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One could say that the 19th century was marked by great advances in fact. Science made enormous strides in understanding the workings of nature. From Pasteur to Darwin, issues of how life arose and continues to change were the stuff of meetings, journals, and great debate. The twentieth century continued that work but its history is marked more by issues of humanity rather than science. From the slaughter of Armenian Christians in 1915 to the demonstrations for civil rights exemplified by the solitary and anonymous man standing in front of a Chinese tank in Tiananmen Square, the last century was more focused on broad issues affecting how civilizations work with each other and with their people. Now, as we move into the twenty-first century, there are clear signs that this time, our time, may be concerned with issues of individuality and individual decision making.

Whether we look to stem cell research, genetic testing, or insurance coverage, the focus is now on the individual. It is the individual’s choice that will cause both the controversy to occur and the consensus to be built. What will be the role of the health professions in this century? Will they sit by and say nothing allowing their individual members to stand alone? Or will they participate, knowing that individual members may not support the position created by the majority? Said another way—what is the role of the individual health professional? Should they be in the forefront of considered debate or not? Many in our own profession would shy away by saying that these issues are beyond the scope of the clinical laboratory.

But—is that so? True, stem cell research is exactly that—research. At the present time, the majority of the clinical laboratorians does not work in research and, as a consequence, some would say that this is not our concern. However, the implications of this research will be seen and performed by clinical laboratorians. Who will provide the testing to determine if a condition is suitable for stem cell therapy? We will. Who will determine if the stem cell therapy is initially successful? We will. Who will monitor the long term consequences of the therapy? We will.

For over fifty years, the state and federal governments of the United States have mandated presymptomatic testing for certain genetic diseases. Who currently performs the tests to determine if a newborn has PKU or sickle cell? We do. Ah, but these tests provide information which can be used prophylactically to correct or mitigate the condition. But what of the other tests for situations less hopeful? Who now performs the test to determine if a child has the gene which inevitably will bring the devastation of Huntington’s Chorea? We do. There is as yet no cure, no successful treatment. Who will perform the tests to determine the subset of cardiac disease a child might have upon reaching adulthood. We will. Who will be performing the tests that determine if a person gets a job or keeps health insurance by virtue of the presence or absence of a single gene? We will.

DNA fingerprinting by such methods as Restriction Fragment Length Polymorphism (RFLP) or Short Tandem Repeat (STR) is becoming more and more common. Witness the number of television programs that highlight it. Who will perform the DNA fingerprinting of persons who have been found guilty of a crime? We or someone like us will. And, that you say, is well and good for they are guilty. After the sentence of the court is completed, should that DNA profile remain available to police so that, for every crime, this person is considered a suspect until proven innocent? Who will perform the DNA fingerprinting of the person who has been accused but not yet brought to court? We will. Who will perform the DNA fingerprinting of the child whose parents want this information in case of the unthinkable? We will. Who should have access to that information? And for how long? Should the DNA taken when a child is three years old be used by the authorities fifty years later?

As we enter this new century still carrying the burdens of the past, should we take up the cause of the individual? While it is our individual civic duty to speak out on issues appropriate to the body politic, is it our special duty to speak out on issues that are of scientific or medical concern? As individuals and as a profession, must we continue explain to an ever increasingly scientifically illiterate society the strengths and limitation of laboratory testing? As individuals and as a profession, must we create and champion a process by which individual clinical laboratory professionals choose whether to practice in a facility that supports these types of tests? Let us use our meetings and our journal for the great debate that must occur in this century for our profession. Regardless of the answers to these questions, we believe that, as individuals and as a profession, we must make these decisions before someone else decides for us or worse, we and those who follow us come to think of us as having been poor stewards of our profession.

Susan Leclair is Editor-in-Chief of Clinical Laboratory Science.
WASHINGTON BEAT

Are You Ready for Some Action?
Giving Voice to the Value and Vision

KATHY HANSEN, DON LAVANTY

An esteemed colleague in ASCLS who is a clinical laboratory science educator recently stated in a presentation that she used to think education was the most important thing that ASCLS did – but now she thinks it’s advocacy. This is not the first time this column has urged activism by ASCLS members and their colleagues, but events in the next year will mandate even more dedication than in the past. With apologies to Monday Night Football – are you ready for some action?

The messages were clear at several sessions at the ASCLS Annual Meeting in Los Angeles July 27–31, 2004. The laboratory community is threatened by a number of current and anticipated initiatives, and we need to come out from our laboratories and be heard as never before.

ASCLS and the Clinical Laboratory Coalition had some success in 2003, as detailed in articles in ASCLS Today. We can be justifiably proud of ASCLS’s advocacy efforts as the House and Senate worked on the massive Medicare Reform Bill, known as the Prescription Drug and Modernization Act of 2003 (HR1). ASCLS worked very hard, in collaboration with other laboratory organizations that are part of the Clinical Laboratory Coalition, to ensure that the proposed 20% co-pay for laboratory tests was removed from the version that eventually passed.

Getting the attention of the House and Senate conferees on one item in such a large complex bill was a real challenge. ASCLS members called, wrote, emailed, attended town meetings, and made visits to their members of Congress (sometimes even at unusual venues like the state fair!) on this issue. For the first time, we went beyond the Government Affairs Committee network of key contacts, and sent broadcast emails to all members in hopes of recruiting more help to get our message out.

The alternative provision was a ten-year freeze in the Medicare Clinical Laboratory Fee Schedule. Even though this is undesirable to say the least, it is less of an administrative and financial burden than administering the 20% co-pay would have been. Through further lobbying, the freeze was reduced to seven years, and finally to five years in the version that became law.

But we cannot rest on our laurels. With record federal budget deficits and huge additional expenses for prescription drug benefits, the costs of the Medicare program will continue to come under scrutiny, no matter which party wins the presidential election. Congress and the administration will be looking for ways to reduce Medicare expenses, the laboratory will be a target once again, and the threats may be more severe than ever before. Some or all of the following will be considered:

- re-consideration of the 20% co-pay for outpatient laboratory tests;
- extending the freeze on the Medicare Laboratory Fee Schedule;
- not just a freeze, but a cut in the Medicare Laboratory Fee Schedule;
- using data from a competitive bidding demonstration project (mandated by the Medicare Modernization Act) to cut or revise the Medicare Laboratory Fee Schedule; and
- possible adoption of the Florida model of competitive bidding for Medicaid laboratory services by other states.

Laboratory professionals must keep current on these and other issues of concern, and be prepared to make their case with lawmakers at both the national and state levels.

Paul Landauer, Director, Health Policy and Payment, Abbott Molecular Diagnostics, gave a thought-provoking session at the ASCLS Annual Meeting. He stated that policy makers must be convinced of the need to invest in the clinical laboratory, the richest source of health and disease status information available. From 1967 until 2001, total Medicare spending increased 382%, while spending for laboratory tests remained flat, due to freezes and reductions in the fee schedule. No one is better qualified to make our case than those who are on the front lines of laboratory service every day.
ASCLS is a leader in the Clinical Laboratory Coalition (CLC) and has had influence and voice far beyond its numbers, comprising less than 5% of the total laboratory workforce. Even the total of the memberships of all the organizations in the CLC is a small minority of those working in the profession. The coming issues will require us to reach out to our colleagues who are not members of any organization, and convince them that they need to participate in this advocacy and not just leave it to others to do. As Paul Landauer said, “As the challenge escalates...the need for teamwork elevates”.

- The advocacy that we need to do is made easier by today’s communication systems. It is easy to call, fax, or email your Senators and Representatives at their Washington and home offices. Go to www.house.gov or www.senate.gov for contact information.

- Many laboratorians live close enough to a home office of their elected officials to call on that office and present their case in person. Follow up that personal visit with an invitation for your elected official and/or their staff to visit your laboratory. (Clear this in advance with your laboratory manager and, in some settings, the person responsible for public relations.)

- Many elected officials hold town meetings around their state to which the public is invited. These are excellent forums to raise concerns about quality and access in laboratory testing, and their impact on patient safety.

- One visit or phone call does not a relationship make—keep up the contact and offer to be available for questions as legislation comes up for discussion and action.

All of these principles apply equally to developing relationships with your state senators and representatives, especially in states that are working toward, or trying to preserve, state licensure laws. Even if you aren’t in a licensure state, state officials have responsibility for Medicaid policy and many other healthcare issues.

We are in this for the long haul—if we don’t convey the value of the laboratory and our visions for excellence and safety in our very important part of patient care, no one will do it. Will you commit to lending your voice and your energy when called upon? Will you spread the word among your colleagues and help them to become committed as well? You WILL be hearing from us!
Monoclonal Anti-CD 20 Antibody Used in Non-Hodgkin’s Lymphoma: A Case Study

KATRINA S MALONE, THOMAS B WIGGERS, LIBBY M SPENCE

ABBREVIATIONS: CD = cluster designation antigen; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy; CT = computed axial tomography; NCI = National Cancer Institute; NHL = non-Hodgkin’s lymphoma.

INDEX TERMS: Non-Hodgkin’s lymphoma; lymphoproliferative disorder; immunophenotyping; Rituxan® therapy.

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A 26-year old gentleman with a history of peptic ulcer disease was admitted in March of 2001 with a two-month history of abdominal pain and a 13 pound weight loss. CT of the abdomen showed a 6 cm x 7 cm pelvic mass and a second 6 cm x 7 cm periaortic mass in the post-hepatic area. The patient underwent exploratory laparotomy and pathology was consistent with diffuse, large-cell lymphoma with immunoblastic features. The operative report and the surgeon described an intra-abdominal cavity consisting of multiple bulky lymphadenopathy in the retroperitoneum, involving the small bowel. Flow cytometry showed a monoclonal population of cells positive for CD19, CD20, and HLA-DR. The patient underwent six cycles of chemotherapy that included Rituxan®. After three cycles of chemotherapy, he had complete resolution of the pelvic and periaortic masses. He still had residual tumor in the post-hepatic area, 5 cm x 6 cm in size. He received three more cycles of chemotherapy with Rituxan® and CT scans were repeated. The CT neck, chest, and pelvis scans were negative; however the post-hepatic mass remained.

MEDICAL HISTORY: Otherwise unremarkable.

LABORATORY FINDINGS: See Table 1.

Immunophenotypic analysis revealed a monoclonal B lymphoid population characterized as CD19+, CD20+, CD5-, CD10+/-, CD23-/+, FMC7-/+, HLA-DR+, and restricted to kappa light chains immunoglobulin with intermediate intensity (Table 1). DNA content analysis of all cells showed a diploid pattern with an increased proliferative rate. The common morphologic features of a B-cell lymphoma are the large cell size, usually four to five times that of a small lymphocyte and a diffuse pattern of growth. The described findings indicated B-cell lymphoma of mixed cell size; an intermediate proliferative rate indicated an aggressive process.

The cells associated with B-cell lymphomas express surface immunoglobulin. All immunoglobulin molecules contain a light chain and a heavy chain. In a random, benign collection of lymphoid cells, the kappa light chains are present on roughly two-thirds of the cells and lambda light chains in one-third. Monoclonal antibody directed at kappa light chain could be used to mark two-thirds of the cells; an antibody to lambda would mark one-third. A malignant collection of lymphoid cells is monoclonal. The cells bear identical surface immunoglobulin molecules with either kappa or lambda light chains, but not a mixture of the two. Monoclonal antibody directed towards a specific kind of light chain would mark either all or none of the cells.
Immunophenotyping refers to the technique of identifying molecules that are associated with lymphoma cells and which help to characterize them. It is helpful because in many instances different kinds of benign and malignant lymphoid cells resemble each other in routinely stained tissue sections and smears. It is especially useful in the case of B-cell lymphomas that express surface immunoglobulin. A lymphoma panel is designed to characterize lymphoproliferative disorders, which are usually comprised of mature B or T cells. The routine lymphoma panel is performed using three-color flow cytometry with the B cell markers CD19, CD20, kappa, and lambda. For B cell malignancies, demonstration of the presence of a monoclonal population by restricted kappa or lambda immunoglobulin light chain expression is diagnostic.

**DISCUSSION**

Non-Hodgkin’s lymphoma (NHL) is the most common cancer involving the lymphatic system. Lymph nodes are found in the underarm, pelvis, neck, and abdomen. Because there are lymph tissues in many parts of the body, NHL can start in almost any part of the body and can spread to almost any organ or tissue in the body. Since the early 1970s, incidence rates for non-Hodgkin’s lymphoma have nearly doubled. The overall five-year survival rate is only 55%. Of the 500,000 Americans with lymphoma, 66% have this form and each year approximately 23,000 Americans will die from the disease.\(^3\)

NHL has shown the greatest increase in incidence in the past 25 years, surpassed only by lung cancer and melanoma.\(^4\) Although recent studies have provided some intriguing clues, the cause of what some experts call the ‘NHL epidemic’ is not known.

According to the National Cancer Institute working formulation, NHLs are classified as low, intermediate, and high grade. This classification scheme accurately predicts the survival of untreated patients, but is not as reliable in predicting the outcomes following treatment. Low grade lymphomas are slow-growing tumors, and some patients can survive for more than a decade without treatment. Although chemotherapy can often shrink low-grade lymphomas, the cancer usually recurs within five years. In contrast, intermediate-grade and high grade lymphomas are fast growing tumors that, without treatment, generally

<table>
<thead>
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<th>Cells expressing</th>
<th>Function</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
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<td>B cell, monocytes, myeloid progenitors, activated T cells</td>
<td>Class II MHC, antigen presentation</td>
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<tr>
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<td>B cells</td>
<td>B cell activation</td>
<td>83</td>
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<tr>
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<td>B cells</td>
<td>Ca(^++) channel B cell activation</td>
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<td>Neutral endopeptidase</td>
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</tr>
<tr>
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<td>T cell activation CD 72 ligand</td>
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<tr>
<td>s Kappa</td>
<td>B cells</td>
<td>Antigen recognition, B cell activation</td>
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<tr>
<td>s Lambda</td>
<td>B cells</td>
<td>Antigen Recognition, B cell activation</td>
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<tr>
<td>CD 23</td>
<td>Activated B cells, CLL</td>
<td>Fc epsilon RII (IgE receptor)</td>
<td>21</td>
</tr>
<tr>
<td>FM C7</td>
<td>The incidence at which FMC7 is positive is &gt;30% of cells in intermediate lymphocytic lymphoma. With mantle cell lymphoma it is usually 40% to 80%; in CLL, it is 10% to 40%.(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD 45</td>
<td>Panhematopoietic</td>
<td>Signal transduction: tyrosine phosphatase</td>
<td>98</td>
</tr>
</tbody>
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\(^3\) FM C7 (IgE receptor) is expressed in >30% of cells in intermediate lymphocytic lymphoma. With mantle cell lymphoma it is usually 40% to 80%; in CLL, it is 10% to 40%.

\(^4\) Other Markers

CD 45 Panhematopoietic Signal transduction: tyrosine phosphatase 98
are fatal within a year or two of diagnosis. Chemotherapy may cure many types of these lymphomas.

Research is also under way to evaluate the safety and effectiveness of monoclonal antibody therapies in NHL patients. Researchers have designed monoclonal antibodies that bind specific epitopes unique to lymphoma cells. The antibodies may be attached to radioactive compounds or toxins that kill the cells. Monoclonal antibody therapy is designed to more selectively target cancer cells, often resulting in less severe side effects than standard therapy. Researchers are also testing the anti-cancer potential of a number of compounds produced by immune cells. These compounds, which include interleukin-2 and alpha interferon, are usually given in addition to standard chemotherapy or radiation therapies.

Presentations at the American Society of Hematology 2003 meeting in San Francisco provided new insights into the diagnosis and treatment of aggressive NHL including the most common subtype, diffuse large B-cell lymphoma. Combining the monoclonal antibody Rituxan with standard chemotherapy is being touted as the first new drug combination in 20 years to improve overall survival in this group of patients. The human chimeric monoclonal anti-CD20 antibody became available under the name Rituxan as the first monoclonal antibody approved by the United States Food and Drug Administration for treating malignancies, including NHL. Rituxan induces apoptosis in target cells with antibody and complement-dependent cellular cytotoxicity. It was less effective in relapsed and advanced disease; however, its specific targeting of B-cells has been further exploited to aim radionuclides at tumor cells while limiting toxicity to normal cells. Rituxan will bind to non-specific CD20 sites and decrease the number of circulating and competing normal B-cells, allowing the infused radionuclides to bind effectively to CD20 sites on the malignant cells. The overall response rate was 87% for low grade and 43% for intermediate NHL. Clearly, the addition of a radionuclide to the specific monoclonal antibody improves the response rate to this treatment, which can also be integrated with chemotherapy and improve the cure rate of NHL.

Rituxan is genetically engineered from portions of mouse and human antibodies and is produced through recombinant DNA technology. Animals, e.g., mice, rabbits, and goats, are immunized with the antigen for which a corresponding antibody is desired. Serum from the immunized animals is collected and purified. The antibodies obtained are polyclonal antibodies. Because they are made in vivo, they consist of many different kinds of antibodies with varying degrees of specificity for the antigen. Individual cells that secrete the desired antibody can be isolated from immunized animals. The selected cell is then fused with a neoplastic plasma cell that also has a natural propensity to make antibodies. This fused hybrid cell, called a “hybridoma”, secretes only one kind of antibody. The serum produced by a culture of the hybridoma cells thus is rich in a monoclonal antibody.

The addition of the monoclonal antibody Rituxan to the standard four-drug combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy leads to significant prolongation of event-free survival in patients without significant additional toxicity. There are fewer adverse additional treatment-related side effects among CHOP-plus-Rituxan patients than CHOP alone. The majority of patients experienced infusion-related symptoms with their first Rituxan infusion. These symptoms include, but are not limited to flu-like fever, chills, nausea, urticaria, headache, bronchospasm, angioedema, and hypertension. These symptoms varied in severity and generally are reversible with medical intervention.

Patients respond much quicker to the combination than to CHOP alone. These results are compelling because standard chemotherapy has shown only a 30% to 40% cure rate in a disease that can be rapidly fatal.

CASE FOLLOW-UP

The patient presented with physical and laboratory findings consistent with an active stage of NHL. Following exploratory surgery and treatment, the patient entered remission. The patient has since returned to the physician for a routine checkup, where laboratory results indicated more abnormal cells in the abdomen. Upon further testing the cells were also found to have entered the lungs. Therapy was resumed, including radiation cycles as well as Rituxan. The patient continues to be treated in this manner.

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Prevalence of Factor V Leiden, Prothrombin G20210A, and MTHFR C677T Mutations in 200 Healthy Jordanians

SUHAIR S EID, GHADA RIHANI

Thrombophilia is now considered a multi-causal condition, with interplay of acquired genetic risk factors. In order to estimate the frequency of the factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations in the Jordanian population, we screened 200 healthy Jordanian individuals. 40% were females. Mean age was 32.1 years for males and 30.0 years for female participants. A PCR method detected 15.0% factor V Leiden (87% heterozygous, 13% homozygous), 2% prothrombin G20210A (100% heterozygous), and 24% MTHFR C677T (67% heterozygous, 33% homozygous).

We conclude that the prevalence of factor V Leiden and MTHFR C677T is elevated in this population of Jordanians. However the incidence of G20210A is relatively low.

Quantification of these genetic thrombosis risk factors in various populations will contribute to a better understanding of the interaction of genetic and environmental risk factors.

ABBREVIATIONS: APC-R = activated protein C resistance; AS-PCR = allele specific polymerase chain reaction; FVL = factor V Leiden; M = mutant; MTHFR = methylene-tetrahydrofolate reductase; PCR = polymerase chain reaction; W = wild.

INDEX TERMS: factor V Leiden; FVL; methylene-tetrahydrofolate reductase; MTHFR; Prothrombin G20210A; thrombophilia.

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Thrombosis risk factors predispose towards thrombosis but, due to the episodic nature of thrombosis, interaction with other components is required before onset of the clinical disorder. A well-established genetic predisposition to thrombosis is a single point mutation in the gene encoding coagulation factor V (G1691A) leading to factor V Leiden (FVL) which was identified as the molecular basis for the phenotype of activated protein C resistance (APC-R) in the majority of affected individuals.1 2 This mutation is associated with a five- to ten-fold risk for heterozygotes and 80-fold risk for homozygotes.3

In 1996, Poort found that the genetic variant in the 3’ untranslated region (a G to A transition at position 20210) is associated with elevated plasma prothrombin levels and an almost three-fold increased risk of venous thrombosis.4

Hyperhomocysteinemia is a risk factor in both arterial and venous thrombosis.5 Recently a common mutation causing C677T in MTHFR coding sequence was observed in individuals with reduced specific MTHFR activity, increased thermolability, and elevated homocysteine concentrations in plasma.6 This coding sequence may be a genetic risk factor, although some studies have failed to show any associations.7 8 Variability in risk ratio among populations studied could be explained by differences in the environmental risk factors or in the genetic makeup of different ethnic origins.

This study aims to establish the frequency of these three genetic mutations in a Jordanian population.

MATERIALS AND METHODS

Blood specimens were collected from 120 male Jordanian soldiers. The mean age was 32.1 years. Eighty specimens were obtained from female volunteer blood donors. The mean age of the female study group was 30.0 years. All of
the individuals assayed were healthy, had no personal or family history of thrombosis, were free of blood coagulation disorders, and none were on any kind of medication.

Blood was collected in 5 mL K$_3$ EDTA (2mg/mL) evacuated tubes. Genomic DNA was extracted from 300 µL ofuffy coat, using the Wizard Genomic DNA Purification kit (Promega, Madison WI, USA). This assay system was designed so that normal alleles were amplified in one reaction tube (‘W’ = wild) while the mutant (M = mutant) alleles were amplified in a second reaction tube (‘M’) for factor V G1691A, G20210A, and C677T each in a separate tube. To detect the six alleles we used six tubes for each patient. In the W reaction, allele-specific primers for the normal prothrombin 20210G, factor V 1691G, and MTHFR 677C alleles potentially direct the amplification of 340bp, 270bp, and 193bp products, respectively, depending on the allele(s) present in the target template. Alternatively, in the M reaction, allele-specific primers for the normal prothrombin 20210A, factor V 1691A, and MTHFR 677T alleles potentially direct the amplification of 340bp, 270bp, and 193bp products, respectively, depending on the allele(s) present in the target template.

Genotyping
Allele-specific amplification (ASA-PCR) was used to amplify genomic DNA. Analysis was accomplished by subjecting samples to ‘W’ and ‘M’ PCR amplifications for wild and mutant types respectively, using the set of primers used by Hessner.9

Procedure
Reactions were performed for prothrombin 20210, Factor V Leiden, and MTHFR mutations separately. FVL ‘W’ and ‘M’ reactions were performed with 180 ng of genomic DNA in 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl$_2$, 0.18M dNTP, 124 ng normal forward primer, 89 ng normal reverse ‘W’ primer, 106 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA). Prothrombin ‘W’ and ‘M’ reactions were performed with 200 ng of genomic DNA in 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl$_2$, 0.18 M dNTP, 47 ng normal forward primer, 71 ng normal reverse ‘W’ primer, 67 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA). MTHFR ‘W’ and ‘M’ reactions were performed with 200 ng of genomic DNA, 10 mM Tris HCl pH 8.3, 50 mM KCl, 0.8mM MgCl$_2$, 0.2 M dNTP, 9 ng normal forward primer, 10 ng reverse normal ‘W’ primer, 18 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA). Reactions were conducted in a total volume of 25 µL. One cycle consisted of 94 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 30 sec, followed by 34 cycles at 94 °C for 15 sec, 64 °C for 15 sec, and 72 °C for 30 sec in a Perkin Elmer 9600 thermal cycler.

The PCR product was analyzed by electrophoresis through 2% agarose gel and visualized by ethidium bromide staining.

RESULTS
Thirty individuals had factor V Leiden (15%), 4 had the prothrombin G20210A mutation (2%), and 48 had the MTHFR C677T mutation (24%). Two individuals showed positive results for all three mutations. Ten individuals were found to have both the FVL and MTHFR mutations (Table 1).

DISCUSSION
The regional and ethnic frequencies of FVL, prothrombin G20210A, and MTHFR C677T mutations have been studied in some European, Asian, African and African-American, and Arab populations.10,11 The only factor studied in a Jordanian population has been FVL.12 The prevalence of factor V Leiden in the general population is variable according to the region and the ethnic group.15

This study evaluates the prevalence of these three mutations in healthy Jordanian individuals. In agreement with the Awidi study, and other studies in the Middle East, the present study showed a high frequency of FVL (15%).11,12,14 These results are similar to some European countries, such as Greece and Turkey, but higher than Saudi Arabia and Egypt.10,13,15,16 The highest prevalence of FVL is usually found among Northern Europeans.17 It is usually less than 1.5% in Southern Europeans.18 A high frequency in the Jordanian population might rise from the consanguinous marriages common in this area of the world. Although it has been reported that the prevalence of FVL in non-Europeans was seven times lower than among Europeans, the presence of high frequency of FVL in European Countries

<table>
<thead>
<tr>
<th>Factor V</th>
<th>Prothrombin G20210A</th>
<th>MTHFR C677T</th>
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<tbody>
<tr>
<td>Heterozygous</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>85</td>
<td>98</td>
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which are near to the Middle East suggests that the distribution of FVL is not only centered in Europe.\textsuperscript{19}

The prevalence of prothrombin G20210A in this study was 2\%, which parallels closely the figures found by Poort and in the Greek population while it appears very rare among African and Asian populations.\textsuperscript{4,10,20}

The world wide distribution of MTHFR 677T variant is not as thoroughly characterized as factor V 1691A. This study found a prevalence of 24\% (67\% heterozygous, 33\% homozygous) compared to 35.3\%, 44.8\%, and 54.5\% reported among Greek, Italian, and Spanish populations respectively, where the present study is similar to a study done in Netherlands.\textsuperscript{10,19,21} It seems from these figures that Jordanians in our study had a lower prevalence of this disorder than those in the other populations we cited in previous protocols.\textsuperscript{5} These data suggest that there is a high degree of heterogeneity in the distribution of the MTHFR 677T allele.

Further studies in more Middle Eastern populations are required for a better breakdown of thrombophilic risk factors in these study groups. Population based studies on the prevalence of this mutation in additional Middle Eastern groups may contribute to a better understanding of the interaction between genetic, ethnic, and environmental risk factors.

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REFERENCES

The Clinical Consequences and Diagnosis of Hypothyroidism

VICKY A. LEGRYS, KATHERINE HARTMANN, JOAN F. WALSH

ABBREVIATIONS: CVD = coronary vascular disease; FT$_3$ = free T$_3$; FT$_4$ = free T$_4$; T$_3$ = triiodothyronine; T$_4$ = thyroxine; TPO = thyroid peroxidase; TPOAb = thyroid peroxidase antibody; TSH = thyroid stimulating hormone.

INDEX TERMS: coronary vascular disease; hypothyroidism.

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Thyroid dysfunction is the most prevalent endocrine disorder, affecting more than 21 million Americans. The annual healthcare costs known to be associated with thyroid dysfunction exceed 10 billion dollars annually. Manifestations of untreated thyroid dysfunction include coronary heart disease, osteoporosis, atrial fibrillation, cognitive impairment, and depression. These sequelae of undiagnosed thyroid dysfunction are among the major causes of mortality, morbidity, and diminished quality of life among older adults. In addition to the individual costs of such morbidity, including functional limitations and disruption of caring giving responsibilities, the healthcare costs associated with caring for individuals with heart disease, stroke, and hip fracture attributed to uncontrolled thyroid dysfunction are substantial. For example, coronary vascular disease (CVD) is the leading cause of mortality in developed countries, with more than 697,000 deaths in 1999 in the United States. In addition, CVD is a leading cause of morbidity, functional limitations, and reduced quality of life among affected individuals.

Thyroid dysfunction can be divided into two general categories: 1) hyperthyroidism, characterized by increased thyroid hormones with decreased thyroid stimulating hormone (TSH) and 2) hypothyroidism, characterized by decreased thyroid hormones with increased TSH. Hypothyroidism is more common; between 10% to 20% of postmenopausal women have evidence of hypothyroidism which can have significant clinical consequences. Hypothyroidism can be further subdivided into overt and subclinical (mild) disorders. Because the signs and symptoms of hypothyroidism are vague and non-specific, many cases are not identified and go undiagnosed, prompting interest in routine screening for thyroid dysfunction. This article will review thyroid gland physiology, pathophysiology, clinical features, prevalence, clinical implications, laboratory diagnosis, treatment, and screening for hypothyroidism, with particular emphasis on subclinical dysfunction.

THYROID GLAND PHYSIOLOGY

The thyroid gland provides the primary control of basal metabolism throughout the body, producing thyroxine (T$_4$) and the more active triiodothyronine (T$_3$). Thyroid hormones circulate in the serum reversibly bound to proteins such as thyroid hormone binding globulin and albumin. Both free T$_3$ (FT$_3$) and free T$_4$ (FT$_4$) enter across the cell membrane, bind to nuclear receptors, and influence gene expression. At the cellular level, thyroid hormone increases carbohydrate and lipid catabolism and stimulates protein synthesis. Among the aspects of homeostasis influenced by thyroid hormone are thermogenesis, glycogen and fat storage, bone resorption and remodeling, bowel motility, blood volume, systemic vascular resistance, cardiac contractility, and heart rate.

Every person has his or her own specific, genetically predetermined FT$_3$/FT$_4$ set-point that is regulated by the hypothalamus and the anterior pituitary via negative feedback (Figure 1). Decreasing levels of FT$_4$/FT$_3$ in the circulation, seen in hypothyroidism, results in increased production of TSH by the anterior pituitary. TSH acts on the epithelium of thyroid gland follicles and increases iodine uptake and thyroxine synthesis. Rising FT$_4$/FT$_3$ suppresses thyrotropin releasing hor-
mone and TSH. Small changes in the individual’s FT₃/FT₄ set-point, while well within the normal range, can trigger an inversely amplified log/linear response in the secretion of TSH from the pituitary gland. Often TSH will be abnormal well before any significant changes in thyroid function are observed.

**PATHOPHYSIOLOGY**

Primary hypothyroidism can be biochemically categorized as overt or subclinical depending on the serum concentrations of FT₃/FT₄. Overt hypothyroidism is characterized by elevated TSH and decreased FT₃/FT₄ concentrations. Subclinical hypothyroidism, in which TSH is also elevated but FT₃/FT₄ are normal, is believed to be a subtle and early indicator of thyroid dysfunction. In fact, subclinical hypothyroidism is a strong predictor of future overt hypothyroidism, suggesting that for some individuals, subclinical hypothyroidism lies along a continuum of thyroid dysfunction. While individuals with subclinical hypothyroidism have FT₃ concentrations within the population reference range, the presence of an abnormal TSH suggests that their FT₃ is not ‘normal’ for them. Based on laboratory testing, subclinical hypothyroidism can be subcategorized by TSH concentration and by the presence of thyroid peroxidase antibodies called TPOAb. The majority of individuals (55% to 85%) have mild elevations of TSH, between 5 mU/L to 10 mU/L." Most people (50% to 83%) in this group do not have significant levels of TPOAb. However, when TSH is greater than 10 mU/L, most individuals (80%) have detectable TPOAb. The presence of high levels of TPOAb (>20 IU/mL) is so strongly predictive of overt disease that it has been proposed that individuals with subclinical hypothyroidism and high TPOAb levels be classified as having ‘impending’ overt disease. The rate of progression from subclinical hypothyroidism to overt disease varies from 3% to 20% per year. Approximately 5.5% of individuals with subclinical hypothyroidism spontaneously regress to having a TSH in the normal range after a year of observation.

The most common cause of primary hypothyroidism in developed countries is autoimmune thyroiditis, also referred to as Hashimoto’s disease. The next most common cause of hypothyroidism is overtreatment of hyperthyroidism with radiation and surgery. Autoimmune thyroiditis involves the gradual destruction of the thyroid gland via abnormal T-cell function. Defective T-cells recognize thyroid antigen in combination with specific major histocompatibility complex antigens. Thyroid targeted T-helper cells present these antigens to B-cells resulting in production of a range of antithyroid antibodies. These antibodies are often directed against both thyroglobulin, the precursor of thyroid hormones stored in the lumen of thyroid follicles, and thyroid peroxidase (TPO), located on the microvilli of the epithelial cells that line the follicle. TPO is required for iodination of the tyrosine residue in thyroglobulin that forms T₃ and T₄, and TPOAb is the most commonly identified antithyroid antibody. The presence of TPOAb is a risk factor for overt disease and TPOAb and TSH levels parallel one another with higher TSH concentrations correlating with higher TPOAb titer and vice versa.

**CASE STUDY**

The following case study illustrates a typical presentation and diagnosis of subclinical hypothyroidism. A 63-year-old Caucasian woman presents to her family medicine physician complaining of fatigue over the last three to five years. She reports that she is so tired after work that she does not have the energy to fix dinner and usually goes bed early without eating. Despite eating less, she has gained 43 pounds...
over the last three years. The physical examination is normal. Her physician tells her that there is nothing to worry about and that her fatigue and weight gain are normal changes for a woman her age. She is advised to exercise more and eat less bread and pasta. Later that month, she attends a health fair at the county health department where she is screened for breast, cervical, and colon cancer, diabetes, hypertension, hyperlipidemia, and thyroid disease. The laboratory results on a fasting sample reveal the following results (Table 1).

She is referred to her local physician for follow up and further blood tests reveal a $FT_4$ of 1.7 ng/dL (reference interval 0.8-2.3 ng/dL). She is placed on 0.05 mg levothyroxine daily. Four months later she returns to the physician, having lost ten pounds and reports that she is has regained her energy and “feels 100% better”. The thyroid function and lipid tests are repeated at that time (Table 2).

The woman will continue to receive daily levothyroxine and return to her physician on a yearly basis.

**CLINICAL FEATURES**
Common symptoms of hypothyroidism include fatigue, depression, cognitive impairment, cold intolerance, dry skin, constipation, and weight gain (Table 3). Patients often have increased total and LDL cholesterol, increased triglycerides, and decreased HDL. Because the signs and symptoms are nonspecific and the onset often insidious, diminished thyroid function is likely to remain undiagnosed because the symptoms are attributed to the inevitable consequences of aging, particularly in post-menopausal women.

**PREVALENCE**
Hypothyroidism is a common disorder, affecting 4.6% of the U.S. population. Subclinical hypothyroidism is more common than overt disease, especially in older females. Several studies have defined the prevalence of subclinical hypothyroidism in women as more than five times that of overt disease, ranging from 8.0% to 21.0% in various population studies. TSH and TPOAb elevation increase with age, are more common among women from the fourth decade on, and are more likely to affect whites and Mexican Americans than African Americans. Individuals with a history of treated hyperthyroidism, neck surgery or neck irradiation, or autoimmune disorders such as type 1 diabetes have an increased risk for developing hypothyroidism. In addition, medications such as lithium, corticosteroids, dopamine, beta-blockers, amiodarone, and interferon can suppress thyroid function.

**CLINICAL IMPLICATIONS**
The primary complication of untreated hypothyroidism is an increased risk for developing atherosclerosis. The link between hypothyroidism and atherosclerosis is multifactorial and includes lipid abnormalities and autoimmune processes. Decreased thyroid hormones increase total and LDL cholesterol by increasing endogenous cholesterol synthesis, decreasing lipoprotein lipase activity, increasing LDL oxidation, and decreasing hepatic LDL receptors. Large population studies of individuals with overt hypothyroidism have noted significantly elevated total cholesterol and LDL cholesterol compared to euthyroid controls. Smaller studies have similar findings with several also noting elevation of triglycerides. The literature on the effect of subclinical hypothyroidism on lipid metabolism and risk for atherosclerosis is often summarized as conflicting. Several groups have identified significant lipid abnormalities in subclinical disease only when TSH was greater than 10 mU/L. Across the spectrum of subclinical hypothyroidism there are trends for higher levels of total cholesterol, LDL, and triglycerides, as well as lower HDL with increasing severity correlated with TSH concentration. The relationship between subclinical hypothyroidism and cardiovascular risk in two large population based studies yielded discordant results. In

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**Table 1. Test results**

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<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference interval</th>
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<tr>
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<td>73</td>
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<tr>
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<td>40-59 mg/dL</td>
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<tr>
<td>Triglycerides</td>
<td>160</td>
<td>1-149 mg/dL</td>
</tr>
<tr>
<td>TSH</td>
<td>11.72</td>
<td>0.46-4.68 mU/L</td>
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</table>

**Table 2. Followup test results**

<table>
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<th>Test</th>
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<td>214</td>
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<tr>
<td>LDL cholesterol</td>
<td>128</td>
<td>1-129 mg/dL</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>60</td>
<td>40-59 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>130</td>
<td>1-149 mg/dL</td>
</tr>
<tr>
<td>TSH</td>
<td>2.57</td>
<td>0.46-4.68 mU/L</td>
</tr>
<tr>
<td>$FT_4$</td>
<td>1.3</td>
<td>0.8-2.3 ng/dL</td>
</tr>
</tbody>
</table>
a 20-year follow-up study of individuals in England with thyroid dysfunction, death from cardiovascular disease was not significantly increased for those with subclinical hypothyroidism when compared to normal controls. However, in a recent study of middle aged women, those with subclinical hypothyroidism were twice as likely to have atherosclerosis compared to normal controls. Variability in the relationship between lipid profile, atherosclerotic risk, and thyroid function may be attributable to divergent definitions of subclinical hypothyroidism, inappropriate control groups, functional limits of older laboratory tests for TSH and thyroid hormones, and diversity within studied populations with regard to age, sex, smoking status, and ethnicity.

In addition to hyperlipidemia as a risk factor for atherosclerosis, individuals with hypothyroidism have evidence of immune-mediated endothelial dysfunction, where the severity is associated with increased TSH level and with increasing TPOAb concentration. In general, autoantibodies and associated immune complexes are associated with hypertension, platelet aggregation, increased vascular permeability, and endothelial dysfunction, all leading to increased risk of thrombosis and atherosclerosis. Endothelial dysfunction is an interrelated process of vascular endothelial damage that includes inflammation, abnormal platelet aggregation, increased adhesion of monocytes, and increased proliferation of vascular smooth muscle cells, all important components in the development of atherosclerosis, intravascular thrombosis, and plaque- and clot-related emboli.

**LABORATORY DIAGNOSIS**

Due to the limited utility of patient history and clinical examination for making a diagnosis, the determination of thyroid function status is based on laboratory tests for serum TSH, FT₄, and TPOAb. Third generation TSH assays with functional sensitivity below 0.02 mIU/L are recommended as the most sensitive and specific tests for detecting thyroid dysfunction in ambulatory individuals. The diurnal fluctuation seen with individual TSH concentrations usually occurs well within the normal reference interval and does not necessitate specific scheduling of specimen collection. TSH is most often measured using non-isotopic immunometric assays on automated analyzers. Interference from heterophilic antibodies can falsely increase TSH concentrations, necessitating the need for dilution studies or repeating the assay using a different method. The recently published document from the National Academy of Clinical Biochemistry (NACB), “Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease” contains guide-
The use of serum TSH to screen for thyroid dysfunction assumes the individual has normal pituitary and hypothalamic function and has stable thyroid status. Pituitary or hypothalamic disease can cause central hypothyroidism in which TSH is abnormally glycosylated resulting in decreased biological activity but normal immunoreactivity in laboratory tests. Rarely, a pituitary tumor may secrete TSH with normal immunoreactivity, but increased biological activity resulting in hyperthyroidism. Recent treatment of, or transition from, hypo- and hyperthyroidism can result in diagnostically misleading TSH concentrations. In addition, hospitalized patients with nonthyroidal illness can have transient abnormalities in TSH concentrations. Individuals receiving levothyroxine replacement therapy for hypothyroidism should wait at least six to eight weeks following initiation of therapy to allow pituitary re-equilibrium before measuring TSH concentrations.

Reflexively testing FT₄ on samples with abnormal TSH represents an efficient and effective way to diagnose thyroid dysfunction and allows the classification into subclinical or overt hypo- and hyper-thyroidism. FT₄, unlike total T₄, is unaffected by common protein binding abnormalities and is diagnostically more accurate. In addition, in ambulatory patients, FT₄ is relatively unaffected by acute and chronic non-thyroidal illness. FT₄ can be measured with direct assays in reference laboratories using physical separation of free and bound hormone by equilibrium dialysis, ultrafiltration, or gel filtration methods. Alternatively, free hormone estimates can be determined indirectly in the clinical laboratory using one or two-step immunoassays or mathematic calculations involving protein uptake methods. Depending on the methodology, the indirect assays can have interferences from abnormal concentrations of serum binding proteins. The limitations of the various assays for FT₄ are discussed in detail in the NACB document.

Because autoimmunity is a hallmark of hypothyroidism, antithyroid antibodies, especially TPOAb, can be used as a marker of disease activity and progression. TPOAb is the most sensitive test for detecting autoimmune thyroid dysfunction with greater than 95% of patients with Hashimoto’s thyroiditis having elevated TPOAb. TPOAb elevation may precede increases in TSH and signal early thyroid gland destruction. The NACB recommends that sensitive, specific, automated TPOAb immunoassays replace the older antimicrosomal antobody agglutination tests. The TPOAb methods currently available vary significantly in sensitivity and specificity, indicating a need for international standardization of this test.

TREATMENT
Hypothyroidism is treated by providing the patient with a daily dose of synthetic thyroid hormone called levothyroxine. Most cases of overt hypothyroidism are treated to decrease the risk of atherosclerosis. The decision to treat subclinical hypothyroidism is controversial, as randomized treatment trials have had varied outcomes. The arguments for treating subclinical hypothyroidism are to prevent the development to overt disease, to improve hyperlipidemia, and to improve patient symptomology. Others argue that the improvement in lipid levels is slight and that over treatment can lead to hyperthyroidism. It has been proposed that patients with subclinical hypothyroidism receive treatment if their TSH is >10 mU/L and TPOAb are present or if a goiter is present.

SCREENING PROGRAMS
Screening at-risk populations (elderly persons, women) for thyroid dysfunction using serum TSH has been proposed because many of the signs and symptoms of mild disease are nonspecific and evidence exists of chronic complications from untreated cases. The American Thyroid Association, a professional association of physicians and scientists, recommends that all adults be screened using serum TSH beginning at age 35 and every five years thereafter. The American College of Physicians supports screening women older than 50 years of age for thyroid disease. Recently, Congress commissioned the National Academy of Science to conduct a study on coverage of routine thyroid screening for Medicare beneficiaries. The study report, “Medicare Coverage of Routine Screening for Thyroid Disease” was published in 2003 by the Institute of Medicine. The report concluded that at the present time, Medicare should not cover screening for thyroid dysfunction due to a lack of sufficient evidence of either benefit or harm. The report recommended that further research be performed to provide definitive answers.

SUMMARY
Hypothyroidism represents a common disorder especially in older women. Left untreated, it can lead to abnormalities in lipid metabolism and subsequent progression to overt hypothyroidism, with significant clinical consequences of myocardial infarction and stroke. More research needs to be performed to investigate the link between subclinical hypothyroidism and cardiovascular disease risk and to evaluate the health and economic outcomes of randomized trials of TSH screening.
REFERENCES


7. Sawin CT, Chopra D, Azizi F, and others. The aging thyroid. increased prevalence of elevated serum thyrotropin levels in the elderly. JAMA 1979;242:247-50.


Assessment of the Graduate Studies Background of CLS Faculty in University-based Programs

RICHARD BAMBERG

OBJECTIVE: To identify the degrees held and the graduate majors or fields of study for faculty teaching full-time and part-time in university-based, baccalaureate-degree clinical laboratory science/medical technology (CLS/MT) programs.

DESIGN AND PARTICIPANTS: A survey and letter of project explanation was sent electronically to the 110 program directors of NAACLS-accredited university-based CLS/MT programs in the United States in May, 2003. Program directors were requested to provide for each full-time and part-time faculty member the following information: titles for all degrees held, major/field of study for each degree held, all specialist certifications held, all other formal degrees or certificates held, and all courses/areas taught in the CLS curriculum.

RESULTS: Information was provided on 288 faculty in 52 CLS/MT programs, for a response rate of 47%. The majority of faculty (75%) described were full-time. A doctorate was held by 43% of the reported faculty, while 46% held a master’s degree as their highest degree, and 11% only a BS in CLS or in biology plus a certificate from a hospital-based CLS/MT program. Graduate degrees in a science major or field represented 52% of the degrees held by the reported faculty, while 48% of the graduate degrees were in education, public health, or administration. Only 13% of the reported faculty held master’s degrees specifically in CLS. Detailed results are provided for degrees held, majors/fields of study, and specialist certifications by specific courses/areas of the curriculum taught.

CONCLUSIONS: The results of this survey indicate that many faculty teaching in university-based CLS/MT programs are extending their preparation as scientists to the graduate level. This should prepare these faculty for their responsibilities in not only teaching but also research. A case cannot be made that a doctorate, as opposed to a master’s degree, is viewed as the ‘terminal degree’ as less than half of the reported faculty in this study as well as others, held a doctorate. The results reported provide a national perspective on the graduate backgrounds of CLS faculty for comparison to an individual program’s faculty during programmatic or institutional accreditation reviews.

ABBREVIATIONS: CLDir = Clinical Laboratory Director; CLS = clinical laboratory science; CLS/MT = clinical laboratory science/medical technology; CLSpH = Clinical Laboratory Specialist in Hematology; CLSup = Clinical Laboratory Supervisor; DABCC = Diplomat of the American Board of Clinical Chemistry; DHS = Doctor of Health Science; DLM = Diplomat in Laboratory Management; DrPH = Doctor of Public Health degree; DSc = Doctor of Science degree; EdD = Doctor of Education degree; MAEd = Master of Arts in Education; MAT = Master of Arts in Teaching; MBA = Master of Business Administration; MEd = Master of Education; MPH = Master of Public Health; MS = Master of Science; MSEd = Master of Science in Education; MSPH = Master of Science in Public Health; MT = medical technology; NAACLS = National Accrediting Agency for Clinical Laboratory Sciences; SACS = Southern Association of Colleges and Schools; SBB = Specialist in Blood Bank; SC = Specialist in Chemistry; SH = Specialist in Hematology; SM = Specialist in Microbiology.

Note: The term clinical laboratory science is used to be synonymous with medical technology, and the abbreviation CLS is used to be synonymous with MT.

INDEX TERMS: CLS faculty; CLS faculty graduate degrees; CLS programs.


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REPORTS AND REVIEWS

BACKGROUND

The impetus for conducting this study was East Carolina University’s reaccreditation site-visit by a team representing the Commission on Colleges of the Southern Association of Colleges and Schools (SACS). More specifically, the SACS team’s review of faculty educational backgrounds and graduate degrees institution-wide was the catalyst for the assessment of graduate studies of CLS faculty teaching in baccalaureate-degrees, university-based programs in the U.S.

SACS, as well as other organizations that award regional accreditation to institutions of higher education, have specific standards that must be met to maintain accreditation. The standards related to the graduate backgrounds of faculty teaching in baccalaureate degree programs vary among the regional accrediting bodies. Based on the author’s review of these standards for six such organizations that are themselves accredited by the Council for Higher Education Accreditation, the SACS standards seem to be slightly more specific and stringent.1

The SACS faculty standards include guidelines that state: “[For] faculty teaching baccalaureate degree courses: a doctoral or a master’s degree in the teaching discipline (minimum of 18 graduate semester hours in the teaching discipline). At least 25% of the discipline course hours in each undergraduate major are taught by faculty members holding the terminal degree – usually the earned doctorate – in the discipline.”2

The other regional accrediting associations that were reviewed have faculty guidelines that could have a more liberal interpretation. The other associations’ faculty guidelines include the following: “It employs a faculty that has earned from accredited institutions the degrees appropriate to the level of instruction offered by the institution.”3; “The institution has an instructional staffing plan that includes a sufficient number of full-time faculty with appropriate backgrounds by discipline and degree levels.”4; “…faculty and other professionals appropriately prepared and qualified for the positions they hold, with roles and responsibilities clearly defined, and sufficiently numerous to fulfill these roles appropriately”; “The faculty is adequately in number and qualifications to meet its obligations toward achievement of the mission’s goals and roles.”; “…faculty are qualified by academic background, degree(s), and/or professional experience to carry out their teaching assignment…”5; and “The preparation and qualifications of all faculty are suited to the field and level of their assignments. Qualifications are measured by advanced degrees held, evidence of scholarship, advanced study, creative activities, and relevant professional experience, training, and credentials.”6

The three full-time Department of Clinical Laboratory Science faculty at East Carolina University (ECU) all hold their graduate degrees in fields other than CLS or MT, with two of the full-time faculty holding a PhD, and the third full-time faculty holding a MS. All three part-time faculty hold a BS in CLS as their highest degrees. Consequently, none of the CLS faculty was viewed by the site-visitors as being “in compliance” with the SACS faculty background requirements. In essence, the Department was being asked to justify to SACS why none of the CLS faculty holds a doctorate in his/her teaching field, i.e., specifically in CLS or MT. Secondary to this question was the requirement to justify the appropriateness of each faculty’s “alternate qualifications” for teaching in CLS and for the specific courses he/she taught. To address these issues, the author sought to obtain information that could be used to compare ECU’s CLS faculty with faculty teaching in the same type of CLS programs, i.e., BS degree and university-based in the U.S.

SUPPORTING INFORMATION

Several approaches to obtaining relevant information were pursued. First, an assessment of the availability of CLS doctoral programs in the U.S. was conducted. A search of the Thomson/Peterson website for Peterson’s Guide to Graduate Programs was performed using the descriptors “clinical laboratory science” or “medical technology”.8 Only two doctoral programs specifically in CLS were found: a PhD in Biomedical Sciences/Track in Medical Laboratory Sciences at Northeastern University, Boston, Massachusetts, and a PhD in Clinical Laboratory Science at Catholic University of America in Washington, DC. Many doctoral degree programs were listed in the fields of “clinical chemistry” and “clinical microbiology” throughout the U.S. Only one doctoral program was found using the descriptors “hematology” or “immunohematology” which was a Doctor of Science program in Hematology and Transplantation Science at the University of Kentucky Medical Center in Lexington. Over 20 PhD programs in pathology with tracks in various CLS fields including clinical chemistry, clinical microbiology, immunology, and toxicology most frequently, and occasionally hematology or immunohematology, were also found. Interestingly, most of the PhD in pathology programs appeared to aim their student selection at predominantly biology and chemistry majors, though a few also listed CLS majors.

The most recent (2002) information from the Association of Schools of Allied Health Profession’s (ASAHP) institutional profile database was accessed. Though these results did not include the fields or majors of graduate study, it did
indicate that of the full-time CLS faculty teaching in the 106 ASAHP-member institutions participating in the survey, 43% held a doctorate as their highest degree, 43% a master's degree, and 14% a baccalaureate degree. The executive offices for both the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS) and the American Society for Clinical Laboratory Science (ASCLS) were contacted for potential information, but both organizations indicated that they did not have CLS faculty backgrounds by graduate fields or majors in any of their databases.

A literature search was performed to identify any previous assessments of CLS faculty backgrounds by fields or majors of study. Searching back to 1980, no published studies with this specific information could be found. The only study with any relevance to the specific information being sought was one conducted in 1987. As one component of the authors’ study, 89 graduate programs in CLS, biomedical and biological fields; health science and public health fields; education; business administration, i.e., MBA, health services administration, computer science; and schools of medicine, dentistry, and veterinary medicine provided information on their students who held the undergraduate major in CLS or MT. The majority (70%) of the responding program directors indicated they had admitted BS-degree CLS students into their graduate programs, while 30% had not or did not know if they had. Most program directors (64%) rated the CLS-degree students as performing average or higher in their programs as compared to students with other degrees. Several studies assessing the research experiences and scholarly productivity of CLS faculty were identified with one national study conducted in 1996 indicating that 46% of such faculty held doctorates and 50% were tenured.

**STUDY PURPOSE**

As no current information on the graduate background of CLS faculty by fields or majors of study could be found after a multiple-source search was conducted, the author chose to obtain this information through an electronic or e-survey. The e-survey was designed to answer the following questions relative to CLS faculty teaching in baccalaureate, university-based programs in the U.S.:

1. What graduate degree(s) held (check all that apply) with the choices being PhD, EdD, DSc, DHS, MD, DDS, MS, MA, MAT, MEd, MAEd, MSEd, MBA, other (specify);
2. major or field of study for master's degree (fill in);
3. major or field of study for doctorate (fill in);
4. specialist certification(s) (list all, fill in);
5. other formal degrees or certificates (list all, fill in); and
6. courses/areas taught in CLS program (check all that apply) with the choices being hematology, hemostasis (coagulation), clinical chemistry, urinalysis, serology, immunohematology (blood bank), microbiology, clinical laboratory management, clinical laboratory teaching/education, molecular diagnostics, instrumentation, other (describe).

The survey was designed as a Microsoft Word 2000 document that could be transmitted by e-mail, completed online, and then returned by e-mail. The survey consisted of a separate table for each full-time faculty member (including the program director or department chair), and each part-time faculty member that consistently taught at least one course each year. The survey did not identify the faculty members by name but anonymously by number. Tables for up to eight full-time and four part-time CLS faculty were provided in the e-survey. For each faculty member, the following information was requested with each point of information being a column of a table:

1. all graduate degree(s) held (check all that apply) with the choices being PhD, EdD, DSc, DHS, MD, DDS, MS, MA, MAT, MEd, MAEd, MSEd, MBA, other (specify);
2. major or field of study for master's degree (fill in);
3. major or field of study for doctorate (fill in);
4. specialist certification(s) (list all, fill in);
5. other formal degrees or certificates (list all, fill in); and
6. courses/areas taught in CLS program (check all that apply) with the choices being hematology, hemostasis (coagulation), clinical chemistry, urinalysis, serology, immunohematology (blood bank), microbiology, clinical laboratory management, clinical laboratory teaching/education, molecular diagnostics, instrumentation, other (describe).

All data were entered and analyzed utilizing SPSS-PC+ version 11.5. Due to the fact that the respondents did not represent over 50% of the population, no statistical analyses were performed other than calculating average number of faculty reported per program, and comparing faculty from institutions within the SACS region versus those not in this region for selected characteristics. The data were analyzed based on frequencies and by cross-tabulations for comparing faculty descriptively by employment status (full-time versus part-time) and by courses/areas taught.

**METHODS**

The survey was sent electronically on May 29, 2003 to the program director for each of the 110 NAACLS-accredited, university-based, i.e., baccalaureate degree CLS programs in the U.S. along with an e-mail explaining the reason for the requested information. The program directors were given the option of returning the completed survey by e-mail, fax, or postal system, and asked to respond within a week. A second request with the e-survey attached, was sent to non-respondents on June 12, 2003.
RESULTS
Almost half (52, 47%) of the program directors returned completed surveys with information on 288 faculty (217 full-time, 71 part-time). An average of 4.2 full-time and 1.4 part-time, or 5.6 total faculty members per CLS program were reported by the program directors. The largest number of reported faculty were in the states of Wisconsin, Texas, and North Carolina.

Upon entering the data, it became evident that that an oversight was that MPH, MSPH, and DrPH were omitted from the list of graduate degree choices, though this was provided by respondents under the “Other (specify)” choice. Faculty profiles by highest degree held, and by majors/fields of study for master’s degrees and for doctorates are displayed in Tables 1 through 3 for full-time faculty, part-time faculty, and total.

<table>
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<td></td>
</tr>
<tr>
<td>Doctorate†</td>
</tr>
<tr>
<td>Master</td>
</tr>
<tr>
<td>Baccalaureate</td>
</tr>
<tr>
<td>* % is percent of n for that column.</td>
</tr>
<tr>
<td>† Includes PhD, EdD, DSc, DrPH, and MD.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Areas of study for CLS faculty holding master’s degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major/field for master’s degree</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Science total</td>
</tr>
<tr>
<td>Clinical laboratory science</td>
</tr>
<tr>
<td>Microbiology</td>
</tr>
<tr>
<td>Biochemistry or clinical chemistry</td>
</tr>
<tr>
<td>Pathology (area not specified)</td>
</tr>
<tr>
<td>Other majors/fields in biological or chemical sciences†</td>
</tr>
<tr>
<td>Public Health total</td>
</tr>
<tr>
<td>Education total</td>
</tr>
<tr>
<td>Medical technology education</td>
</tr>
<tr>
<td>Allied health education</td>
</tr>
<tr>
<td>Health education</td>
</tr>
<tr>
<td>Other majors/fields in education† m</td>
</tr>
<tr>
<td>Administration total</td>
</tr>
<tr>
<td>Major/field for master’s degree not provided</td>
</tr>
<tr>
<td>Total master’s degrees</td>
</tr>
</tbody>
</table>

* % is percent of n for that column. Percent is rounded to the nearest whole number. Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Column percents do not add to 100% because some faculty had a baccalaureate degree as the highest degree, or they matriculated directly to the doctorate without receiving a master’s degree.
† Majors/fields listed under Other are each held by <1% of the reported faculty category.
sample. The program directors reported 32 faculty (12 full-
time, 20 part-time)(11%) who held the BS as their highest
degree but were teaching in a baccalaureate program in CLS/
MT. Majors/fields of study are categorized into science, public
health, education, and administration. Degree title is not
displayed as it was not found to offer useful information.

For faculty holding the BS as their highest degree, 28 of the
32 baccalaureate degrees were in CLS with the remaining BS
degrees being 3 in biology and 1 in microbiology. Only the
most frequently listed specific majors under the science and
education categories are provided for graduate degrees in Tables
2 and 3. Specific majors/fields in public health and adminis-
tration are not listed due to the small number of faculty holding
graduate degrees in these categories.

Specialist certification was held by more of the full-time fac-
ulty (66, 30%) than the part-time faculty (13, 18%), or by 79
(27%) of the total faculty. Specialist certification in blood bank
(SBB), hematology (SH or CLSpH), clinical chemistry (SC
or DABCC), and microbiology (SM) were the most com-
monly held with each of these specialties held by 6% to 7% of
the total faculty. One percent or less of the faculty held
each of the following specialty certifications: immunology (SI),
clinical laboratory management (DLM, CLSup, or CLDir),
molecular biology (CLSpMB or MP), and virology (SV).

A primary focus of the data analyses was the degree level, the
specific graduate majors or fields of study, and specialist cer-
tifications in relation to the CLS course(s) or curriculum area(s) taught. For those curriculum areas where a technical
specialist certification exists, none of these areas were taught
by faculty with the majority of them holding the specialist
credential. The curriculum areas with the highest propor-
tion of faculty holding the specialist certification were immu-
nohematology, i.e., SBB, with 18 (30%) of the 61 fac-
ulty teaching in this area holding the SBB and one addi-
tional person holding the SI; and hematology with 18 (25%)
of the 68 faculty teaching this area holding a SH or CLSpH.
For the 83 CLS faculty teaching microbiology (includes

<table>
<thead>
<tr>
<th>Major/field for doctorate</th>
<th>Full-time faculty</th>
<th>Part-time faculty</th>
<th>Total faculty</th>
</tr>
</thead>
<tbody>
<tr>
<td># (%)*</td>
<td># (%)*</td>
<td># (%)*</td>
<td></td>
</tr>
<tr>
<td>n = 217</td>
<td>n = 71</td>
<td>n = 288</td>
<td></td>
</tr>
<tr>
<td>Science total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microbiology and immunology</td>
<td>16 (7%)</td>
<td>6 (8%)</td>
<td>22 (8%)</td>
</tr>
<tr>
<td>Biochemistry or clinical chemistry</td>
<td>10 (5%)</td>
<td>2 (3%)</td>
<td>12 (4%)</td>
</tr>
<tr>
<td>Pathology (area not specified)</td>
<td>6 (3%)</td>
<td>4 (6%)</td>
<td>10 (3%)</td>
</tr>
<tr>
<td>Other majors/fields in biological or chemical sciences†</td>
<td>35 (16%)</td>
<td>8 (11%)</td>
<td>43 (15%)</td>
</tr>
<tr>
<td>Public Health total</td>
<td>1 (—)</td>
<td>0 (—)</td>
<td>1 (—)</td>
</tr>
<tr>
<td>Education total</td>
<td>26 (12%)</td>
<td>1 (1%)</td>
<td>27 (9%)</td>
</tr>
<tr>
<td>Higher education</td>
<td>7 (3%)</td>
<td>0 (—)</td>
<td>7 (2%)</td>
</tr>
<tr>
<td>Curriculum and instruction</td>
<td>4 (2%)</td>
<td>1 (1%)</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Other majors/fields in education†</td>
<td>15 (7%)</td>
<td>0 (—)</td>
<td>20 (7%)</td>
</tr>
<tr>
<td>Administration total</td>
<td>4 (2%)</td>
<td>0 (—)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Major/field for doctorate not provided</td>
<td>5 (2%)</td>
<td>1 (1%)</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>Total doctoral degrees</td>
<td>103 (47%)</td>
<td>22 (31%)</td>
<td>125 (43%)</td>
</tr>
</tbody>
</table>

* % is percent of n for that column. Percent is rounded to the nearest whole number. Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Percent is not provided if rounding to nearest whole number is <1%, denoted by (—).
courses in bacteriology, mycology, and/or parasitology), 12 (15%) held a SM and 1 additional faculty member held the SV. Only 13 (17%) of the 77 faculty teaching clinical chemistry held either the SC or DABCC. The curriculum areas with the lowest proportion of faculty holding the specialist certification were serology with only 3 (5%) of the 59 teaching this area having the SI; and only 2 (3%) of the 73 faculty teaching clinical laboratory management having the DLM, CLDir, or CLSup certification. Of 56 faculty teaching courses in molecular diagnostics, 2 (4%) held the CLSpMB or MP.

The highest degree held by faculty in relation to the courses or curriculum areas taught, is provided in Table 4. Microbiology, clinical chemistry, hematology, and immunohematology are traditionally viewed as the primary curriculum areas in a university-based CLS program with a full-time faculty member usually devoted to each of these areas. In none of the primary curriculum areas do the majority of faculty teaching that area hold a doctorate. More of the faculty teaching microbiology (48%) and clinical chemistry (46%) hold doctorates as compared to hematology (37%) or immunohematology (36%) faculty. Faculty teaching laboratory information systems and immunohematology have the highest percents with a BS as their highest degree at 50% and 15%, respectively.

Secondary curriculum areas/courses include urinalysis, hemostasis, serology, clinical laboratory management, clinical laboratory teaching/education, and CLS research. Molecular diagnostics, though not a primary curriculum area, is becoming more integrated into the CLS curriculum as is laboratory information systems. When tabulating the multiple courses taught by full-time faculty, faculty teaching microbiology most often also taught molecular diagnostics and serology. The hematology faculty most often also taught hemostasis, clinical laboratory management, and clinical laboratory teaching/education, while the faculty teaching clinical chemistry most often also taught clinical laboratory instrumentation and urinalysis. Immunohematology faculty who did teach a second course most often also taught serology.

Faculty graduate degree majors/fields were cross-tabulated with courses taught for the primary curriculum areas. These

---

**Table 4. Highest degree level of CLS faculty by curriculum courses/areas taught**

<table>
<thead>
<tr>
<th>Course/area in CLS curriculum</th>
<th>Doctorate # (%)*</th>
<th>Master’s # (%)*</th>
<th>Baccalaureate # (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiology† (n = 83)</td>
<td>40 (48%)</td>
<td>38 (46%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Clinical chemistry (n = 77)</td>
<td>35 (46%)</td>
<td>37 (48%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Clinical laboratory instrumentation (n = 56)</td>
<td>22 (39%)</td>
<td>28 (50%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Hematology (n = 68)</td>
<td>25 (37%)</td>
<td>36 (53%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Hemostasis, i.e., coagulation (n = 59)</td>
<td>23 (39%)</td>
<td>30 (51%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Urinalysis (n = 67)</td>
<td>24 (36%)</td>
<td>39 (58%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Immunohematology (i.e., blood bank) (n = 61)</td>
<td>22 (36%)</td>
<td>30 (49%)</td>
<td>9 (15%)</td>
</tr>
<tr>
<td>Serology (n = 59)</td>
<td>28 (47%)</td>
<td>30 (51%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Clinical laboratory management (n = 73)</td>
<td>28 (38%)</td>
<td>42 (58%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Clinical laboratory teaching/education (n = 63)</td>
<td>28 (45%)</td>
<td>31 (49%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Molecular diagnostics (n = 56)</td>
<td>38 (68%)</td>
<td>18 (32%)</td>
<td>0 (—)</td>
</tr>
<tr>
<td>Immunology (n = 11)</td>
<td>5 (45%)</td>
<td>5 (45%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Clinical laboratory science research (n = 9)</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td>0 (—)</td>
</tr>
<tr>
<td>Laboratory information systems (n = 4)</td>
<td>1 (25%)</td>
<td>1 (25%)</td>
<td>2 (50%)</td>
</tr>
</tbody>
</table>

* % is percent of total for that row. Percent is rounded to the nearest whole number. Percent is not provided if rounding to nearest whole number is <1%, denoted by (—).
† Microbiology includes courses in bacteriology, mycology, and/or parasitology.
results are displayed in Table 5 for master's degrees and in Table 6 for doctoral degrees.

Faculty in institutions within the SACS accreditation region (n = 116) were compared to faculty in institutions not in this region (n = 172). The two groups were compared for total number of faculty, number of part-time faculty, and number of full-time faculty by the independent samples T-test with no significant differences in means found at p < .05. Cross-tabulation with calculation of the Pearson Chi-square statistic was performed for 15 nominal variables comparing the SACS versus non-SACS faculty and, again, no significant differences were found at p < .05.

DISCUSSION
The percentage of CLS faculty with doctorates as their highest degree (43%) reported in this survey was identical or similar to that found for CLS faculty in the 2002 ASAHP-member institutional profile (43%) and in a 1996 assessment of university-based CLS faculty (46%).9,13,14 The proportion with doctorates increases to 47% for this survey if only full-time CLS faculty are examined. The 11% of reported faculty with a baccalaureate as the highest degree were found to be predominantly part-time faculty.

Relative to the specific graduate degree majors/fields, 69% of the faculty held at least one graduate degree in a biological or chemical science, while 29% held a degree in a field of education. The number of faculty with graduate degrees in either of these categories may be higher as 21% of the faculty did not provide the major/field for his/her master’s degree. For master’s degrees, 39% of the CLS faculty held the degree in a biological or chemical science, 20% in a field of education, and 9% in a public health or administration field.

<table>
<thead>
<tr>
<th>Table 5. Areas of study for CLS faculty holding master’s degrees by curriculum courses/areas taught</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Master’s degree major/field</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Clinical laboratory science</td>
</tr>
<tr>
<td>Microbiology or microbiology and immunology</td>
</tr>
<tr>
<td>Clinical chemistry or biochemistry</td>
</tr>
<tr>
<td>Pathology (area not specified)</td>
</tr>
<tr>
<td>Medical biology/clinical chemistry track</td>
</tr>
<tr>
<td>Medical biology/hematology track</td>
</tr>
<tr>
<td>Immunohematology</td>
</tr>
<tr>
<td>Other major/field in biological or chemical sciences</td>
</tr>
<tr>
<td>Public health major/field</td>
</tr>
<tr>
<td>Medical technology education</td>
</tr>
<tr>
<td>Other major/field in education&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Administration major/field</td>
</tr>
<tr>
<td>Major/field for master’s degree not provided</td>
</tr>
</tbody>
</table>

<sup>*</sup> Microbiology includes courses in bacteriology, mycology, and/or parasitology.

<sup>†</sup> % is percent of n for that column. Percent is rounded to the nearest whole number. Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Column percents do not add to 100% because some faculty had a baccalaureate degree as the highest degree.

<sup>‡</sup> Majors/fields included under Other are each held by <1% of the reported faculty category.
Only 13% of reported faculty held their master’s degree specifically in CLS. At the doctoral level, 30% of the reported faculty held a PhD or ScD in a science, 9% a PhD or EdD in a field of education, and 2% a PhD or DrPH in an administration or public health major. In comparing full-time versus part-time faculty, there were no noteworthy differences in type of graduate degrees by major/field.

The most prevalent science degrees at the master’s level were ones in CLS and in microbiology, while master’s degrees in education were most frequently in allied health education, MT education, or health education. As there are only three doctoral programs in CLS or a primary CLS curriculum area currently in the U.S., faculty usually must pursue programs in either another science or education at the doctoral level. The most prevalent science doctorates were ones in microbiology or microbiology and immunology, followed by ones in clinical chemistry or biochemistry and in pathology. The doctorates in education were most frequently in higher education or in curriculum and instruction.

Relative to primary curriculum areas or courses taught, no faculty group had more than 50% with a doctorate. Some of the secondary curriculum areas or courses did have faculty with more than half holding the doctorate including molecular diagnostics (68%) and CLS research (78%). These two areas may be taught by faculty from other departments such as biology or biostatistics where close to 100% of the faculty traditionally hold the doctorate.

Relative to having a master’s degree specifically in CLS, faculty teaching clinical chemistry had the lowest proportion (9%) with this graduate major, while hematology faculty had the highest proportion (19%). Faculty teaching microbiology and immunohematology each had 15% with a master’s degree in CLS. Less than a third of the reported faculty teaching each primary curriculum area held specialist certification in that technical area.

Faculty teaching microbiology had the highest percentage with a doctorate in the teaching field of microbiology (22%), followed by clinical chemistry faculty who held a doctorate

<table>
<thead>
<tr>
<th>Doctorate major/field</th>
<th>Microbiology*</th>
<th>Clinical chemistry</th>
<th>Hematology</th>
<th>Immunohematology</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 83</td>
<td># (%)†</td>
<td># (%)†</td>
<td># (%)†</td>
<td># (%)†</td>
</tr>
<tr>
<td>Microbiology or microbiology and immunology</td>
<td>18 (22%)</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Clinical chemistry or biochemistry</td>
<td>1 (1%)</td>
<td>8 (11%)</td>
<td>0 (—)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Pathology (area not specified)</td>
<td>1 (1%)</td>
<td>3 (4%)</td>
<td>3 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hematology</td>
<td>0 (—)</td>
<td>0 (—)</td>
<td>2 (3%)</td>
<td>0 (—)</td>
</tr>
<tr>
<td>Immunology</td>
<td>0 (—)</td>
<td>1 (1%)</td>
<td>0 (—)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Other major/field in biological or chemical sciences‡</td>
<td>9 (11%)</td>
<td>14 (18%)</td>
<td>5 (7%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Public health major/field</td>
<td>0 (—)</td>
<td>0 (—)</td>
<td>1 (2%)</td>
<td>0 (—)</td>
</tr>
<tr>
<td>Education major/field</td>
<td>8 (10%)</td>
<td>7 (9%)</td>
<td>11 (16%)</td>
<td>11 (18%)</td>
</tr>
<tr>
<td>Administration major/field</td>
<td>1 (—)</td>
<td>1 (1%)</td>
<td>2 (3%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Major/field for doctorate not provided</td>
<td>2 (3%)</td>
<td>0 (—)</td>
<td>0 (—)</td>
<td>0 (—)</td>
</tr>
</tbody>
</table>

* Microbiology includes courses in bacteriology, mycology, and/or parasitology.
† % is percent of n for that column. Percent is rounded to the nearest whole number. Percent is not provided if rounding to nearest whole number is <1%, denoted by (—). Column percents do not add to 100% because not all faculty held a doctorate.
‡ Majors/fields included under Other are each held by <1% of the reported faculty category.
in clinical chemistry or biochemistry (11%). Faculty teaching in each of these two curriculum areas had 35% of faculty with a doctorate in a science and only about 10% with doctorates in an education major/field. Faculty teaching hematology or immunohematology each had 16% with doctorates in a science, though in only two instances was the degree in hematology, and 16% and 18% respectively, with doctorates in an education major/field.

The graduate science majors/fields included under “other” were quite varied, though only one or two faculty held degree(s) in each field, and included analytical chemistry, toxicology, biomedical science, cell biology, genetics, nutrition, physiology, anatomy, and molecular biology. Graduate degrees in pathology also offer a viable option for CLS faculty as these degrees usually have clinically-relevant tracks in areas such as clinical chemistry, toxicology, and hematology, though these degrees are not as widespread as microbiology or biochemistry doctorates. The graduate degrees in administration were most often either a master of business administration or in health services administration. The public health degrees included majors/fields in public health laboratory science, epidemiology, and health policy and administration. Majors/fields for education degrees combined under “other” included science education, adult education, educational psychology, educational leadership, instructional technology, counseling, and distance education.

CONCLUSIONS
The application of these results to education settings for the preparation of CLS/MTs is limited to university-based programs, and further limited by the fact that faculty information was provided by slightly less than half of this population of CLS programs. Within these limitations, the results do offer a national perspective of the graduate backgrounds of CLS university-based faculty to which individual programs can compare their faculty for self-review related to programmatic or institutional accreditation.

Though the percentage of the reported faculty holding a doctorate was not higher than previous assessments of such faculty, this data indicates that almost half of the reported CLS faculty in U.S. universities are preparing themselves as scientists for their roles in teaching and, increasingly, in research. Slightly over half (52%) of the total graduate degrees reported for the 288 CLS faculty were in a field of biological or chemical science as opposed to fields of education, public health, or administration, but only 13% had a master’s degree specifically in CLS. This cadre of basic science CLS faculty should aid in CLS academic units becoming integral components and peers with their colleagues in other professional units. The wide variety of degrees reported and the lack of a substantial number of doctorates in CLS or in primary CLS curriculum areas, does not support a doctorate in the teaching field as the standard for faculty teaching in baccalaureate CLS programs.

REFERENCES
10. Personal communication with Dr. O. M. Kimball, Chief Executive Officer, National Accrediting Agency for Clinical Laboratory Sciences, May 1, 2003.
11. Personal communication with Ms E Passiment, Executive Vice President, American Society for Clinical Laboratory Science, May 1, 2003.
Rapid Detection of West Nile Virus in Birds Using the VecTest™ WNV Antigen Assay

GAY HENSON, PAUL HICOCK

OBJECTIVE: To determine if the VecTest™ West Nile Virus Antigen Assay (for testing mosquitoes) could be adapted to detect West Nile virus (WNV) rapidly and accurately in birds for screening purposes.

DESIGN: Cloacal swabs and tissue (kidney and spleen) were harvested from 40 fresh dead birds. The VecTest was used for each swab specimen for detection of WNV; PCR was used for each tissue specimen for confirmation of WNV.

SETTING: Mississippi Veterinary Diagnostic Laboratory (MVDL) in Jackson Mississippi and College of Veterinary Medicine-Mississippi State University (CVM-MSU) in Starkville Mississippi.

SPECIMENS/SUBJECTS: Forty birds of the Corvid family (31 blue jays and 9 American crows) were included in the study. Fresh dead birds that died from no obvious cause were submitted for testing.

RESULTS: VecTest results were 35 positives and 5 negatives. PCR results were 35 positives and 5 negatives.

CONCLUSION: The VecTest showed 100% accuracy.

ABBREVIATIONS: CVM-MSU = College of Veterinary Medicine-Mississippi State University; MVDL = Mississippi Veterinary Diagnostic Laboratory; PCR = polymerase chain reaction; PFU = plaque forming unit; RT-PCR = reverse transcription-nested polymerase chain reaction; SLE = St Louis encephalitis; VI = virus isolation; WNV = West Nile virus.

INDEX TERMS: West Nile virus; virus testing.

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This article was written while the author was a student at the University of Mississippi Medical Center, School of Health Related Professions, Department of Clinical Laboratory Sciences, 2500 North State Street, Jackson MS. It was the winner of the ASCLS Education Scientific Assembly (ESA) 2003 competition for CLS/CLT student research papers.

West Nile virus (WNV) is an arbovirus that can cause encephalitis in humans and horses, as well as death in birds. In 1937, the first case of WNV was isolated from a febrile adult woman in the West Nile District of Uganda.1 In the early 1960s, the first noted equine disease was found in Egypt and France.1 The virus has been described in Africa, Europe, the Middle East, west and central Asia, Oceania, and most recently, North America in 1999.1 Cases have been reported in humans, horses, and birds.1 The first outbreak of WNV in humans, horses, and birds in the United States occurred in 1999 in New York.2 From 1999 through 2003, the virus spread south and west at a rapid rate. As of January 20, 2004, the virus has been documented in 45 states and the District of Columbia with cases in humans, horses, and birds.1 There have been an estimated 9100 human cases with over 400 deaths.1

WNV belongs to the family Flaviviridae, genus Flavivirus. Several other viruses fall in this genus, such as St. Louis encephalitis, Kunjin, and Japanese encephalitis. They are single-stranded RNA enveloped viruses.

WNV is monitored through the use of sentinel birds such as crows and blue jays.3 The virus is maintained in nature by birds.3 Since birds are the principal reservoir hosts, the virus should occur earlier and more frequently in them than in humans and horses. Therefore, the use of sentinel birds helps determine where the virus is present sooner, which in turn, is used for implementing human and veterinary interventions when necessary. Bird species that are most susceptible to the
virus should be used as sentinels. With regard to WNV infec-
tion, crows and blue jays seem to be the most susceptible.

STATEMENT OF PROBLEM/RATIONALE FOR STUDY
The primary purpose of this project was to determine if the
VecTest (Medical Analysis Systems Inc, Camarillo CA) West
Nile Virus Antigen Assay, which is used for detection of
WNV in mosquitoes, could be adapted to detect the virus
rapidly and accurately in birds for screening purposes. This
 assay would not replace any current testing protocols for
WNV. It would be used only for screening purposes to de-
tect the virus quickly. PCR, virus isolation (VI), and ELISA
assays are used for detecting WNV in birds presently. These
procedures work well, but they are time consuming. They
can take three days to several weeks for results. The VecTest
takes 20 to 30 minutes to complete. This quick assay would
allow the epidemiologist to know in which areas the virus
exists and intervention in the human and veterinary popu-
lation could take place sooner. Presently all dead birds col-
crated from across the state are sent to Mississippi Veteri-
nary Diagnostic Laboratory (MVDL). Some of these birds
are so deteriorated and covered in maggots and ants by the
time they arrive at the laboratory that no tissue can be col-
lected to perform PCR testing. Technicians could perform
the VecTest assay in the field where the birds are found. By
performing the test in the field, the epidemiologist would at
least have some data if the birds were too deteriorated to
provide tissue for further testing when they arrive at the lab-
atory. The VecTest would benefit all laboratories and espe-
cially those that currently have to send samples out to refer-
ence laboratories for results. Reference laboratories with
which MVDL was acquainted were backlogged with WNV
samples and would only accept ten samples per month from
any given laboratory. In this situation, MVDL needed a
screening test such as the VecTest.

METHOD USED IN RESEARCH
Postmortem specimens were collected from 40 corvids (9
American crows and 31 blue jays) that had died from no
obvious cause. Kidneys and other organs were harvested, and
postmortem cloacal swabs were collected (using sterile-cot-
ton tipped applicators). The organs harvested were frozen at
–20 °C until assayed for WNV with PCR. The cloacal swabs
were assayed on the same day they were collected.

The assay of the cloacal swabs was done at MVDL and the
PCR on the tissue was done at the College of Veterinary
Medicine–Mississippi State University (CVM-MSU). The
procedure for the cloacal swab was done using the VecTest.
The materials provided in the kit included: VecTest WNV
Antigen Assay dipsticks, grinding solution, culture tubes,
conical tubes, and tube racks. The materials required but
not provided included: timing device, pipette and tips, vor-
tex machine, and sterile cotton-tipped applicators. The fol-
lowing procedure was followed when assaying cloacal swabs
and tissue.

1. Dispense 1,000 µL of grinding solution into each plast-
ic culture tube.
2. Place a cloacal swab specimen into each plastic culture tube.
3. With the swab still in the tube, vortex for 30 seconds.
4. Dispense 300 µL of the mixed solution into a conical tube.
5. Insert a test strip into the conical tube with the arrows
pointing down.
6. Incubate for 20 minutes at room temperature and de-
termine results.

The presence of only a control line on the dipstick indicates
a negative test result. The presence of two lines (control and
WNV line) indicates the presence of WNV antigen. If the
control line on the dipstick is not present, the test is invalid
and needs to be run again.

The tissue samples (kidneys) that were collected from the
postmortem birds were used for confirmation testing. These
samples were tested for WNV using the RT-PCR assay pre-
viously described.4

RESULTS/STATISTICS
Cloacal swabs (screening)
Cloacal swab specimens were collected from 40 fresh dead
birds (blue jays and crows). These samples were tested for
WNV using the VecTest™ WNV Antigen Assay. Of these
40 samples, 35 were positive and 5 were negative.

Tissue samples (confirmation)
Tissue samples (kidneys) were collected from the same 40
birds. These samples were tested for WNV using the RT-
PCR assay as previously described.4 Of these 40 samples, 35
were positive and 5 were negative.

Both methods revealed the same results showing 100% ac-
curacy in the correlation between the two assays.
DISCUSSION
The goal of this research project was to determine if the VecTest™ WNV Antigen Assay for testing mosquitoes could be modified and used as a screening test to detect WNV in fresh dead birds. At the beginning of the project, the laboratory (MVDL) and epidemiologist decided to implement the assay as a screening test if 85% or higher accuracy was achieved between the VecTest and the standard PCR assay. The VecTest assay would not replace any current testing methods for WNV. PCR-based molecular methods are the 'gold standard' for WNV identification. According to the manufacturer, the VecTest assay uses monoclonal antibodies against WNV and the Flavivirus group to identify the presence or absence of viral antigen specific to WNV.5

Sensitivity/specificity studies were performed during assay development in a laboratory setting.5 The manufacturer stated the sensitivity of the WNV assay is 10^3 fold dilution of a culture antigen, and 10^5 PFU/mL WNV in laboratory infected mosquito pools when the individual WNV assay was tested.5 Also, the manufacturer stated there was no cross-reactivity between WNV and SLE antigens during development.5 The assay is specific for WNV.5 This assay is a rapid, one step assay, providing rapid results requiring no specialized equipment, and easily stored at 4 °C.

Blue jays and crows, which belong to the Corvid family, were used in this project. The virus seems to be more detrimental in this family of birds. There is a need for a quick and accurate screening test to detect the virus in birds to help the public health departments identify the location of the virus and possibly predict where human cases may occur. Since 1999, surveillance of dead birds has become a standard method for detecting the spread of WNV transmission in the U.S.6 CDC states that birds with acute WNV infection frequently shed the virus in cloacal or oral cavities.6

In this project, cloacal swab and kidney specimens were collected from 40 fresh dead blue jays and crows. The VecTest™ WNV Antigen Assay was used to screen for WNV using cloacal swab specimens. RT-PCR was the method used for confirmation using the kidney specimens collected. The goal was to see if the VecTest would give the same results as PCR, the ‘gold standard’. The correlation between the two assays yielded 100% accuracy; therefore, the VecTest could be used as a screening test for WNV in crows and blue jays.

CONCLUSION/SUMMARY
The results of this study indicate that the VecTest can be used as a screening test on cloacal swabs from fresh dead birds (crows and blue jays) with 85% or higher accuracy. This has been and will continue to be very beneficial to Mississippi health departments and epidemiologists by helping to quickly determine where to expect human and animal cases of WNV to occur rather than waiting a few days or weeks for results. Studies need to be done on other species of birds using the VecTest. Other species could carry the virus and never show any clinical signs. Also, the possibility of using the VecTest out in the field should be addressed. This would allow field technicians to perform the assay on birds on the spot and at least give the epidemiologist some data in case the birds are too deteriorated to provide samples when arriving at the laboratory.

MVDL incorporated the VecTest to screen dead birds for WNV into its surveillance program. By using the VecTest as a screening assay for WNV in birds, the laboratory has been able to report preliminary results to the epidemiologist sooner. This has allowed the laboratory and epidemiologist to know the locations where the virus is present. Thus intervention or precautionary measures can be implemented sooner in the human and animal population.

ACKNOWLEDGEMENTS
Dr David Fowler, Dr Libby Spence, Dr Lanny Pace, Dr Sally Slavinski. Dr Kurti Dave (Medical Analysis Systems Inc) kindly provided the VecTest kit used for this study.

REFERENCES
Advances in Understanding the Molecular Pathogenesis of Neoplastic Hematologic Disorders

J LYNNE WILLIAMS

Clin Lab Sci 2004; 17(4):221

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Focus Continuing Education Credit: see pages 247 to 249 for learning objectives, test questions, and application form.

The 45th Annual Meeting of the American Society of Hematology was held December 6–9, 2003, at the San Diego Convention Center in California. As with past meetings, there were exciting new developments, comprehensive educational programs, and phenomenal exhibits. There was also an ever increasing ASCLS presence at the meeting, as more hematology/hemostasis educators and researchers are finding their way to this very excellent scientific meeting.

A group of us who attended the meeting would like to share with our colleagues who were unable to attend some of the new information in select areas, covered in the following two articles with a third to follow in the next issue of CLS. While a comprehensive summary of the entire meeting would be desirable, the scope of the meeting makes such an endeavor unfeasible. Consequently, we have chosen the areas of myeloproliferative disorders and myelodysplastic syndromes, acute lymphocytic leukemias, and acute myelocytic leukemias.

This is an exciting and rapidly changing time in the study of malignant and premalignant hematologic disorders. As we evolve from a morphology-based assessment of these disorders to an era in which we are beginning to understand the basic underlying mechanisms, the development of new diagnostic and prognostic tests, as well as the development of interventions specifically targeted at the molecular defects unique to individual disease processes are being realized. By understanding the molecular mechanisms of these diseases, and the genetic and epigenetic changes that underlie their evolution and progression, it may be possible in the not too distant future, to not only detect (and possibly eliminate) the disease at its earliest phases of development but also to modulate or control its behavior in terms of its evolution to a lethal phenotype.1

There has been a veritable revolution in our understanding of cancer since the early 1970s. As more has been learned about the pathogenesis of cancer, it has become clear that specific molecular events underlie the malignant process.2 There are many different changes within cells that contribute to tumorigenesis. A genetic change is an aberration in nucleotide sequences that cause a particular disease phenotype. The vast majority of these genetic alterations has been found to involve one of two broad classes of genes: tumor suppressor genes and proto-oncogenes. The former normally function to inhibit inappropriate growth of the target cell, and ‘loss of function’ alterations of these genes can promote cancer. Proto-oncogenes, by contrast, stimulate growth of the cell, prevent growth arrest, or inhibit apoptosis. Activated proto-oncogenes (called oncogenes) also contribute to inappropriate cell growth and may promote cancer. Point mutations, gene deletions, and chromosomal translocations often underlie these functional changes. The classic example of a genetic mutation, and the first identified in the study of human malignancy, is the Philadelphia chromosome, characteristic of chronic myelocytic leukemia.

However, it is now becoming clear that there are also many epigenetic changes (stable, inheritable) which can also cause
or contribute to a disease phenotype in the absence of nucleotide aberration. The Presidential Symposium for this year’s meeting, “Epigenetics in Hematology”, provided the background for understanding some of the new discussions concerning disease mechanisms. Arthur L Beaudet MD of the Baylor College of Medicine began with an overview of genetic disease vs. epigenetic disease, and Stephen Baylin, MD of Johns Hopkins University followed with a presentation on the fundamental role of epigenetics in cancer.3

Epigenetics (meaning literally “on top of genetics”) refers to the fact that there is another layer of stable or semi-stable gene regulation on top of that controlled by the primary nucleotide sequences. Epigenetics is the study of the changes in gene function that are stably inheritable (or potentially inheritable), which do not entail a change in DNA sequence. One of the most common epigenetic changes found in the human genome involves the methylation of certain cytosine nucleotides within genes and/or their promoter regions. Cytosine nucleotides particularly susceptible to methylation are those found adjacent to a guanine nucleotide, the so-called “CpG dinucleotide”.

CGATCGATCGAT → CMGATCMGATCMGAT

These methylations or epigenetic changes can be translated across mitosis or meiosis, and thus become incorporated into the heritable genetic/epigenetic regulatory mechanisms of the organism. Although the methylation of CpG dinucleotides is a potentially reversible process, approximately 70% to 75% of CpG dinucleotides in our genome are methylated. In addition, CpG dinucleotides are often clustered in ‘CpG islands’, many of which are in and around the promoter regions of genes. The unmethylated state of the promoter regions of genes favors a ‘transcription ready status’ or accompanies active transcription. Typically, methylation of the promoter regions is associated with ‘gene silencing’ and is part of the normal terminal differentiation process seen in many diverse tissue types.

Cancer is a complex disorder of DNA methylation. One may see demethylation of the genome in regions where it should be methylated, or methylation of regions of the genome which are typically unmethylated. There is a growing list of genes, somewhere along the road to tumorigenesis, which acquire hypermethylation of CpGs in their promoter regions. This change is associated with transcriptional silencing of these genes, and is the explanation for one of the most common causes of loss of function of key tumor suppressor genes.

Extensive information can also be encoded in the protein component of the chromatin, in what is now being called the “histone code”. Modifications of the histone proteins include lysine acetylation, serine phosphorylation, lysine methylation, and arginine methylation. These modifications can be stably passed from one cell generation to the next as well. These modifications, constituting the histone code, play an essential role in the very complex system responsible for regulating the potentially reversible euchromatin to heterochromatin transitions. Various types of malignant cells utilize enzymes called histone deacetylases (HDACs) to modify the histone code, and induce transcription of genes which favor cell growth over differentiation.

Hematology researchers have been trying to define the genes whose genetic and epigenetic changes contribute to the evolution of a malignancy. One result of this evolving process has been a realignment and redefinition of the malignant and premalignant hematologic diseases, proposed by the WHO working group.5 An understanding of the relevant mechanisms underlying cancer will result in not only the development of improved clinical and laboratory tools for the detection, diagnosis, and prognosis of cancer, but will also lead to the development of better and more targeted therapies for the treatment, and perhaps even the prevention, of cancer. This is truly an exciting time of exponential expansion of our scientific understanding of these diverse and often confusing diseases.

REFERENCES
FOCUS: NEOPLASTIC HEMATOLOGIC DISORDERS

The Myelodysplastic Syndromes and Myeloproliferative Disorders

J LYNNE WILLIAMS

LEARNING OBJECTIVES:
1. Compare and contrast the FAB and WHO classification systems for the myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD).
2. Discuss the rationale for the use of antiangiogenic therapy in MDS.
3. Explain epigenetic alterations of DNA, and the functional role of DNA methyltransferase and histone deacetylase (HDAC) in these changes.
4. Describe the role of molecular assays for PRV-1 and MPL in the diagnosis of MPD.
5. Discuss the role of neoangiogenesis in idiopathic myelofibrosis.
6. Explain why Imatinib (Gleevec) (the bcr-abl tyrosine kinase inhibitor) is effective in some patients with myelofibrosis.

ABBREVIATIONS: AML = acute myelocytic leukemia; APL = acute promyelocytic leukemia; CMML = chronic myelomonocytic leukemia; CMPD = chronic myeloproliferative disorders; ET = essential thrombocythemia; FAB = French American British; FDA = Food and Drug Administration; HDAC = histone deacetylase; HDACi = HDAC inhibitor; H SCT = hematopoietic stem cell transplantation; HU = hydroxyurea; IMF = idiopathic myelofibrosis; MDS = myelodysplastic syndromes; PDGF = platelet derived growth factor; PRV-1 = polycythemia rubra vera-1; PV = polycythemia vera; RA = refractory anemia; RARS = refractory anemia with ringed sideroblasts; RCMD = refractory cytopenia with multilineage dysplasia; RAEB = refractory anemia with excess blasts; RCMD-RS = RCMD with ringed sideroblasts; TGF-β = transforming growth factor beta; TNF-α = tumor necrosis factor alpha; TPO = thrombopoietin; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor; WHO = World Health Organization.

INDEX TERMS: hematopoietic stem cells; myelodysplastic syndromes; myeloproliferative disorders.

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Focus Continuing Education Credit: see pages 247 to 249 for learning objectives, test questions, and application form.

The myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD) are a diverse group of hematologic diseases characterized by deregulation of the CD34+ hematopoietic stem cell and with a propensity to transform to acute myeloblastic leukemia (AML). As is true for the acute leukemias, there has been intense interest in determining the molecular mechanisms underlying the cellular deregulation, and the development of more targeted therapies based on unique molecular phenotypes.

In 1997, the Clinical Advisory Committee of the World Health Organization (WHO) published a revised classification of neoplastic diseases of the hematopoietic and lymphoid tissues. This new WHO classification system incorporated morphologic, biologic, and genetic information into a working nomenclature that had clinical relevance, and replaced the previous French-American-British (FAB) classification which was predominantly a morphologic classification.
This paper will not attempt a comprehensive discussion of the WHO classification system, but will summarize the significant changes, and then discuss selected aspects of the MDS/MPD's presented at the 45th Annual Meeting of the American Society of Hematology.

The WHO classification of the chronic myeloproliferative diseases lists seven disease entities (Table 1). The major changes from the FAB classification are:

1. Only the Philadelphia chromosome+ cases (or those with the BCR/ABL fusion gene) are called chronic myelocytic leukemia (CML) by the WHO system. The Ph-cases, which show myelodysplastic signs, and are known to have significantly worse prognosis, are called atypical CML (aCML), and belong to the newly created myelodysplastic/myeloproliferative group.

2. There are two newly recognized entities which were not included in the FAB classification, chronic neutrophilic leukemia (CNL) and chronic eosinophilic leukemia (CEL)/hypereosinophilic syndrome (HES).

The WHO classification of the myelodysplastic syndromes incorporates many of the definitions of the FAB system, but updates and refines the definition of some subtypes and thus improves their clinical relevance (Table 2). The WHO classification recognizes eight subtypes, in contrast to the five listed by the FAB system. The major changes include:

1. The definitions of the lower grade diseases, refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) have been refined, and a new category refractory cytopenia with multilineage dysplasia (RCMD) has been introduced (Table 3). In the WHO classification, RA and RARS are defined as diseases in which dysplasia is morphologically restricted to the erythroid lineage. If there is multilineage dysplasia (10% or more dysplastic cells in two or more of the myeloid lineages) the diagnosis is RCMD. In cases of RCMD with at least 15% ringed sideroblasts, the diagnosis is RCMD-RS.

2. Refractory anemia with excess blasts (RAEB) is divided into two subgroups, RAEB-1 (with 5% to 9% blasts) and RAEB-2 (with 10% to 19% blasts), reflecting a difference in median survival and rate of transformation to acute leukemia for these two groups of patients.

3. The most significant change was the lowering of the blast threshold for the diagnosis of acute myelocytic leukemia from 30% to 20% blasts in the blood or bone marrow. As a result, the FAB category RAEB-T is eliminated from the WHO classification.

4. A new subgroup, the 5q-syndrome (loss of the long arm of chromosome 5) is defined by the presence of a specific cytogenetic abnormality.

5. Chronic myelomonocytic leukemia is eliminated from the MDS category and placed in a group of myeloid disorders with features of both myelodysplasia and myeloproliferative diseases, MDS/MPD (Table 4).

6. If a myelodysplastic disease lacks findings appropriate for classification as RA, RARS, RCMD/RCMD-RS, or RAEB, it is in the category myelodysplastic syndrome, unclassifiable (MDS-U).

<table>
<thead>
<tr>
<th>Table 1. Chronic myeloproliferative diseases: comparison of the FAB and WHO classifications*</th>
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<tbody>
<tr>
<td>Chronic Myeloproliferative Diseases (M PD)</td>
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<tr>
<td>Chronic myelocytic leukemia (CML)</td>
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<tr>
<td>Agnogenic myeloid metaplasia with myelofibrosis (Idiopathic myelofibrosis)</td>
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<tr>
<td>Polycythemia vera (PV)</td>
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<td>Essential thrombocythemia (ET)</td>
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</table>

* http://www.cancer.gov/templates/doc.aspx?viewid=f3133a91-a7e0-4d6c-acf2-06f61b7ba66&version=1&sectionID=77
FOCUS: NEOPLASTIC HEMATOLOGIC DISORDERS

Table 2. Myelodysplastic syndromes: comparison of the FAB and WHO classifications*

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Myelodysplastic Syndromes (MDS)</td>
<td>Myelodysplastic Syndromes (MDS)</td>
</tr>
<tr>
<td>Refractory anemia (RA)</td>
<td>Refractory anemia (RA)</td>
</tr>
<tr>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts (RAEB)</td>
<td>Refractory anemia with excess blasts (RAEB-I and RAEB-II)</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts in transformation (RAEB-t)</td>
<td>Acute myeloid leukemia (AML) as “AML with multilineage dysplasia following a myelodysplastic syndrome”</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia (CMML)</td>
<td>MDS/myeloproliferative disorders (MDS/M PD)</td>
</tr>
</tbody>
</table>

Reclassified from MDS to:

*http://www.cancer.gov/templates/doc.aspx?viewid=f3133a91-a7e0-4d6c-acf2-06f6e1f7baa6&version=1&sectionID=77

MYELODYSPlastic SYNDROMES

Myelodysplastic syndromes (MDS) are clonal stem cell disorders, characterized by dysplasia and ineffective hematopoiesis, involving one or more of the myeloid lineages. The result is a decline in one or more peripheral blood cell counts, and generally a hypercellular bone marrow, although occasionally the marrow may be normocellular or hypocellular. An increase in myeloblasts is often present, though by definition they comprise less than 20% of the differential count, in either peripheral blood or bone marrow. In contrast to the myeloproliferative disorders, organomegaly is not seen. Typically, these are disorders of older patients, a factor which may complicate therapeutic strategies.

Until recently, the mainstay of the treatment of patients with MDS has been entirely supportive, with blood and platelet transfusions as needed. The development of targeted therapies for MDS has been limited by the lack of understanding of the fundamental genetic and biologic abnormalities in MDS progenitor cells. Despite this barrier, several new classes of drugs with reasonable biochemical rationale have proved promising in early clinical development and will likely alter the current standard of care for patients with MDS. The focus of the educational program on MDS at this year’s ASH meeting was the emergence of novel therapeutic strategies for this group of disorders, and the rationale for their use.

To develop effective therapies for MDS, it is first necessary to “find the engine that drives the train”. Unlike chronic myelocytic leukemia (CML) or acute promyelocytic leukemia (APL) in which a specific cytogenetic abnormality has been recognized to be causally associated with the disease, and for which specific targeted therapies have been developed, a variety of genetic and epigenetic components may contribute to the evolution of the MDS disorders. A variety of new therapeutic strategies are in progress.

The MDS/M PD category includes myeloid disorders that have both dysplastic and proliferative features at the time of initial presentation and that are difficult to assign to either the myelodysplastic or myeloproliferative group of diseases (Table 4). The three recognized disorders in this group are chronic myelomonocytic leukemia (CMML), atypical chronic myelocytic leukemia (aCML), and juvenile myelomonocytic leukemia (JMML). Myeloid disease that shows features of both MDS and MPD but which does not meet the criteria for any of the three major MDS/M PD entities is designated as myelodysplastic/myeloproliferative disease, unclassifiable (MDS/M PD-U). The FAB classification scheme did not contain this ‘overlap category’.
### Table 3. WHO classification and criteria for the myelodysplastic syndromes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Blood findings</th>
<th>Bone marrow findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory anemia (RA)</td>
<td>Anemia</td>
<td>Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td>No or rare blasts</td>
<td>&lt;5% blasts</td>
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<tr>
<td></td>
<td></td>
<td>&lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
<td>Anemia</td>
<td>Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td>No blasts</td>
<td>15% ringed sideroblasts</td>
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<tr>
<td></td>
<td></td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
<td>Cytopenias (bicytopenia or pancytopenia)</td>
<td>Dysplasia in 10% of cells in two or more myeloid cell lines</td>
</tr>
<tr>
<td></td>
<td>No or rare blasts</td>
<td>&lt;5% blasts in marrow</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>No Auer rods</td>
</tr>
<tr>
<td></td>
<td>&lt;1 x 10^9/L monocytes</td>
<td>&lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)</td>
<td>Cytopenias (bicytopenia or pancytopenia)</td>
<td>Dysplasia in 10% of cells in two or more myeloid cell lines</td>
</tr>
<tr>
<td></td>
<td>No or rare blasts</td>
<td>15% ringed sideroblasts</td>
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<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 x 10^9/L monocytes</td>
<td>No Auer rods</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts-1 (RAEB-1)</td>
<td>Cytopenias</td>
<td>Unilineage or multiligneage dysplasia</td>
</tr>
<tr>
<td></td>
<td>&lt;5% blasts</td>
<td>5% to 9% blasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>No Auer rods</td>
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<tr>
<td></td>
<td>&lt;1 x 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory anemia with excess blasts-2 (RAEB-2)</td>
<td>Cytopenias</td>
<td>Unilineage or multiligneage dysplasia</td>
</tr>
<tr>
<td></td>
<td>5% to 19% blasts</td>
<td>10% to 19% blasts</td>
</tr>
<tr>
<td></td>
<td>Auer rods ±</td>
<td>Auer rods ±</td>
</tr>
<tr>
<td></td>
<td>&lt;1 x 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Myelodysplastic syndrome, unclassified (MDS-U)</td>
<td>Cytopenias</td>
<td>Unilineage dysplasia in granulocytes or megakaryocytes</td>
</tr>
<tr>
<td></td>
<td>No or rare blasts</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>No Auer rods</td>
</tr>
<tr>
<td>MDS associated with isolated del(5q)</td>
<td>Anemia</td>
<td>Normal to increased megakaryocytes</td>
</tr>
<tr>
<td></td>
<td>&lt;5% blasts</td>
<td>&lt;5% blasts</td>
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<tr>
<td></td>
<td>Platelets normal or increased</td>
<td>No Auer rods</td>
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<td>Isolated del(5q)</td>
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clinical trials which may offer, for the first time, sustained benefit and possibly curtail the relentless progression of the disease.

ANTIANGIOGENIC THERAPIES
Interest in antiangiogenic molecules began several years ago with the recognition that angiogenesis plays a role in MDS. Evidence indicates that clonal expansion and apoptotic response in MDS arise from an interaction between the malignant clone and its microenvironment. Autocrine production of angiogenic molecules has been implicated in the self-renewal of MDS precursors, particularly vascular endothelial growth factor-A (VEGF-A). VEGF-A as well as its high affinity receptor (VEGFR-1 and/or VEGFR-2) is over-expressed by myeloblasts and monocytes derived from the abnormal clone. Studies indicate an autocrine role for VEGF as a mitogenic cytokine supporting myeloblast self-renewal in MDS. Additionally, VEGF-A elaborated by the MDS clone induces the release of inflammatory cytokines, e.g., TNF-α, from VEGFR+ stromal cells within the microenvironment, which potentiates ineffective hematopoiesis by suppressing proliferation of normal, VEGF receptor negative, hematopoietic progenitors. Consequently, small molecule inhibitors of angiogenic cytokines have emerged as a promising class of therapeutics for MDS.

The first agent studied in MDS was thalidomide (Thalomid, Celgene Inc). Thalidomide displays both antiangiogenic and TNF-α inhibitory properties, and has been shown to have some degree of activity in these patients, primarily a reduction in red blood cell transfusion dependence. The major problem with thalidomide was its toxicity, with 40% to 70% of patients withdrawing from the studies before the end of 12 weeks.

The demonstration of biologic (erythropoietic) activity with thalidomide led to the search for novel, more potent thalidomide analogues with lower toxicity profiles, and several have recently entered clinical trials. CC5013 (Revimid, Celgene Inc) is a more potent immunomodulatory derivative (IMiD) of thalidomide that lacks the neurological toxicities of the parent compound. CC5013 inhibits the trophic response to VEGF in myeloblasts and endothelial cells, while augmenting adhesion of hematopoietic progenitors to bone marrow stroma (thus promoting sustained growth arrest and extinction of the myelodysplastic clone). In an early study, 62% of MDS patients with symptomatic or transfusion-dependent anemia experienced an erythroid response. Many patients maintained the response (transfusion independence and near normal hemoglobin levels) as long as one year following cessation of treatment. Not only does the drug have the capacity to promote erythropoiesis, but there is evidence that it also can suppress cytopenic abnormality. Of the 13 patients with an abnormal karyotype at the beginning of the study, 62% had a complete cytogenetic remission. CC1088, a member of a second functional class of analogs termed selective cytokine inhibitory drugs (SelCIDs) appears significantly less active in preliminary clinical studies in MDS.

A second class of antiangiogenic agents being investigated are small molecule inhibitors of the VEGF receptor tyrosine kinases. These agents impair ligand-induced activation of the VEGFR. Clinical trials are underway, but to date, no data are available on their effectiveness.

Arsenic trioxide (ATO) (TrisenoxTM, Cell Therapeutics) has been approved by the Food and Drug Administration (FDA) for treatment of relapsed APL. ATO has broad biological properties derived from its ability to bind to and deplete sulfhydryl-rich proteins such as glutathione. ATO inhibits glutathione peroxidase, thus potentiating peroxide generation, disrupting mitochondrial membrane integrity and respiration, repressing anti-apoptotic proteins, and initiating caspase-mediated apoptotic responses. In MDS, the anti-proliferative effects of ATO relate in part to its ability to suppress myeloblast elaboration of VEGF-A. The results of several clinical trials indicate that ATO has activity in MDS, with approximately a third of patients having experienced hematological improvement.

FARNESYL TRANSFERASE INHIBITORS
The second class of targeted therapeutics being investigated in MDS are farnesyl transferase inhibitors. While activating mutations of the RAS proto-oncogene are detected in only about 20% of patients with MDS, they are common in CML (40% to 70% of patients). The RAS gene superfamily encodes guanosine triphosphate hydrolases (GTPase) that function as critical regulatory elements in signal trans-

Table 4. WHO classification of the myelodysplastic/myeloproliferative diseases

| Chronic myelomonocytic leukemia (CMML) |
| Atypical chronic myeloid leukemia (aCML) |
| Juvenile myelomonocytic leukemia (JMML) |
| Myelodysplastic/myeloproliferative disease, unclassifiable |
FOCUS: NEOPLASTIC HEMATOLOGIC DISORDERS

The constitutive activation of RAS can result from either mutations within RAS alleles or from reciprocal translocations deregulating receptor tyrosine kinases. A subset of CMML patients have a characteristic translocation involving the platelet-derived growth factor receptor beta chain (PDGFRβ) gene on the long arm of chromosome 5 (5q33). The partner chromosome in this reciprocal translocation may vary, although the most common is chromosome 12 (the TEL gene locus, 12p12-13). An exciting recent discovery is that patients harboring the 5q33 translocation respond to imatinib (Gleevec). Imatinib binds to the PDGFRβ receptor analogous to its interaction with BCR/ABL, to act as a potent inhibitor of receptor kinase activity. Among five patients reported to date, each achieved rapid hematological control and sustained complete cytogenetic remission with imatinib therapy.

THERAPIES TARGETED AT EPIGENETIC ALTERATIONS

Recent interest in the treatment of neoplastic cells by targeting epigenetic changes to restore normal gene transcription has been intense. Unlike genetic changes (mutations, deletions) which are irreversible outside of the introduction of new genetic information, epigenetic changes represent potentially reversible modifications to DNA. The potential reversibility of epigenetic changes makes them attractive targets for cancer therapeutics.

Histones (DNA packaging proteins) have lysine tails, which can be acetylated in a post-translational modification step. When acetylated, histones interact loosely with DNA so that the chromatin is open and genes downstream from the acetylated histone can be transcribed. Hypoacetylation is associated with heterochromatin formation and gene silencing. DNA methyltransferase is the enzyme responsible for imparting a parent cell's methylation pattern to daughter cells. Abnormalities of cytosine methylation constitute some of the best characterized and most common epigenetic changes in cancer. The DNA of neoplastic cells may be characterized by global hypomethylation, dysregulation of DNA methyltransferase, and regional methylation of CpG dinucleotide clusters in gene promoter regions. These CpG clusters (CpG islands) are normally protected from methylation in normal cells. In cancer cells, CpG islands are heavily methylated. Methylated CpG islands attract transcriptional inhibitory complexes which include histone deacetylases (HDAC). The enzyme HDAC deacetylates the lysine tail of histones, resulting in the tight association of the histone with DNA, forming a transcriptionally repressive configuration and inhibiting gene transcription.

Extensive studies have demonstrated promoter methylation, associated with transcriptional silencing, of a wide variety of cell regulatory genes in many cancers. These epigenetic changes are associated with phenotypic abnormalities in the malignant cells. Because promoter methylation is relatively "cancer-specific," DNA methylation is an attractive therapeutic target. In malignant myeloid cells, the most widely reported methylated gene is the cyclin-dependant kinase inhibitor p15INK4B. Methylated p15 promoter has been demonstrated in 68% of primary AML samples, and 35% of MDS; the frequency of methylation increases with MDS disease progression. A variety of other genes are methylated in myeloid neoplasms, including E-cadherin, p73, and RARβ.

The DNA methyltransferase inhibitors which have been most extensively characterized for the treatment of AML and MDS are 5-azacitidine (5AC) and 5-aza-2'-deoxycytidine (decitabine, DAC). A Phase III trial of DAC demonstrated an overall hematologic response rate of 60%, including a 7% complete remission rate and a 16% partial remission rate. Similar response rates have been reported utilizing DAC. Current data suggest that the two drugs as currently studied are equivalently effective in the treatment of MDS. The relationship of DNA methyltransferase inhibition to the clinical activity of 5AC and DAC in the treatment of MDS remains a critical question. Although a subset of patients treated with DAC did exhibit reversal of p15 methylation, associated with re-expression of p15 protein, a link between changes in methylation and clinical response could not be ascertained.

A variety of HDAC inhibitors (HDACi) are under clinical investigation, including sodium butyrate, sodium phenylbutyrate, valproic acid, suberoylanilide hydroxamic acid (SAHA), and vorinostat (SAHA). HDACi are known to disrupt transcriptional silencing by reactivating repressed genes, and it is believed that these agents may also reverse aberrant histone modifications. The HDACi clinical trial data suggest that HDACi alone and in combination therapies may be effective for hematologic malignancies. As these agents are rapidly advancing to the clinic, HDACi will likely provide new therapeutic opportunities.
Focus: Neoplastic Hematologic Disorders

Acid (SAH A), and FK 228 (depsipeptide). Once it was recognized that one of the primary mechanisms by which methylated DNA repressed transcription was through recruitment of HDAC, investigators became interested in whether it was possible to augment gene expression by combining a methytransferase inhibitor with a histone deacetylase inhibitor. Currently, the only data available is a Phase I dose-finding study of 5AC followed by phenylbutyrate. The combined therapy was well tolerated, and significant sustained clinical responses were achieved.

Novel Differentiation Approaches

Although growth factors are often potent differentiation inducers, the proliferation-inducing effects of these cytokines often outweigh their differentiation effects. Most of the agents that have been used to induce terminal differentiation in vitro are antiproliferative; they induce the cell cycle inhibitor p21, and thus cell cycle arrest. In vitro data suggested that the pairing of such cytostatic agents with myeloid growth factors might lead to the predominance of differentiation activity of the growth factor over the proliferative effects. The protein kinase C activator bryostatin has been paired with GM-CSF in a Phase I trial, with promising results.

Transplantation and Immunosuppressive Therapy in MDS

There has been debate on whether hematopoietic stem cell transplantation (H SCT) was a curative option for patients with MDS. Autologous H SCT in MDS is theoretically feasible only in a small proportion of patients who achieve a complete remission following induction chemotherapy and in whom a suitable autologous harvest can be collected. However, there is a high relapse risk of up to 72%, and enthusiasm for this approach is limited.

Conventional myeloablative allogeneic H SCT has a significantly lower relapse rate than autografts, but the transplant-related complications increase in frequency and severity with advancing age. However, allogeneic H SCT is the only therapeutic modality at present that is potentially curative in MDS. Allogeneic H SCT replaces recipient dysplastic hemopoiesis with healthy donor hemopoiesis. Its applicability, however, is limited by the availability of a suitable HLA-matched donor and by the toxicity of the conditioning regimen, which is directly proportional to the age of the recipient. As the majority of patients with MDS are of advanced age, often with concurrent medical conditions that effectively preclude standard conditioning for allogeneic H SCT, various strategies have been adopted in order to attempt to reduce the toxicities associated with the transplant procedure. Recently there has been significant interest in a reduced-intensity or ‘nonmyeloablative’ conditioning which can result in stable donor hemopoietic engraftment, without the toxicity associated with conventional H SCT.

Even in a clonal disease like MDS, there is a subgroup of patients in whom, for whatever reasons, there is evidence of immune dysfunction. Such evidence may include abnormal CD4:CD8 ratios and increased activated cytotoxic T-cells. Immunotherapeutic agents that inhibit these immune mechanisms play an important role in the management of the immune-mediated marrow failure syndrome in MDS. Antithymocyte globulin has been shown to produce clinically meaningful responses in patients with MDS, with 34% to 64% of patients becoming transfusion independent. The administration of cyclosporine in patients with hypoplastic MDS has also resulted in prolonged partial hematologic improvements.

Levels of the cytokine tumor necrosis factor alpha (TNFα) have been demonstrated to be elevated in patients with MDS and have been shown to play a major role in the apoptosis of hemopoietic cells in MDS. The inhibition of TNFα therefore appears to be a legitimate target for directed therapy. At present, there are 2 anti-TNFα agents available for clinical use, with limited data available in MDS.

Chronic myeloproliferative disorders

The chronic myeloproliferative disorders (CMPD) are characterized by proliferation within the bone marrow of granulocytes, erythrocytes and/or megakaryocytes. Under the new WHO classification, the CMPD include Philadelphia chromosome-positive chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytopenia (ET), chronic idiopathic myelofibrosis (IM F), chronic neutrophilic leukemia (CN L), and chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES) (Table 1). This article will focus on the Philadelphia chromosome-negative CMPD, primarily PV, ET, and IM F. CML is to be covered by a separate article in another issue of the journal.

The diagnosis, and management, of patients with PV, ET, and IM F has been difficult for a number of reasons. They share many overlapping clinical features. In addition to their phenotypic mimicry, there is a lack of specific molecular diagnostic markers, a lack of understanding of their molecular basis, and a paucity of controlled, prospective therapeutic trials for treatment decisions. As a result, currently there are...
still significant unresolved issues concerning the CMPD, including whether these disorders are truly different, and/or how they are related.

There are a number of features which are common to the CMPD. There is involvement of a multipotential hematopoietic progenitor cell, and usually clonal dominance of the abnormal clone over normal hematopoietic progenitor cells. The disorders share abnormalities of chromosomes 1, 8, 9, 13, and 20, although a consistent cytogenetic abnormality as described for CML or APL is not found. In general, they result in a hypercellular bone marrow, and the various cell lines involved generally show relatively normal maturation, although the megakaryocytic lineage may display dysplastic features. Hematopoiesis is generally considered ‘effective’, as elevated cell counts in the peripheral blood are observed. The CMPD are often associated with organomegaly (enlarged spleen and/or liver), as a result of either cell sequestration, extramedullary hematopoiesis, or leukemic infiltration. Thrombotic and hemorrhagic diatheses are common. The CMPD disorders have a varying tendency to progress either to a stage of bone marrow failure (decline of peripheral blood cell counts and evolving marrow fibrosis) or to undergo transformation to acute leukemia.

In addition to the phenotypic mimicry among these disorders, there is also overlap with nonclonal hematopoietic disorders. Many patients with idiopathic thrombocytosis go on to develop ET, IMF, or PV, and many patients with idiopathic erythrocytosis go on to develop PV (Figure 1).

Despite the extensive overlap in characteristics for these disorders, it is important to appreciate that they are not monolithic, but actually show both clinical and genetic heterogeneity. For example, the increase in bone marrow reticulum (myelofibrosis) occasionally seen as a complication in PV must be distinguished from the bone marrow failure state associated with IMF. Myelofibrosis is a histologic condition, and the increase in marrow fibrosis in PV generally has minimal effect on erythropoiesis (most patients continue to have increased hemoglobin and packed cell volume).

Recently, two new molecular assays have been described which may prove useful for the diagnosis of CMPD. Polycythemia rubra vera-1 (PRV-1), a novel member of the urokinase-type plasminogen activator receptor (uPAR) superfamily, has been reported to be overexpressed in mature peripheral blood granulocytes from patients with PV, but not in a variety of controls including healthy individuals, patients with secondary erythrocytosis, and patients with CML. CD177, the current designation for the gene that encodes PRV-1, was found to be the only gene strongly overexpressed in polycythemia neutrophils. While CD177 is almost universally overexpressed in polycythemia vera, the mechanisms of overexpression are not known. No abnormalities of the CD177 gene have been found. Also, although the expression of CD177 mRNA is markedly elevated in patients with PV, the neutrophil protein encoded by CD177 (NB1 glycoprotein) is similar to that of health subjects. Decreased expression of Mpl, the receptor for thrombopoietin, has been described in platelets and mega-
Thrombocytosis (platelet count higher than 1500 \( \times 10^9 \) /L), a previous thrombotic event, and long duration of thrombocytosis are major risk factors for thrombosis. The biological basis appears to be either alternative splicing of Mpl mRNA, or a single nucleotide polymorphism. The number of patients expressing increased quantities of CD177 mRNA, or decreased Mpl, varies among the disease type and among studies (Table 5). However, both have become important molecular markers of myeloproliferative disorders.

**CYTOREDUCTIVE THERAPY IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA**

PV and ET are chronic MPDs in which there is a thrombohemorrhagic diathesis, and a variable incidence of progression to myelofibrosis or acute myeloid leukemia (AML). The main cause of morbidity and mortality in these disorders is thrombosis, which occurs more frequently in older patients or in those with previous vascular complications. Severe bleeding is relatively rare, and limited to patients with a very high platelet count and to those taking antiplatelet drugs. It has been suggested that propensity for vascular complications can be reduced by cytoreductive treatment. While cytoreductive therapy is effective in preventing thrombosis, there is concern that some myelosuppressive agents may increase the rate of transformation to acute leukemia. Thus, the aim of treatment of both PV and ET must be to reduce/prevent thrombosis and hemorrhage without increasing the risk of acute leukemia. It has been suggested that patients with ET or PV be stratified into low, intermediate, and high risk groups (in terms of their relative risk for thrombohemorrhagic events), and cytoreductive therapy be given only to high risk patients.

Major risk factors for thrombosis are age (greater than 60 years), a previous thrombotic event, and long duration of thrombocytosis (platelet count higher than 1500 \( \times 10^9 \) /L).

In addition, patients with impaired expression of Mpl in bone marrow megakaryocytes or with over expression of PRV-1 were at higher risk for vascular complications. Both prospective and retrospective studies confirmed that thrombotic deaths are rare in low-risk ET patients. Thus, because of the low risk of thrombotic complications, and the potential leukemogenicity of cytotoxic drugs, it has been suggested that chemotherapy be withheld for young (<60 years), asymptomatic ET patients with a platelet count below 1500 \( \times 10^9 \) /L and with no additional risk factors for thrombosis.

Several platelet count-lowering agents have been investigated. Hydroxyurea (HU) has emerged as the treatment of choice in high risk patients with ET because of its efficacy in preventing thrombosis and rare acute toxicity. However, the incidence of acute leukemic transformation is higher in patients with ET who have cytogenetic abnormalities or who are receiving multiple cytotoxic drugs. Alternatives to HU include interferon and anagrelide. Both are effective in reducing platelet counts without leukemic transformation, but the high cost and side effects reduce their attractiveness for some patients. Generally, HU is recommended for older high-risk ET patients, while anagrelide or interferon is recommended for younger patients because of possible leukemogenicity associated with long-term use of HU.

The approach to treatment of patients with PV follows a similar stratification based on probability of developing thrombotic complications. The mainstay of treatment for all patients has not changed in several decades—phlebotomy is recommended for all patients to keep the hematocrit below 0.45. Stable patients at low risk for thrombosis (age <60 years, no history of thrombosis) might not require additional cytoreductive therapy. In patients at high risk of thrombosis or with a very high phlebotomy requirement, myelosuppressive therapy is indicated. As with ET, the choice of cytoreductive agent is age-adapted. Typically, busulfan or interferon is used for older patients (>70 years), HU is the agent of choice in middle-aged patients (50 to 70 years of age), and interferon is preferred in younger patients (<50 years).

An ongoing question has been the decision whether or not to treat PV patients with aspirin in an attempt to reduce the incidence of major thrombotic events. Although thrombotic complications in patients receiving cytoreductive treatment are far less frequent than in untreated patients, they still remain a major cause of morbidity and mortality. The efficacy and safety of antithrombotic drugs is unclear. An initial study suggested that aspirin increased the risk of bleeding.

### Table 5. Molecular markers for Philadelphia chromosome-negative CMPD

<table>
<thead>
<tr>
<th>Marker Description</th>
<th>Percentage Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRV-1 Overexpression in CMPD:</strong></td>
<td></td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>69% to 100%</td>
</tr>
<tr>
<td>Idiopathic myelofibrosis</td>
<td>50% to 100%</td>
</tr>
<tr>
<td>Essential thrombocytopenia</td>
<td>33% to 67%</td>
</tr>
<tr>
<td><strong>Impaired Mpl Expression in CMPD:</strong></td>
<td></td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>30% to 95%</td>
</tr>
<tr>
<td>Idiopathic myelofibrosis</td>
<td>67% to 75%</td>
</tr>
<tr>
<td>Essential thrombocytopenia</td>
<td>35% to 68%</td>
</tr>
</tbody>
</table>
with no reduction of thrombotic complications, and resulted in the avoidance of the use of aspirin in treating patients with PV.\textsuperscript{45} Subsequent studies using low-dose aspirin did document an improved clinical outcome (decrease of vascular deaths) and aspirin has become an accepted treatment modality in patients with PV.\textsuperscript{45}

**IDIOPATHIC MYELOFIBROSIS**

Idiopathic myelofibrosis (myelofibrosis with myeloid metaplasia) has remained one of the least understood of the MPDs. Aspects of the disease which remain inadequately explained include the cause of the blunting of hematopoiesis with the development of anemia and thrombocytopenia, and the cause of the displacement of hematopoiesis from the bone marrow to extramedullary organs.

IMF has been reported to be a clonal disorder involving erythroblasts, megakaryocytes, granulocytes, monocytes, and B and T lymphocytes, originating from a pluripotent progenitor. The number of CD34\textsuperscript{+} progenitor cells in the bone marrow of patients with IMF are increased in the early hypercellular stages of the disease, indicating a higher proliferative activity of the precursor cell pool. When the disease evolves into an overt fibroblastic stage, bone marrow progenitor cells are usually reduced in number.\textsuperscript{46} Simultaneously, hematopoietic stem cells mobilize and exit the bone marrow, traveling via the peripheral blood to colonize the spleen and other organs. The clonal cells excessively produce hematopoietic, fibrogenic, and angiogenic growth factors.\textsuperscript{47}

Megakaryocytes have been linked to the induction of an abnormal cytokine environment that is critical for the stimulation of fibroblasts, causing collagen fibrosis. The megakaryocyte lineage has been shown to have a proliferative advantage, demonstrated by the elevated growth of progenitor cells in vitro, by their enhanced sensitivity to thrombopoietin (TPO), or by their autonomous growth. Platelets and megakaryocytes of patients with IMF express an abnormal TPO-receptor (Mpl) isoform that is paradoxically poorly expressed on the cell surface, yet associated with an enhanced response to TPO and a proliferative advantage.

Mouse models of IMF have documented that TGF-\(\beta\) and osteoprotegerin (OPG) are essential for the development of myelofibrosis and osteosclerosis, respectively. Murine hematopoietic cells in which the TGF-\(\beta\) gene has been ‘knocked-out’ (TGF-\(\beta\)-\textsuperscript{-/-}) cannot produce myelofibrosis. OPG is a molecule secreted by TGF-\(\beta\) activated osteoblasts, which blocks a cytokine signaling pathway for the activation of osteoclast precursors to osteoclasts. In IMF, there is increased production of OPG, in response to elevated TGF-\(\beta\) which is directly responsible for the osteosclerosis seen in this disorder.\textsuperscript{48}

An interesting finding in patients with IMF is the observation of excessive and pathologic emperipolesis of polymorphonuclear leukocytes (PMN) into the megakaryocyte. It has been proposed that PMN entering the megakaryocyte release proteases which cause cell lysis and leaking of TGF-\(\beta\) and other \(\alpha\)-granular proteins.\textsuperscript{49}

Neoangiogenesis, or formation of new vessels, has emerged as a hallmark of IMF. Immunohistochemical staining demonstrates that 70% of patients with IMF had a substantial increase in bone marrow microvessel density.\textsuperscript{50} Neoangiogenesis has now been documented as an integral component of medullary and extramedullary hematopoiesis. There is also a correlation between angiogenesis and bone marrow cellularity and the amount of spleen and blood CD34\textsuperscript{+} hemopoietic stem cells.\textsuperscript{48,50} Recently, increased serum levels of VEGF have been demonstrated in most patients with IMF, suggesting a cytokine-mediated stromal reaction inducing angiogenesis in IMF.\textsuperscript{51}

**Therapeutic options in IMF**

The conventional therapeutic options for IMF included supportive care, chemotherapy, or biologic-modifying agents. Allogeneic stem cell transplantation has usually been used in the setting of advanced and refractory disease, often after the failure of standard therapy. New approaches being investigated in the treatment of IMF include some of the same ones being used for other MPD and MDS. Reduced-intensity conditioning stem cell transplantation appears to reduce transplantation-related mortality while maintaining a graft-versus-leukemia reaction potentially sufficient to obtain disease eradication.\textsuperscript{48} Thalidomide is being studied for its antiangiogenic action and modulation of cytokines, particularly TNF-\(\alpha\). In preliminary studies, thalidomide has been reported to ameliorate anemia, thrombocytopenia and splenomegaly in a subset of patients.\textsuperscript{51}

Imatinib mesylate (STI571, Gleevec) is a potent and selective tyrosine kinase inhibitor with significant in vitro activity against c-abl and bcr-abl. Imatinib also inhibits two other tyrosine kinases: c-kit (CD117), which is highly expressed on CD34\textsuperscript{+} cells in IMF, and the platelet derived growth factor receptor, believed to play a role in the pathogenesis of the fibrosis. Imatinib is being investigated as a potential therapy in IMF, with mixed early results.\textsuperscript{48}
SUMMARY

Our understanding of the exact molecular and genetic alterations responsible for the evolution of the diverse diseases included under the myelodysplastic and myeloproliferative disorders lags behind that of the acute leukemias and CML. However, progress is being made, and new tests for molecular markers (increased PRV-1/CD177, decreased Mpl expression) are being developed. While in the past, treatment for both groups of diseases was primarily supportive, an improved understanding of the underlying pathobiology has led to new treatments with promising preliminary results. The laboratory's role in the diagnosis, prognosis, and determination of efficacy of treatment will continue to expand as new tests become available.

REFERENCES

FOCUS: NEOPLASTIC HEMATOLOGIC DISORDERS


TEXAS: Faculty Position

Faculty position, full-time twelve month tenure-track position in the Clinical Laboratory Science and Molecular Pathology Programs at the Texas Tech University Health Sciences Center. Qualified candidates must be certified in clinical laboratory science and have a graduate degree (doctorate preferred). Responsibilities include teaching clinical immunology and immunohematology (blood banking), development of relevant preceptorship materials, evaluation of policies and procedures within the program, evaluation of curriculum and program effectiveness, and development of scholarly activities to include publications, presentations, and research.

A letter of application, current vitae, and official transcripts should be sent to:

Lori Rice-Spearman, Chair of Search Committee, TTUHSC 3601 4th Street, Mail Stop 6281, Lubbock, Texas 79430
Phone: (806) 743-3252 or email: lori.ricespearman@ttuhsc.edu.

Review of applications will begin immediately and continue until position is filled.

AA/EOE/ADA1
FOCUS: NEOPLASTIC HEMATOLOGIC DISORDERS

Advances in Acute Lymphoblastic Leukemia

TIM R RANDOLPH

DATA SOURCES: Current literature

DATA SYNTHESIS: Acute lymphoblastic leukemia (ALL) is a stem cell disorder characterized by an overproduction of lymphoblasts in the bone marrow that eventually spill into circulation, producing lymphocytosis. As with the other acute leukemias, the most common symptoms experienced by patients include fatigue, bleeding, and recurrent infections resulting from the suppression of normal hematopoiesis in the bone marrow by the accumulating blasts. ALL primarily affects children and exhibits the best response to standard chemotherapy as compared to acute myeloblastic leukemias (AML). Further, remission rates are highest among ALL patients, many of whom are experiencing sustained remissions suggesting cure. In light of early treatment successes, researchers began to investigate modifications of standard treatment regimens to accommodate variability in weight, age, and response to therapy among children with ALL. Individualized treatment plans were implemented where some patients received a reduced intensity course of therapy to minimize drug toxicity while others received drug intensification to maximize response. More recently, research efforts have been directed at the elucidation of leukemogenic mechanisms implicated in ALL to identify specific protein mutants that can be used to design drugs tailored to interfere with the activity of these mutant protein targets. Identification of chimeric proteins produced from chromosomal translocations and gene expression profiles from microarray analyses are the primary techniques used to identify the potential therapeutic targets.

CONCLUSION: Several reliable prognostic indicators have been identified and are being used to improve therapeutic planning and outcome prediction in ALL patients. Individualized treatment regimens have been developed based on the specific characteristics of each patient to minimize treatment related adverse events and maximize response. Through the use of cytogenetic, molecular, and microarray testing, ALL classification schemes have improved and potential therapeutic targets have been identified. It is anticipated that the next major advance in the treatment of ALL will involve the use of designer therapies developed to specifically interfere with particular molecular abnormalities producing the leukemogenic aberration to the normal signal transduction pathways.

ABBREVIATIONS: 6MP = 6-mercaptopurine; ABL = abelson oncogene found in a strain of mouse leukemia virus; ALL = acute lymphoblastic (lymphocytic) leukemia; BCR = breakpoint cluster region; CBF = core-binding factor; CCG = Children’s Cancer Group; CDK = cyclin-dependent kinases; EFS = event free survival; FAB = French-American-British; HAT = histone acetyltransferase; HDAC = histone deacetylase; MLL = mixed-lineage leukemia; MRD = minimum residual disease; OPAL1 = outcome prediction for acute leukemia number 1; POG = Pediatric Oncology Group; RB = retinoblastoma; RFC = reduced folate carrier; TPMT = thiopurine methyltransferase; WHO = World Health Organization.


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Shirlyn B McKenzie PhD CLS(NCA) is the Focus: Neoplastic Hematologic Disorders guest editor.

Focus Continuing Education Credit: see pages 247 to 249 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES

1. Briefly outline the FAB classification of acute lymphoblastic leukemia (ALL), the immunological revisions to the classification of ALL and the proposed changes to ALL classification schemes by the World Health Organization (WHO).
2. Discuss the prognostic indicators of ALL to include:
   a. clinical indicators.
   b. molecular indicators.
   c. therapeutic response indicators.
3. Briefly describe patient stratification strategies using:
   a. risk assessment (as predicted by clinical and cytogenetic data).
   b. response to therapy.
   c. microarray profiling.
4. Identify potential therapeutic targets with a focus on chimeric genes produced from chromosomal translocation and mutations in proteins affecting tumor suppressor gene pathways.

Acute lymphoblastic (lymphocytic) leukemia (ALL) is a stem cell disorder in which the bone marrow produces an increased number of blasts that accumulate and eventually spill into circulation. It is thought that the disease begins with genetic mutations that occur in a hematopoietic stem cell. These mutations accumulate in the stem cell to produce three general functional impairments: increased rate of proliferation by increasing self-renewal and resistance to negative growth controls, loss of differentiation beyond the blast stage, and reduced apoptotic death. The types of genetic mutations identified thus far in ALL can be placed into one of three general categories: aberrant expression of proto-oncogenes, controls, loss of differentiation beyond the blast stage, and reduced apoptotic death. The types of genetic mutations identified thus far in ALL can be placed into one of three general categories: aberrant expression of proto-oncogenes, chimeric protein kinases and transcription factors resulting from chromosomal translocations, and hyperdiploidy involving more than 50 chromosomes. These cellular aberrations cause patients to present with many of the peripheral blood findings commonly associated with most forms of acute leukemia to include increased WBC count, blasts in circulation, neutropenia, anemia, and thrombocytopenia. The presence of blasts in the peripheral blood is due to their release from the overcrowded bone marrow accounting for the elevated WBC count. The neutropenia, anemia, and thrombocytopenia result from the inhibition of normal hematopoiesis by the accumulating bone marrow blasts that deprive the normal hematopoietic tissue of space, nutrients, and growth factors. Failure of bone marrow or other organ systems resulting from the deposition and accumulation of blasts is responsible for the morbidity and mortality experienced in ALL patients.

ALL is primarily considered a leukemia of childhood but it can occur in individuals of any age. The prevalence of ALL is bimodal with the majority of cases occurring between the ages of 2 and 10 years and the remaining minority occurring in the elderly. Prior to the 1960s, treatment success was dismal in all forms of acute leukemia, to include ALL, both in children and in adults. However, the introduction of chemotherapeutic agents significantly improved outcomes for patients with ALL compared to the other acute leukemias. Today, the five-year event-free survival (EFS) rate in children with ALL is 80% while in adult ALL patients it is 40%. Children presenting with one type of ALL known to have a good prognosis are experiencing complete remission rates of 90% with approximately 60% of those being potentially cured. In contrast, adults experience a complete remission rate of 68% to 91% with an estimated cure rate of 25% to 41%.

Classification of ALL has changed over the years and is still evolving. The first universally accepted classification scheme was the French-American-British (FAB) system that relied primarily on morphologic criteria. Once cytochemical staining confirmed theblast lineage as lymphoid, ALL patients could be classified into one of three categories; L1, L2, or L3, based on the morphology of the lymphoblasts. Generally, L1 lymphoblasts were small and homogeneous, L2 lymphoblasts were large and heterogeneous, and L3 lymphoblasts were large, homogeneous, dark staining, and heavily vacuolated. The L1 morphology accounted for 71% of ALL cases, L2 represented 27%, and L3 morphology was observed in the remaining 3% of patients. After only a few years of using the system it became clear that the L1 and L2 morphologic groups did not predict patient age, lymphocyte subtype (T-cell vs. B-cell), response to treatment, or outcome, and it was eventually considered clinically irrelevant. Although the L3 group did prove to be a reliable predictor of a true ALL subtype (mature B-cell), another classification system was needed.

A more clinically relevant system of ALL classification was accomplished by immunologic detection of cell surface and cytoplasmic proteins known as immunophenotyping. By using a dozen or more monoclonal antibodies specific to individual protein markers, the presence or absence of the corresponding protein resulted in the identification of at least four different subtypes of ALL; early pre-B (progenitor-B), precursor-B, mature-B, and T-cell (Table 1). Two additional subclasses, common acute lymphoblastic leukemia (cALLa) and precursor-T cell have also been described. The cALLa subtype is very similar to progenitor-B cell ALL, differing by only one surface marker (CD10) causing some to fold cALLa ALL into the progenitor-B ALL category creating early pre-B ALL. Similarly, some have chosen to collapse the precursor-T cell ALL and mature T-cell ALL into one category citing no clinical relevance to the distinction.
The newest classification system was introduced by WHO and focuses on immunologic, cytogenetic, and molecular measures instead of morphologic features. Regarding ALL, the WHO group retained two of the immunologic groups (mature B-cell and T-cell) and subdivided the precursor-B cell groups into four distinct cytogenetic subtypes. The FAB group, L3, was found to be consistent with the immunologic group, mature-B cell ALL, and is represented in the WHO classification system as Burkitt cell (B-cell) ALL. The immunologic group, T-cell ALL, was also retained in the WHO classification system as precursor T-cell ALL. The two precursor B-cell ALL subtypes described in the immunological classification system were subdivided into four distinct subtypes based on the following cytogenetic abnormalities: t(9;22)-BCR/ABL; t(v;11q23)-MLL rearrangement; t(1;19)-E2A/PBX1; and t(12;21)-ETV/CBFα. The specifics of these four translocations will be discussed later.

Hematologists have long recognized that discussions of ALL are viewed as bitter sweet. On the one hand, treatment success and the potential for cure in ALL patients has outpaced all other acute leukemias bringing excitement and hope to the field. However, the realization that the majority of patients are children brings regret as we witness the morbidity resulting from treatment sequelae and inevitable mortality in a subset of patients who fail to respond to therapy. The focus of recent advances in ALL are directed at identifying predictors of treatment success so patients can be stratified into groups that can be offered a reduced treatment protocol to minimize sequelae and those requiring more intensified treatment regimens to improve outcomes. The future of ALL will involve the development of new therapeutic drugs tailored to target particular molecular abnormalities that produce aberrant signal transduction pathways with the hope of improving outcomes in every subtype of ALL.

Table 1. ALL classification based on immunophenotyping

<table>
<thead>
<tr>
<th>Marker</th>
<th>Early pre-B cell</th>
<th>Pre-B cell</th>
<th>Mature-B cell</th>
<th>T-cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD34</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD10</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>CD20</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cytoplasmic μ (cμ)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Surface Ig (sIg)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ig gene rearrangement</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD3</td>
<td>-</td>
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<tr>
<td>CD5</td>
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<td>+</td>
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<tr>
<td>CD7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
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<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TCR gene rearrangement</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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</tbody>
</table>

PROGNOSTIC INDICATORS

Both patients and physicians are most interested in disease indicators that will best predict therapeutic responses and prognostic outcomes. Drug selection, when more than one type is available for a given disorder, and appropriate dosing are of utmost concern. Selection of an inferior drug or a dosing schedule that is too low to be effective or so high as to produce adverse events are to be avoided. Above all, physicians and patients desire to be empowered with sufficient information to accurately identify the disorder, predict how well the disease will respond to the chosen therapy, and predict the patient outcome. Several prognostic indicators will be discussed to include clinical indicators, genetic and molecular indicators, and therapeutic response indicators (Table 2).

CLINICAL INDICATORS

To date, the most reliable predictors of treatment success for all forms of ALL are patient age and white blood cell count (WBC) at presentation. These two variables have maintained their reliability over the past forty years even into the age of molecular biology. A threshold has been set for both variables by the National Cancer Institute (NCI)/Rome placing all patients into one of two categories for each variable. The threshold for age is ten years while the cut-off for WBC count is 50,000/uL. Patients who meet both criteria (<10 years of age and WBC <50,000/uL) are considered standard risk and those who are either ≥10 years of age or present with a WBC count of ≥50,000/uL are considered higher risk. These criteria are not only reliable but are also easily obtained in almost all clinical settings. One exception to the rule involves patients less than one year of age who consistently have a poor prognosis. WBC count remains a reliable prognostic pre-
dictor because the number of WBCs reflects tumor burden and is suggestive of leukemic growth rate and aggressiveness.

Other prognostic indicators include gender, blast phenotype, and presence of disease within the central nervous system. Girls consistently show a more favorable response compared to boys regardless of treatment intensity. Historically, mixed lineage ALL blasts coexpressing myeloid markers (My+) have a less favorable prognosis: however, changes in therapy have equalized the prognosis of mixed lineage ALL to that of pure B-cell ALL. Patients with a T-cell type ALL have a poorer prognosis than those with B-cell ALL. Patients with a T-cell type ALL have a poorer prognosis but also express poor prognostic indicators like older age, mediastinal mass, and lymphadenopathy. In addition to differences in prognosis, patients with T-cell ALL are placed into a separate category because of differences in response to certain chemotherapeutic agents. T-cell blasts are more sensitive to asparaginase and 2-amino-6-methoxypurine arabinoside (506U), while being less sensitive to methotrexate. The drug 506U is a water-soluble prodrug converted to ara-G by adenosine deaminase. Blasts in the cerebrospinal fluid (CSF) is considered a poor prognosis even when the CSF WBC count is normal.

GENETIC AND MOLECULAR INDICATORS

Blast hyperdiploidy is a consistently positive prognostic indicator and is observed in about one third of patients with childhood ALL. These patients show hyperdiploidy (chromosome numbers around 50) in their blast cells that exhibit an increase in apoptosis in vitro and sensitivity to various chemotherapeutic agents. Patients with trisomies of chromosomes 4, 10, and 17 express event-free survivals (EFS) of between 75% to 90%, which improves to >90% in patients with triple trisomies. In contrast, patients with hypodiploidy and trisomies of chromosome 5 have a poorer prognosis. EFS is approximately 40% (±18%) for patients with 33 to 44 chromosomes in their blasts and it drops to 25% (±22%) with chromosome numbers of less than 28.

Another one-third of the ALL population exhibit one of four chromosomal translocations in the absence of increases in chromosomal number which can predict outcome. Three of the four translocations: t(1;19), mixed-lineage leukemia (MLL) translocations, and the t(9;22) Philadelphia chromosome are associated with a poor prognosis while the most common translocation, t(12;21), predicts a favorable response. The t(1;19) translocation fuses the E2A and Pbx1 genes and is observed most often in pre-B ALL in which cytoplasmic µ heavy chains are identified. Translocations involving the MLL gene are observed in approximately 6% of ALL patients and can involve over 40 different chromosome partners. The most common MLL translocation is the t(4;11) that predominates in the infant ALL population.

The Philadelphia chromosome is present in approximately 3% to 5% of children and 20% of adults with ALL. Although Philadelphia positive ALL produces the BCR/ABL fusion gene, most noteworthy in chronic myelocytic leukemia (CML), a different breakpoint in the BCR produces a fusion protein that makes the disease more difficult to treat. As in CML, the chimeric fusion protein produced in ALL is

<table>
<thead>
<tr>
<th>Table 2. Prognostic indicators for ALL</th>
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<tbody>
<tr>
<td><strong>Prognostic Indicator</strong></td>
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<tr>
<td>WBC count</td>
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<tr>
<td>Age at presentation</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Blast phenotype</td>
</tr>
<tr>
<td>Abnormal karyotypes</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>BM blast count during induction therapy</td>
</tr>
</tbody>
</table>
a constitutive protein kinase that abnormally phosphorylates and activates multiple signaling pathways resulting in loss of controls on cell proliferation, survival and self-renewal. Even therapy involving the tyrosine kinase inhibitor imatinib mesylate that has revolutionized CML treatment does not produce a favorable response in ALL.

The most common translocation observed in childhood ALL, the t(12;21), brings together the TEL and AML1 genes and is observed in approximately 25% of patients with precursor B ALL. The translocation is associated with sensitivity to asparaginase and predicts an excellent prognosis.

THERAPEUTIC RESPONSE INDICATORS

Evidence is compounding that dramatically lowered blast counts achieved following seven days of conventional induction chemotherapy is predictive of five-year EFS rates. A Children’s Cancer Group (CCG) study reported that at day 7 of therapy, 52% of children who achieved a markedly lowered marrow blast count (M1) had an 80% (±1%) EFS. Of the remaining patients, 23% achieved a moderate reduction (M2) in the marrow at day 7 with an EFS rate of 74% (±2%) while the remaining 25% only experienced a mild reduction (M3) in marrow blasts and an EFS rate of 68% (±2). Another CCG study reported improved EFS rates in ALL children who were given intensified therapy regimens beginning at day 7 of conventional therapy when slow early response rates (SER) were observed. More aggressive courses with methotrexate and asparaginase produced EFS rates of 75% (±3.8%) compared to 55% (±4.5%) for those remaining on standard therapy. This evidence suggests that rigorous assessment of blast counts at day 7 of conventional therapy will not only predict outcomes but it could also govern therapy decisions. Patients achieving dramatically lowered blast counts may receive a reduction in therapy, patients with a moderate reduction may remain on standard therapy, and those achieving only mild reductions may be given an intensified regimen. More sensitive methods of measuring blast counts using flow cytometry and molecular techniques are redefining minimum residual disease (MRD) for children with ALL. Patients with no detectable MRD at day 7 of therapy showed >90% EFS at three years while children with high MRD exhibited a 25% three year EFS rate. These types of assessments will continue to shape therapy decisions and impact prognosis.

RISK CATEGORIES

Using the clinical, genetic, and therapeutic response indicators, children with precursor-B ALL can be grouped into one of four risk categories; low, standard, high, and very high. Low risk is defined as patients who present with the standard risk clinical criteria (<10 years of age; WBC <50,000/uL), low risk cytogenetic markers (t(12;21), TEL/AML1 or trisomies of chromosomes 4, 10, and 17) and dramatic reductions in bone marrow blasts at day 7 of induction chemotherapy. Approximately 22% of cases of precursor-B ALL meet the criteria for low risk and are eligible for a reduction in therapy intensity.

Standard risk children present in one of three ways. First, they may exhibit standard clinical risk factors but do not show either low risk or high risk cytogenetic markers. Second, standard risk children may exhibit high risk clinical indicators and low risk cytogenetic indicators. Third, patients may present with standard risk clinical indicators and low risk cytogenetic markers but exhibit slow clearance of bone marrow blasts following seven days of induction chemotherapy. The standard risk group comprises about half of the patients with precursor-B ALL and is a heterogeneous group. While some patients could achieve the same long standing outcome with reduced intensity therapy, a significant number will relapse following remission.

Another 30% of children with precursor-B ALL will fall into the high risk category in which patients present with both high risk clinical features and high risk cytogenetic indicators like t(4;11) and t(1;19). A very high risk group represents the remaining 3% of children with precursor-B ALL and is characterized by the presence of the Philadelphia chromosome [t(9;22)] or hypodiploidy (<45 chromosomes).

TREATMENT GROUPS

In light of the early successes experienced with chemotherapy, physicians began to recognize that childhood ALL holds great promise in achieving dramatic outcome improvements by implementing individualized treatment strategies. While over 75% of children with ALL have been cured through medication alone, the remainder will ultimately succumb to their disease. Because there is great diversity in patient age, liver function, and physical size, the maintenance of drug levels within the narrow therapeutic range requires close attention at more frequent intervals. Drug levels that rise above therapeutic range can produce drug related adverse events while inappropriately low levels are less effective. In addition, dose escalation in slow early responders has proven successful in achieving EFS in many such patients.

A newly organized panel of experts called the Children’s Oncology Group, representing a merger of the Pediatric Research Group and the Children’s Cancer Group, has proposed a single treatment stratification strategy. The panel developed four
distinct treatment groups: T-cell, infant, high risk precursor-B, and standard risk precursor-B. These four treatment groups represent an amalgamation of prognostic indicators that classify patients at the time of diagnosis based on the following criteria: age, presenting WBC count, and immunophenotype. Patients less than 12 months of age are placed into the infant category, a poor prognostic indicator. Immunophenotyping will divide the remaining patients into T-cell and B-cell types. The B-cell group is further divided into high risk and standard risk based on the WBC count at presentation. The two precursor-B groups are reassessed at day eight, following the one-week induction period, and at days 15 and 29 to determine molecular abnormalities in the blasts, response to therapy, and bone marrow morphology. Quantitation of minimum residual disease is also assessed at day 29. At each assessment point, all precursor-B ALL patients are assigned to one of four prognostic groups: low risk, standard risk, high risk, and very high risk based on the definitions previously described. Treatment strategies will be adjusted appropriately as patients move among prognostic groups. The emergence of MLL mutations will prompt treatment intensification while t(12;21)-TEL/AML1 may justify relaxation of the treatment regimen.

Mutations in genes that code for drug metabolizing proteins will also reduce drug clearance resulting in toxic sequelae. The chemotherapeutic agent 6-mercaptopurine (6MP) is an important drug in the treatment of ALL but requires the enzyme thiopurine methyltransferase (TPMT) to accomplish normal drug metabolism. Inherited point mutations in TPMT genes reduce the metabolism of 6MP by increasing the natural proteolytic degradation of the TPMT enzyme. Approximately 1 in 300 people have a deficiency of TPMT with 10% expressing heterozygosity at the TPMT locus.Individuals expressing inactivating mutations on both TPMT alleles cannot inactivate 6MP through normal methylation, resulting in toxic accumulation of active thioguanine nucleotides. Many patients experience life-threatening toxicities from normal doses of 6MP. Studies have confirmed that dose reductions are most often indicated in homozygous TPMT mutants, required less often in heterozygous TPMT mutants, and rarely needed in wild-type patients. However, since slowed metabolism from TPMT mutations results in elevated 6MP blood levels, reduced doses still provide therapeutic plasma drug levels needed to produce the desired antileukemic effect.

Point mutations in genes that code for other detoxifying enzymes can also affect antileukemic drug metabolism prompting dose modifications to produce the desired antileukemic effect without the accompanying toxicity. Polymorphisms in the number of tandem repeats in the enhancer of the thymidylate synthetase gene increase the expression of the corresponding protein resulting in excess metabolic turnover of methotrexate and a poorer outcome. Mutations in the RFC1 gene (reduced folate carrier), produces a protein that is unable to effectively transport methotrexate from the blood stream into ALL blast cells.

**ALL SUBTYPES USING MICROARRAYS**

Microarray is a new and powerful technology that is used to determine gene expression profiles in cell populations. Gene expression patterns have the potential to produce a specific signature for a particular cancer type or subtype. Microarray testing slides are prepared by spotting either PCR amplified complementary DNA (cDNA) molecules or synthesized oligonucleotides complementary to specific genes onto a specially coated glass slide. Hundreds to as many as 50,000 gene sequences can be spotted onto a single microarray slide. The cells to be tested for gene expression are separated from contaminant sequences and total RNA is isolated from the cell population. All mRNA species are reverse transcribed into cDNA using three regular and one fluorescently labeled deoxynucleotide triphosphate (dNTP) that bears a particular fluorescent color. In most applications this process is applied to both a population of normal and abnormal cells using two different fluorescent labels so to distinguish their respective gene expression profiles by color differences. When performing microarrays to evaluate leukemias, normal and leukemic cells found in blood or bone marrow are first separated by density gradient centrifugation. The fluorescently labeled cDNA molecules are then generated such that the normal cells emit one fluorescent color and the leukemic blasts emit another. The gene expression patterns of the leukemic blasts can be compared to the normal counterpart.

Researchers performing experiments to analyze gene expression panels in ALL patients using microarrays are proposing alternate ALL classification schemes. One report studied gene expression profiles on 327 pediatric ALL samples and proposed 7 distinct ALL subtypes: T-ALL; t(1;19) E2A-PBX1; t(9;22) BCR/ABL; t(12;21) TEL/AML1; MLL rearrangements; hyperdiploid (>50 chromosomes); and a novel subtype (Figure 1). Most of the earlier analyses were performed on pre-spotted arrays containing 10,000 genes while the latter analyses were performed on similar arrays containing 33,000 genes. Another investigator using microarray gene expression profile data proposed that MLL expressing leukemias be classified as a separate entity distinct from both ALL and AML citing the existence of a unique expression profile.
involving FLT3 and certain HOX genes.\textsuperscript{27} It is known that the HOX genes represent a family of transcription factors that regulate expression of genes important in both embryogenesis and in self-renewal and proliferation of hematopoietic stem cells.\textsuperscript{28,29} The reliability of using gene expression profiles on microarrays to accurately classify or subtype leukemias is still in question. However, by using statistical approaches to identify class discriminating genes and sophisticated computer-assisted supervised learning algorithms to determine if the class discriminating genes are useful in leukemia classification, some groups are reporting an accuracy of 96\% in the subtyping of various pediatric ALL patients.\textsuperscript{29} Using this approach it is estimated that as few as 20 genes, analyzed in parallel, may be sufficient to diagnose the different ALL subtypes proposed. In addition, consistent gene expression patterns suggest potential links to leukemogenic mechanisms of transformation. However, caution must be exercised in making such assumptions because many inappropriately expressed genes from the leukemic clone may retain important diagnostic value, but prove not to be leukemogenic.

Another group, led by Mosquera-Caro, used microarrays to analyze 127 cases of infant leukemias that were classified as either ALL or AML by traditional methods (Figure 2). Infant leukemias were defined based on a patient population of less than 356 days of age which characteristically show poor survival rates of <25%. Pre-spotted microarrays containing 12,625 genes were used and expression results were analyzed using sophisticated statistical approaches and computer-assisted nonsupervised clustering analysis. In nonsupervised cluster analysis the computer algorithm will identify aggregates, or clusters, of like data and place them into groups that were not predetermined. This process is able to create categories that may not have previously existed. Following the analysis, three biologically distinct groups emerged but none clustered according to the AML vs. ALL divisions nor was clustering based on the expression of MLL rearrangements. For example, the first group contained 21 cases in which 16 were previously categorized as ALL and the remaining 5 as AML. The types of genes expressed (EPOR, AML1, KIT, CD34, FLT1, and HOX) were consistent with gene profiles found in very primitive hemato-

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**Figure 1.** Supervised microarray analysis of pediatric ALL patients

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>327 pediatric ALL samples</td>
<td>↓</td>
</tr>
<tr>
<td>Isolate mRNA</td>
<td>↓</td>
</tr>
<tr>
<td>Reverse transcribe to cDNA</td>
<td>↓</td>
</tr>
<tr>
<td>Labeled with fluorescent dNTP</td>
<td>↓</td>
</tr>
<tr>
<td>Hybridize to 10,000 – 33,000 pre-spotted genes</td>
<td>↓</td>
</tr>
<tr>
<td>Perform supervised computer analysis</td>
<td>↓</td>
</tr>
<tr>
<td>As few as 20 genes can segregate samples into 7 categories</td>
<td>↓</td>
</tr>
<tr>
<td>T-ALL</td>
<td>↓</td>
</tr>
<tr>
<td>t(1;19) E2A/PBX1</td>
<td>↓</td>
</tr>
<tr>
<td>t(9;22) BCR/ABL</td>
<td>↓</td>
</tr>
<tr>
<td>t(12;21) TEL/AML1 mutations</td>
<td>↓</td>
</tr>
<tr>
<td>MLL hyperdiploid &gt;50 chr.</td>
<td>↓</td>
</tr>
<tr>
<td>novel type</td>
<td>↓</td>
</tr>
<tr>
<td>Possibly unique leukemia</td>
<td>↓</td>
</tr>
<tr>
<td>not ALL or AML</td>
<td>↓</td>
</tr>
</tbody>
</table>

ABL = ablleson oncogene; BCR = breakpoint cluster region; cDNA = complementary DNA; dNTP = deoxynucleotide triphosphate; E2A = a.k.a. TCF3 (transcription factor 3); MLL = mixed-lineage leukemia; mRNA = messenger RNA; PBX1 = pre-B-cell transforming factor; TEL = ETS protein (oncogene from E26 erythroblastosis virus).
poietic stem cells. The second cluster contained 52 cases, 51 of which were previously categorized as ALL. This group exhibited a more homogeneous gene expression pattern: CD19, IgM, BCL7 and t(4;11) that resembled committed precursor-B cells. The last cluster consisted of 54 cases in which 42 were previously diagnosed as AML and 12 cases as ALL. This group exhibited a more heterogeneous gene expression pattern mainly involving genes in or related to the RAS family: ECGF, CCR1, MAPKAPK3. Interestingly, members in all three groups expressed MLL rearrangements, albeit different types of rearrangements, with varying levels of penetrance.20

A retrospective pediatric study by Mosquera-Caro, using microarrays on a large cohort of pediatric ALL patients from two Pediatric Oncology Group (POG) studies, identified nine distinct biologic clusters in ALL. The patients tested by microarray analysis were segregated into six groups: t(4;11), t(9;22), t(1;19), t(12;21), monosomy 7, and monosomy 21 based on cytogenetic abnormalities, and five additional demographic categories. The retrospective design involved patients who were past the fourth year of therapy so they could be subdivided into two treatment groups, those who failed therapy and those who achieved complete remission. A similar design as was used in the infant ALL studies previously described was also applied to the pediatric study, except the analytical approach was supervised because the patients were first segregated into groups. The supervised approach is looking for gene expression patterns that characterize previously existing categories. The microarray results exhibited similar gene expression patterns in each cluster. As expected, each cluster had in common the cytogenetic abnormality used to originally subdivide the patient cohort. However, when a full unsupervised approach (not segregated into cytogenetic groups) was performed on 147 target genes, nine distinct biologic clusters emerged. These nine biologic clusters included two T-cell clusters and seven precursor-B cell clusters. Although there were over 100 unique genes that characterized these nine clusters, surprisingly there were no cytogenetic abnormalities that defined any of the clusters. In addition, three novel genes were discovered and named G0, G1, and G2. The G0 gene showed the greatest power to divide the cohort into good and poor outcome groups. The G0 gene was cloned and named OPAL1, which stands for outcome prediction for acute leukemia.

Figure 2. Nonsupervised microarray analysis of infant ALL patients

<table>
<thead>
<tr>
<th>127 infant ALL samples (&lt;1 year old)</th>
<th>↓</th>
<th>Isolate mRNA</th>
<th>↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcribe to cDNA</td>
<td>↓</td>
<td>Labeled with fluorescent dNTP</td>
<td>↓</td>
</tr>
<tr>
<td>Hybridize to 12,625 pre-spotted genes</td>
<td>↓</td>
<td>Perform nonsupervised computer analysis</td>
<td>↓</td>
</tr>
</tbody>
</table>

3 clusters: Group #1 (n = 21) Group #2 (n = 52) Group #3 (n = 54)
Leukemia type: ALL=16, AML=5 ALL=51, AML=1 ALL=12, AML=42
Genes: EPOR, AML1, KIT, CD34, FLT1, HOX CD19, IgM, BCL7, ECGF, CCR1, MAPKAPK3 t(4;11)

AML1 = acute myelocytic leukemia; BCL7 = B-cell leukemia/lymphoma; CCR1 = chemokine receptor; cDNA = complementary DNA; dNTP = deoxynucleotide triphosphate; ECGF = endothelial growth factor; EPOR = erythropoietin receptor; FLT1 = fms related tyrosine kinase 1; HOX = homeobox transcription factor; KIT = tyrosine kinase receptor for stem cell factor (feline sarcoma virus oncogene); MAPKAPK3 = mitogen-activated protein kinase-activated protein kinase 3; mRNA = messenger RNA.
As stated earlier, t(12;21) is the most common translocation identified in ALL. Therefore, therapy intensification may be warranted in pediatric ALL patients with low OPAL1 expression even if the other indicators suggest low risk disease. Overall, these data suggest that microarrays may produce ALL subtypes that provide physicians better therapeutic guidance and outcome prediction capabilities than the cytogenetic groups previously defined.

It seems clear that while still in the age of traditional chemotherapy, patient stratification should begin with the clinical indicators of age and WBC count. Therapy can then be adjusted based on bone marrow response at day 7, adverse drug reactions, emergence of certain karyotypes like MLL, and the identification of mutated drug metabolism genes. However, as microarrays and other molecular approaches begin to identify mutations in important proteins central to aberrant signal transduction pathways, new patient stratification systems may be developed around treatment protocols tailored to these mutants.

**FUTURE THERAPEUTIC TARGETS**

Although therapeutic responses in patients with ALL are generally better than other acute leukemias, the identification of new therapeutic targets has the potential of improving outcomes and reducing treatment related sequelae. Current therapeutic strategies are based largely on traditional chemotherapeutic approaches. Most chemotherapeutic drugs produce a cytotoxic effect on malignant cells by interfering with normal DNA replication or protein synthesis systems. In this way, rapidly dividing cells are targeted and eliminated. Unfortunately, some malignant cells will often escape destruction by being quiescent during courses of therapy or being physically separated from the chemotherapeutic agent. In contrast, many normal cells will also succumb to death from chemotherapy due to exposure to the chemotherapeutic agent while in the cell cycle. A therapeutic approach that is more specific to aberrations unique to the malignant clone has the potential to selectively target the leukemic cells while sparing normal cells. A better understanding of normal signal transduction pathways and the aberrant messengers produced by cancer causing mutations observed in ALL, will likely identify future therapeutic targets.

**ABERRANT MESSENGERS CAUSED BY CHROMOSOMAL TRANSLocations**

As stated earlier, t(12;21) is the most common translocation observed in childhood ALL, accounting for approximately 25% of patients with precursor-B ALL. The translocation predicts an excellent prognosis due to its sensitivity to asparaginase. The t(12;21) translocation joins the TEL and AML1 genes from chromosomes 12 and 21, respectively. This translocation creates a chimeric gene in which the head is composed of the 5’ end of the TEL gene and the tail houses the majority of the 3’ end of the AML1 gene. The TEL gene product normally functions as a transcription factor providing a protein/protein interacting domain that is essential in the homing of hematopoietic stem cells to the bone marrow. The normal AML1 gene codes for core-binding factor α (CBFα), the alpha subunit of the heterodimeric transcription factor core-binding factor (CBF), composed of CBFα (AML1) and CBFβ. The CBF heterodimer functions as a transcription factor by binding specific DNA sequences (core enhanced sequence) and recruiting a protein complex with histone acetyltransferase (HAT) activity. HAT proteins activate transcription by acetylating lysine residues in the core histones that unpackage chromatin allowing RNA polymerase to bind and transcribe genes. These genes are instrumental in regulating hematopoietic stem cell growth probably through the action of the HOX genes. By virtue of the retained DNA binding domain of the AML1 moiety, the TEL/AML1 transcription factor is able to dimerize with CBFβ forming the heterodimer complex. This complex will bind the same core enhanced sequence as AML1 preventing normal AML1 from binding. Further, when the TEL/AML1/CBFβ complex binds DNA it recruits proteins with histone deacetylase activity (HDAC) that remove the acetyl groups from the lysine residues causing the chromatin to repack, preventing transcription. Therefore, the t(12;21) translocation produces a hybrid transcription factor that forms a complex preventing the transcription of proteins critical in the regulation of hematopoietic stem cells that alters both self-renewal and differentiation. Small-molecular inhibitors of histone deacetylase are currently in clinical trials and are exhibiting some antileukemic activity when used alone but appear to exert a greater effect when administered in combination with other therapeutic agents.

Approximately 6% of ALL patients possess translocations involving the MLL gene (mixed-lineage leukemia) that can partner with over 40 different chromosomes. The t(4;11) is the most common MLL translocation that predominates in the infant ALL population. The MLL gene codes for a nuclear binding protein that is required to maintain the transcription of HOX genes, particularly HOXA7 and HOXA9, whose importance have already been described. Various translocations involving the MLL gene each result in a fusion protein...
with MLL at the 5’ head and another partner at the 3’ tail. Translocations involving the MLL gene causes overexpression of MLL fusion proteins that enhances the activity of the downstream HOX genes affecting self-renewal, proliferation, and differentiation of stem cells and committed progenitors.  

The t(1;19) translocation is observed in 5% of childhood ALL but occurs in 25% of pre-B ALL in which cytoplasmic μ heavy chains are identified. The translocation fuses the E2A and PBX1 genes from chromosomes 1 and 19, respectively. Both E2A and PBX1 normally function as transcription factors. PBX1 is involved in the regulation of HOX gene expression and the E2A target genes are thought to play some role in hematopoiesis. The protein product of the fusion gene is a chimeric protein that also acts as a transcription factor. The E2A/PBX1 transcription factor disrupts the normal expression of HOX genes and E2A target genes resulting in aberrant stem cell growth patterns. Since the HOX genes are downstream messengers common to the t(12;21), t(4;11) and t(1;19) translocations, they have become attractive targets for the development of therapeutic interventions.

Philadelphia chromosome, caused by the t(9;22) translocation, is well established in chronic myelocytic leukemia (CML) but is also found in about 3% of childhood ALL patients. However, the breakpoint in the BCR gene from chromosome 9 usually occurs in a different position than in CML creating a larger fusion protein that is not sensitive to imatinib therapy. The presence of the Philadelphia chromosome is considered a poor prognostic indicator. Actually, three of the four chromosomal translocations discussed, those involving MLL, t(1;19) and t(9;22), are associated with a poor prognosis while the t(12;21) has a favorable prognosis.

**MUTATIONS IN TUMOR SUPPRESSOR GENES**

Mutations in the proteins involved in the various pathways controlled by the retinoblastoma protein (RB) are common in ALL. The retinoblastoma protein functions as a tumor suppressor by inhibiting transcriptional factors that stimulate expression of proteins necessary for cells to enter the S-phase of the cell cycle. Cell surface signals designed to stimulate proliferation, induce the expression of D-type cyclins like D1, D2 and D3. In general, cyclins are proteins that regulate the cell cycle by combining with cyclin dependent kinases (CDKs) to form an enzymatic complex. These complexes phosphorylate proteins that affect how cells move between phases of the cell cycle. D-cyclins bind with cyclin dependent kinases (CDKs), like CDK4 and CDK6, and phosphorylate the RB protein. Phosphorylation of RB removes the inhibitory activity of the RB protein which, in turn, releases transcription factors like E2F from inhibition. These transcription factors are then free to stimulate the expression of genes that synthesize proteins which allow the cell to enter S-phase of the cell cycle. Thus, lack of RB phosphorylation inhibits cell proliferation. Mutations to RB genes are rare in ALL, but mutations in proteins that affect RB phosphorylation, like p16 and p15 are common in T-cell ALL but also occur in B-cell ALL.

Like the RB protein, p53 is a tumor suppressor that is rarely mutated in ALL patients but whose function is frequently altered by mutations to genes that code for proteins that regulate p53 function. Whereas RB prevents excessive proliferation by inhibiting cells from entering S-phase, p53 triggers the arrest of the cell cycle, also known as apoptosis. Activation of p53 occurs in response to cells that have acquired DNA damage that may be engaged in aberrant cell proliferation. Mutations to proteins that regulate p53 function, like HDM2, p14, and p21, are frequent findings in ALL.

A more thorough understanding of the normal signal transduction pathways involved in the control of cell proliferation, cell differentiation, and natural cell death will provide the framework to determine the manner in which mutated proteins alter these pathways and impact cell growth. Those mutant proteins that have the greatest impact on abrogating normal cell growth patterns become favorable targets for the development of tailored drug therapy. Most experts believe that the next major advance in ALL will lie in the stratification of patients based on particular mutation or gene expression patterns and the development of drugs designed to target the products of these mutations that disrupt the pathways that control normal cell growth.

**REFERENCES**


The Editors of *Clinical Laboratory Science* solicit your assistance in selecting the next recipient(s) of the *CLS* Distinguished Author Award. You are invited to participate in the selection process by completing this ballot and sending it to the editorial office no later than February 1, 2005. The award will be presented at the ASCLS annual meeting in July 2005.

ASCLS members, *CLS* readers, and *CLS* editors will choose the recipient(s) of the award. Nominations should be based on originality and quality of writing, relevance to the laboratory science profession, and integration of theory and application.

Please indicate your selection of the best article for 2004 from the four eligible issues of *CLS*, volume 17, issues 1 through 4. The nominated article can be from any section of the journal.

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Send this completed ballot to: *CLS* Editorial Office, PO Box 5399, Coralville, IA 52241; or fax to (319) 351-2927. Ballot deadline: February 1, 2005
Continuing Education Questions

To receive 3.0 contact hours of intermediate level P.A.C.E. credit for the Neoplastic Hematologic Disorders questions, insert your answers in the appropriate spots on the immediately following page; then complete and mail the form as directed.

NOTE: There may be more answer spaces on the answer sheet than needed. If so, leave them blank. Make sure the number of the answer space you fill matches the number of the question you are answering.

LEARNING OBJECTIVES
1. Compare and contrast the FAB and WHO classification systems for the myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD).
2. Discuss the rationale for the use of antiangiogenic therapy in MDS.
3. Explain epigenetic alterations of DNA, and the functional role of DNA methyltransferase and histone deacetylase (HDAC) in these changes.
4. Describe the role of molecular assays for PRV-1 and Mpl in the diagnosis of MPD.
5. Discuss the role of neoangiogenesis in idiopathic myelofibrosis.
6. Explain why Imatinib (Gleevec) (the bcr-abl tyrosine kinase inhibitor) is effective in some patients with myelofibrosis.
7. Briefly outline the FAB classification of acute lymphoblastic leukemia (ALL), the immunological revisions to the classification of ALL and the proposed changes to ALL classification schemes by the World Health Organization (WHO).
8. Discuss the prognostic indicators of ALL to include:
   a. clinical indicators.
   b. molecular indicators.
   c. therapeutic response indicators.
9. Briefly describe patient stratification strategies in ALL using:
   a. risk assessment (as predicted by clinical and cytogenetic data).
   b. response to therapy.
   c. microarray profiling.
10. Identify potential therapeutic targets for ALL with a focus on chimeric proteins produced from chromosomal translocations and mutations in proteins affecting tumor suppressor gene pathways.

CONTINUING EDUCATION QUESTIONS
1. Which of the following diseases is included in the WHO classification of myeloproliferative diseases, but was not included in the FAB classification?
   a. Chronic myelocytic leukemia
   b. Polycythemia vera
   c. Chronic neutrophilic leukemia
   d. Essential thrombocytopenia
2. Which of the following diseases was eliminated from the FAB classification of myelodysplastic syndromes by the new WHO classification?
   a. Refractory anemia
   b. Refractory anemia with ringed sideroblasts
   c. Refractory anemia with excess blasts
   d. Refractory anemia with excess blasts in transformation
3. The activity of thalidomide in the treatment of MDS and MPD is as an:
   a. farnesyl transferase inhibitor.
   b. antiangiogenic therapy.
   c. histone deacetylase inhibitor.
   d. methyltransferase inhibitor.
4. Hemopoietic stem cell transplantation has limited application in the treatment of MDS and MPD because:
   a. the advanced age of the majority of patients increases the frequency and severity of transplant-related complications.
   b. there is a high relapse risk.
   c. there is difficulty in obtaining suitable transplant material (insufficient autologous harvest, or availability of HLA-matched donor).
   d. All of the above.
5. TGF-β and osteoprotegerin (OPG) are believed to be involved in the pathobiology of:
   a. idiopathic myelofibrosis.
   b. refractory anemia.
   c. polycythemia vera.
   d. refractory anemia with excess blasts.
6. DNA methyltransferase and histone deacetylase (HDAC) have which of the following effects on the functioning of cells?  
   a. They interfere with RAS oncogene signaling.  
   b. They promote heterochromatin formation and gene silencing.  
   c. They block the action of angiogenic cytokines.  
   d. They promote cellular proliferation.

7. Imatinib mesylate (Gleevec) is effective in some patients with myelofibrosis because:  
   a. those patients express the bcr-abl tyrosine kinase.  
   b. those patients have a Philadelphia chromosome+ form of myelofibrosis.  
   c. Gleevec inhibits the c-kit and PDGFR tyrosine kinases found on progenitor cells in myelofibrosis.  
   d. Gleevec blocks the action of the cytokines TGF-β and osteoprotegerin.

8. Molecular assays for PRV-1 and Mp1 may be useful in the diagnosis of:  
   a. the acute myelocytic leukemias.  
   b. myelodysplastic syndromes.  
   c. chronic myelocytic leukemia.  
   d. the myeloproliferative disorders PV, IMF, and ET.

9. Which of the following are the two most reliable clinical indicators that predict treatment success in childhood ALL?  
   a. Mutations in RB and CDKs  
   b. Patient age and WBC count  
   c. Presence of the t(9;22) and t(12;21) translocations  
   d. Hyperdiploidy and OPAL1

10. Select the translocation that predicts the MOST favorable prognosis in ALL.  
    a. Translocations involving MLL  
    b. t(1;19)  
    c. t(12;21)  
    d. t(9;22)

11. Which ALL subtype has remained consistent through the FAB, immunologic, and WHO classifications systems?  
    a. T-cell ALL  
    b. Early precursor-B cell  
    c. L2  
    d. B-cell ALL (Burkitt’s type)

12. Which of the following translocations found in ALL is LEAST likely to exert leukemogenic activity by altering HOX gene activity?  
    a. Translocations involving MLL  
    b. t(1;19)  
    c. t(12;21)  
    d. t(9;22)

13. Expression of the OPAL1 gene in ALL patients may be used to:  
    a. predict good outcome when expression is high.  
    b. predict reduced detoxification of methotrexate.  
    c. identify a new ALL classification group.  
    d. classify ALL as a T-cell type.

14. Therapy intensification may be warranted in children with ALL in all the following situations EXCEPT:  
    a. blast counts that do not fall by day 7 of therapy.  
    b. emergence of MLL mutations.  
    c. patients with homozygous thiopurine methyltransferase (TPMT) mutations.  
    d. low OPAL1 expression.

15. ALL has a bimodal prevalence that occurs primarily in which two age groups?  
    a. Infants and children between 2 and 10 years of age  
    b. Children between 2 and 10 years of age and in the elderly  
    c. Infants and the elderly  
    d. Children between 2 and 10 and young adults

16. Mutation in the thiopurine methyltransferase gene (TPMT) affects treatment success with the chemotherapeutic drug 6-mercaptopurine by which of the following mechanisms?  
    a. TPMT mutations create abnormal enzymes that are more sensitive to proteolytic degradation thus reducing 6-mercaptopurine metabolism.  
    b. TPMT mutations increase the binding affinity of the TPMT enzyme to 6-mercaptopurine increasing its chemotherapeutic activity.  
    c. TPMT mutations inhibit the activity of tyrosine kinases that are responsible for regulating 6-mercaptopurine activity.  
    d. TPMT mutations create TPMT enzymes that increase the rate of enzymatic degradation of 6-mercaptopurine.
Continuing Education Registration Form

To earn continuing education (P.A.C.E.®) credit, (1) complete the form below, (2) record your answers, and (3) tear out and mail this form with a check or money order ($18 for ASCLS members, $28 for non-members for all articles) to:

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A certificate and credit will be awarded to participants who achieve a passing grade of 70% or better. Participants should allow eight weeks for notification of scores and receipt of certificates.

Focus: Neoplastic Hematologic Disorders carries 3.0 hours of intermediate level credit. This form can be submitted for credit for up to one year from the date of issue.

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Answers

Circle correct answer (questions are on previous two pages).

1. a b c d e 8. a b c d e 15. a b c d e 22. a b c d e
2. a b c d e 9. a b c d e 16. a b c d e 23. a b c d e
3. a b c d e 10. a b c d e 17. a b c d e 24. a b c d e
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7. a b c d e 14. a b c d e 21. a b c d e 28. a b c d e

Participant Information

Please circle the most appropriate answers.

1. Is this program used to meet your CE requirements for:
   (a) state license (b) NCA (c) employment (d) other

2. Specialty: (a) biochemistry/urinalysis (b) microbiology (c) lab administration (d) hematology/hemostasis (e) education (f) immunology (g) immunohematology

3. Workplace: (a) hospital over 500 beds (b) hospital 200–499 beds (c) hospital 100–199 beds (d) hospital under 100 beds (e) private lab (f) community blood bank (g) group practice (h) private physician (i) clinic (j) other

4. Salary range: (a) under $10,000 (b) $10,000 to $20,000 (c) $20,000 to $30,000 (d) $30,000 to $40,000 (e) over $40,000

5. Did these articles achieve their stated objectives?
   (a) yes (b) no

6. How much of these articles can you apply in practice?
   (a) all (b) some (c) very little (d) none

7. Employment status: (a) full time (b) part time (c) student (d) not employed (e) retired

8. How long did it take you to complete both the reading and the quiz? __________ minutes

9. What subjects would you like to see addressed in future Focus articles?
TRENDS AND TECHNOLOGY

Trends and Technology: Fall 2004

MARY JANE GORE

ONLINE
Greatlabdeals.com is a site of potential interest to laboratories that are seeking discounted equipment. I have not ordered from this site, nor can I vouch for it, but it appears to be a customer-friendly site, with many opportunities for smaller, private laboratories or hospital laboratories on tight budgets. BestLabDeals is an e-commerce site at which clinical laboratory scientists, researchers, educators, safety and industrial professionals, and others can purchase laboratory equipment and supplies at low prices, "typically 50 to 70% less than retail", according to the site. “Inventory is made up of brand-name overstocked, gently used, or cosmetically challenged merchandise” from leading industry manufacturers, they note. My search of the ten available clinical analysts showed this to be true—names like Kodak, Nova Biomedical, and Dade appeared. The product descriptions don’t say (or I didn’t see easily) whether the items are new and marked down or previously used. Items are categorized on a left-side frame alphabetically, e.g., analyzers, clinical. Typically available are instruments, equipment, supplies, workstations, and chemicals used by research laboratories; diagnostic instruments, test materials, and related products for clinical laboratories; monitoring apparatus, protective clothing, and other materials for controlled environments and occupational health and safety applications; and teaching aids for science education. Online purchasing is available with a shopping cart mechanism. The site lists its bricks-and-mortar location as a 32,000-square-foot facility in Raleigh, NC. The company welcomes appointments: phone number is (919) 661-8030.

NEW PRODUCTS
To enhance general competence and user skills for Mettler Toledo products, the company has introduced Insight! 2004, an educational program for laboratory scientists. The broad range of application seminars and courses is designed equally for users of both the company’s and other companies’ instrumentation. The seminars are held in convenient locations across North America. For more information, contact Tom Butta at (800) 638-8537, x 7168.

The new Mettler Toledo XP precision balance delivers QM effectiveness and the higher resolution performance needed by laboratory environments. Built in applications allow the balance to control calibration, testing, and optimization, while the extremely quick weighing cycle increases laboratory productivity. The color touch-screen’s configurable display allows customization for individual users, while optional hands free sensors allow users to work according to application demands. When using the Mettler Toledo SmartSens sensors, sample, batch, and up to two other identification numbers can be stored. All displayed information can be easily printed out utilizing an optional printer or wireless technology. Accuracy of the XP Balance can be checked at any time using the Reprocheck feature. Contact Sheila St. Jean at (614) 438-4936.

ESA Inc., a leader in bio-analytical instrumentation, has successfully utilized its flagship electrochemical (EC) systems in conjunction with a Mass Spectrometer (MS) to greatly expand the number and types of molecules for analysis. The EC systems (Coulochem™, CoulArray™, or the new DiscovArray™, easily integrated into virtually any Mass Spectrometer or LCMS system) can be used as a powerful tool to oxidize/ionize many different types of compounds. For more information, contact John Christensen, ESA Inc, (978) 250-7175 or email info@esainc.com; visit www.esainc.com.

The new, full-color, 864-page VWR Production Supplies for Controlled Environments Catalog offers over 13,300 products for a wide range of industries and applications – from research laboratories to production facilities. This selection of production chemicals and supplies is one of the most extensive available, representing more than 228 leading manufacturers and showcasing over 8,300 new prod-
Ortho-Clinical Diagnostics, a Johnson & Johnson company, is launching its VITROS 350 Chemistry System. This system introduces several new features that enhance laboratory productivity while reducing labor requirements. The VITROS 350 System will feature a new flat panel, touch sensitive monitor with adjustable positions for increased operator ergonomics and ease of use. Additional software updates are expected to provide improved throughput and greater levels of productivity. The VITROS 350 System software will support the VITROS Chemistry Products dHDL Slides. Contact Mary Richardson at (267) 679-4220.

Dade Behring has announced that it has established a partnership with Tecan, a leading participant in the healthcare supply industry, to co-market Tecan’s Genesis FE 500 pre-analytical workstation. The partnership will enable Dade Behring customers to automate essential steps in the pre-analytical work area for samples entering the laboratory. Contact Melissa Ziriakus at (847) 236-7038.

Carstens Inc, a leading provider of medical charting systems, now has available an optional wire storage basket to further increase the point-of-care efficiency of its WALKaroo III mobile PC cart. Carstens WALKaroo III mobile PC cart is expressly designed to help healthcare facilities more efficiently and effectively implement point-of-care computerized patient charting and secure expensive electronic charting equipment. To guard against theft and tampering, yet allow convenient access for authorized maintenance, the PC notebook is secured under WALKaroo III’s large work surface by a high-security tubular locking system. After the notebook has been locked in place, a full-sized, adjustable keyboard tray allows for easy, user-friendly documentation entry. The gray, epoxy-coated wire basket is easily attached to the base column of WALKaroo III and provides a convenient way for keeping ringbinder chartholders, equipment, and other supplies easily accessible at the point-of-care. Visit www.carstens.com.

A study determined that use of the Dade Behring Stratus® CS Acute Care™ Diagnostic System for cardiac troponin I testing in the coronary care and cardiac short stay units, versus testing done in the central laboratory, demonstrated significant cost savings when the instrument was used in those settings, with a mean 25% reduction among all charge variables. A poster detailing the product was presented at the 2004 American Association for Clinical Chemistry meeting in July. Contact Melissa Ziriakus at (847) 236-7038.

Leica Microsystems has introduced a new level of sectioning precision, the Leica RM2245 semi-automated rotary microtome. This microtome embodies the latest technological innovations in microtomy for the modern high-throughput histology laboratory. Contact Molly Lundberg at (847) 405-0123 or visit www.leica-microsystems.com.

FDA NEWS

Roche Diagnostics received clearance from the U.S. Food and Drug Administration (FDA) for the Elecsys® Troponin T STAT test as an aid in the differential diagnosis of acute coronary syndrome, for risk stratification in patients with acute coronary syndrome, and for cardiac risk in patients with chronic renal failure. The test may also be useful for the selection of more intensive therapy and intervention in patients with elevated levels of cardiac Troponin T. The Elecsys Troponin T test measures the level of Troponin T, a cardiac-specific protein, in a patient’s blood. Troponin T is released into the blood when heart cells die, and its presence can help a physician diagnose heart attack. Contact Lori LeRoy at (317) 521-7159.

In late July, Dade Behring Holdings received FDA clearance to sell its NT-proBNP (N-terminal pro brain natriuretic peptide) assay in the U.S. NT-proBNP is a key cardiac marker, which will be used on the company’s Dimension® instruments as an aid in the diagnosis of individuals suspected of having congestive heart failure. Contact Pattie Overstreet-Miller at (847) 267-5426.

Bayer HealthCare LLC Diagnostics notes that its BNP test has received FDA clearance for two additional claims – prediction of survival in patients after

(continued on inside back cover)
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AB = Abstract  
CP = Clinical Practice  
DD = Dialogue and Discussion  
FO = Focus  
LE = Letter to Editor  
RR = Reports and Reviews  
RS = Research  
TT = Trends and Technology  
WB = Washington Beat

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CUTTING EDGE
Genstruct Inc, a knowledge-driven discovery company, presented findings from one of its independent discovery programs at IBC Life Sciences’ 9th Annual Drug Discovery Technology World Congress held August 8–13 in Boston. Dexter Pratt, project leader for Genstruct’s Oncology Program, outlined the program’s findings describing both probable molecular causes and mechanisms involved in androgen-dependent prostate carcinoma. To define the molecular causes of prostate carcinoma, Genstruct has utilized its Molecular Epistemics™ discovery platform to build and interrogate an Oncology Knowledge Assembly™ model using computer-aided causal reasoning. The model includes the knowledge of cell cycle, cell signaling, vesicle trafficking, metabolism, and gene regulation. It is the largest and most complete computable model of prostate carcinoma ever assembled. Contact Karen Higgins at (610) 831-5723 or khiggins@aandecomm.com.

With more than $20 billion distributed to states and localities to help them upgrade their security efforts, and another $1 billion earmarked by the interagency Technical Support Working Group as seed funding for technology projects around the country, the homeland security effort has fostered a new wave of technological innovations. Many of these new technologies were the subject of discussion and on display at The McGraw-Hill Companies’ Homeland Security Summit & Exposition in Washington DC. From bioterrorism technology, such as Genstruct’s Molecular Epistemics™ discovery platform to build and interrogate an Oncology Knowledge Assembly™ model using computer-aided causal reasoning, the model includes the knowledge of cell cycle, cell signaling, vesicle trafficking, metabolism, and gene regulation. It is the largest and most complete computable model of prostate carcinoma ever assembled. Contact Karen Higgins at (610) 831-5723 or khiggins@aandecomm.com.

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During the opening ceremony at the International Analytica Trade Fair in Munich, Germany, the German Society for Biochemistry and Molecular Biology awarded the “Molecular Bioanalytics” prize for 2004. This year, the prize went to American Dr Stephen PA Fodor, British Professor Sir Edwin Southern, and Russian Professor Andrei Mirzabekov (posthumously) for their fundamental contributions to the development of microarray technology (DNA chip). Individual DNA segments are affixed to a glass surface, thereby functioning as so-called probes. Each probe detects a specific gene sequence. The sequences bind to the probe by hybridization. Gene chips are a premier example of miniaturization and automation in bioanalytics and medicine.
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