is that if the 75% cure rate seen today was established with only modest insight into the underlying biology of ALL, then we should expect nothing short of 100% cure as well as successful preventive strategies in the decades to come.

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JAKing up hematopoietic proliferation

Mutations that deregulate proliferation and survival pathways have emerged as a common molecular theme in the pathogenesis of myeloproliferative disorders (MPDs). Three studies now report an amino acid substitution in the JAK2 kinase in most patients with polycythemia vera as well as in some cases of essential thrombocythemia and chronic idiopathic myelofibrosis. Functional analysis demonstrates that this mutation confers erythropoietin-independent growth in vitro, deregulates signaling pathways downstream of JAK2, and causes polycythemia in mice. These results open new avenues for diagnosing and classifying patients with these disorders, and identify a new molecular target for drug discovery.

Myeloproliferative disorders (MPDs) are clonal malignancies characterized by overproduction of one or more hematopoietic lineages with relatively normal differentiation (Van Etten and Shannon, 2004). The World Health Organization (WHO) classifies chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (CIMF), and the related disorders chronic eosinophilic leukemia (CEL) and idiopathic hypereosinophilic syndrome (HES) as distinct MPDs. Atypical CML, chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML) comprise a related group of "overlap" disorders in which myeloproliferation is prominent, but the bone marrow also shows aberrant maturation (myelodysplasia). Laboratory and clinical observations such as de novo chromosomal translocations (e.g., t[9;22] in CML and t[5;12] in some cases of CMML), an increased risk of JMML in children with neurofibromatosis and Noonan syndrome, and the unexpected responses of some patients with HES to imatinab mesylate provided clues that facilitated identifying molecular lesions that play a central role in the pathogenesis of MPDs and "overlap" diseases (Figure 1). Aberrant activation of kinase signaling cascades and hyperactive Ras have emerged as common biochemical themes in these disorders, and studies in animal models strongly imply that many of the mutations found in human patients can initiate MPD-like diseases in vivo (Van Etten and Shannon, 2004).

A paper by Levine et al. (2005) in this issue of *Cancer Cell*, and data published in the *Lancet* and *Nature* (Baxter et al., 2005; James et al., 2005), report *JAK2* point mutations in most patients with PV and in a substantial proportion of ET and CIMF. These results are satisfying, as they follow logically from the known role of the JAK2 kinase in hematopoietic proliferation and are consistent with previous studies of PV patient samples. The four mammalian Janus (JAK) kinases are recruited by ligand binding to cytokine receptors, where they are activated by *trans*-phosphorylation and, in turn, phosphorylate critical tyrosine residues on the receptor that can then serve as docking sites for members of the STAT (signal transducer and activation of transcription) family and for other signaling molecules (O'Shea et al., 2002). Specific cytokine receptors recruit and activate distinct pairs of JAK and STAT proteins. JAK2 is the primary tyrosine kinase activated by erythropoietin (EPO), and is essential for definitive erythropoiesis (Parganas et al., 1998). Many of the effects of JAK2 are mediated through the recruitment of STAT5 to phospotyrosyl residues on the EPO, interleukin 3 (IL-3), and granulocyte-macrophage colony stimulating factor (GM-CSF) receptors. Interestingly,