Meeting Report

2001 Association for Molecular Pathology Annual Meeting

The Association for Molecular Pathology (AMP) held a very successful annual meeting November 15–18, 2001 in Philadelphia, Pennsylvania. AMP was very pleased to open its seventh annual meeting by presenting its Award for Excellence to Dr. Bert Vogelstein of John Hopkins University. This award, sponsored by Visible Genetics, Inc., (Toronto, Canada) is presented annually to a scientist who has made major contributions affecting our field. Dr. Vogelstein has been instrumental to our current level of understanding of the molecular events leading to development of colorectal cancer. In his address, he covered the genomic alterations uncovered in early studies, molecular mechanisms of genomic micro- and macroinstability, and newer studies examining gene expression changes in colorectal tumors. He discussed several examples of genes involved in regulation of angiogenesis, and presented technical approaches, such as “digital PCR,” used in his lab to improve sequencing results and detection of heterozygous mutations. We would like to congratulate Dr. Vogelstein and thank him for attending the meeting.

Genetics Session

During the first evening of the meeting, Genetics subsection Chair Dr. Richard Press (Oregon Health Sciences University) organized a Late Breaking Update session on “Laboratory Issues in Population Based Cystic Fibrosis Carrier Screening.” The discussion was sparked by questions from Dr. Tony Killeen on CHAMP, “the AMP list-serv,” as to how labs were planning to handle the new recommendations from the American College of Medical Genetics, National Institutes of Health, and American College of Obstetrics and Gynecology for testing pregnant Caucasian women for cystic fibrosis (CF) (see [http://www.acmg.net/Pages/ACMG Activities/policy statements pages/current/CF3](http://www.acmg.net/Pages/ACMG%20Activities/policy%20statements/pages/current/CF3) see Retired list below for CF1 and CF2 Cystic Fibrosis Carrier Screening Laboratory Standards and Guidelines for Population based.htm for details). Several AMP members and guests, including Bert Gold, Jean Amos, Paul Rothberg, Adam Guenther, and Barb Zehnbaauer, participated in a well-attended roundtable exploration of specific methodologies, mutation alleles included, annual specimen accrual, turnaround time, and test cost. The consensus approach involves some type of reverse dot blot format, either commer-

cial or home-brew, with most people using non-isotopic detection of allele-specific hybridization. An important aspect that elicited active audience input concerned the interpretive text that lab directors include with the primary assay results. The alleles included in each application and the sensitivity of the assay were influenced by the ethnic origin of the patient and whether the test was requested for the purpose of disease diagnosis or carrier detection. Keen audience interest in constructing reliable, accurate test interpretations for CF and other genetic disorders will be further addressed at next year's meeting. The Late Breaking session on topics of particular interest to AMP members was also deemed a valuable forum to include in future annual meetings.

The Genetics Workshop I session targeted “Genetics of Common Complex Diseases.” The first speaker was Dr. Alexander (Steve) Whitehead from the University of Pennsylvania School of Medicine who focused on the functional enzyme variants in the metabolism of folate and homocysteine. His expertise in pharmacogenetics, with particular interest in MTHFR, provided valuable insights to the clinical utility of molecular genetic testing as an accurate predictor of drug responses. His lab has developed multiplexed polymerase chain reaction (PCR) approaches to genotyping for several enzymes in folate pathways. Many contend that measuring levels of homocysteine may be a more accurate predictor of clinical status than mutation testing for risk assessment. Also included were aspects of clinical response to the chemotherapy agent methotrexate as affected by MTHFR mutations and 5-fluorouracil metabolism influenced by functional polymorphisms in the thymidylate synthase.

The second speaker was Dr. Ron McGlennen from the University of Minnesota who provided a broader view of the complexities of devising diagnostic approaches to complex, multigenic disorders. He included his experience in dealing with the challenges of high-throughput, population-based studies, automation and robotics, as well as miniaturization and point of care technologies.

The Genetics Workshop II session brought together representatives of several federal and professional groups to discuss the regulatory oversight of genetic testing. Dr. Debra Leonard, past-President of AMP, has been actively involved in this area and moderated the session. This issue has been addressed during the re-

cent past by Clinical Laboratory Improvement Act Committee (CLIA), Secretary's Advisory Committee on Genetic Testing (SACGT), and the Food and Drug Administration (FDA). Dr. Joe Boone of the Centers for Disease Control (CDC) outlined the issues leading up to the current discussions of increased regulation, including moves by CLIA, the formation of SACGT, and the recent CDC notice of intent. CLIA has proposed increased oversight of pre- and postanalytic phases of genetic testing, such as appropriate levels of informed consent and reporting mechanisms, as well as professional training requirements for molecular genetics laboratory directors and staff. These issues were covered by Judy Yost of HCFA (Health Care Financing Administration, recently renamed CMS). Dr. Joseph Hackett from the FDA outlined the mechanisms that might be used in the review of in-house developed genetic tests, and discussed the template that has been proposed as a tool to gather information on such tests. Dr. Andrea Ferreira-Gonzalez of Virginia Commonwealth University and Chair of the newly formed AMP Public Relations Committee, presented some of the concerns of increased regulation, including the broad definitions of genetic testing being used in ongoing discussions. Dr. Walter Noll of Dartmouth-Hitchcock Medical Center also addressed concerns with the proposed oversight, suggesting an alternative approach based on improvements in existing laboratory oversight by improving lab inspections and expanding proficiency testing. We would like to thank all of the presenters for their willingness to cover the issues relating to increased oversight at the AMP meeting as this remains a controversial topic.

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Hematopathology Session

The 2001 Hematopathology Plenary session was truly outstanding. Organized by Adam Bagg, M.D. (University of Pennsylvania) and Steven Schichman, M.D., Ph.D. (University of Arkansas Medical School), the session featured two internationally known researchers, with substantive contributions in the field of acute leukemia. Dr. Todd Golub, M.D., Director of Cancer Genomics at the Whitehead/MIT Center for Genome Research, presented his work on the molecular profiling of leukemias. Using Affymetrix gene chips with probes for 6817 human genes, Dr. Golub determined that the gene activity of acute leukemias fell into two distinct clusters that clearly identified the leukemia type. Differential genome expression patterns were 100% accurate in distinguishing between acute myelogenous leukemia and acute lymphoblastic leukemia. The microarray datasets on this series of acute leukemias are available through Dr. Golub's website (www.genome.wi.mit.edu). Dr. Pier Paolo Pandolfi, M.D., Ph.D., Head of the Molecular and Developmental Biology Lab at the Sloan-Kettering Institute, was the second Plenary session speaker. He gave a very dynamic overview of his work over the past several years to define the molec-

ular pathogenesis of acute promyelocytic leukemia. His studies with transgenic mice have provided new insights into the leukemogenic pathogenesis of the different PML/RAR α and PZLF/RAR α oncoproteins.

The first hematopathology workshop, "Conversion of PCR-Based Assays from Conventional Gels to Capillary Electrophoresis: Technical and Interpretative Issues," was organized by Dr. Adam Bagg. The speakers included Karen Berg, M.D. from Johns Hopkins and Daniel Sabath, M.D., Ph.D. from the University of Washington. There has been a rapid increase over the past two years in molecular labs using capillary electrophoresis (CE) techniques to perform analysis of antigen receptor gene rearrangements, and Drs. Berg and Sabath discussed their experiences in implementing CE-based clinical assays for antigen receptor clonality. Both speakers also presented results of assays from several clinical cases for discussion, which was highly interesting to participants. Dr. Berg also presented results of her recent CHAMP e-mail survey of interpretation of CE assays in a series of cases of benign and neoplastic lymphoproliferative processes. This workshop highlighted the increasing use of this technology in the diagnosis of hematological malignancies, and the urgent need for development of guidelines for interpretation of CE assays used for diagnostic purposes.

The second hematopathology workshop, "Disease Burden Assessment by Quantitative PCR: How and Why?" was also organized by Dr. Adam Bagg, and included presentations by Rajyalakshmi Luthra, Ph.D. from M.D. Anderson Cancer Center and Richard Press, M.D., Ph.D. from Oregon Health Sciences University. Dr. Luthra presented her results of quantitative Taqman PCR (Applied Biosystems, Foster City, CA) analyses of the t(14;18), t(11;14), and cyclin D1 in follicular and mantle cell lymphomas. Dr. Press discussed the results of a study on BCR-ABL quantitation in blood and marrow following treatment with ST1571 therapy, using a commercially available LightCycler kit (Roche Molecular Biosystems, Indianapolis, IN). There was some discussion of different variables that can affect these assays, and of potential pitfalls of this kind of assay. Both speakers stressed the importance of interpreting the results of these assays cautiously; results on multiple specimens from a patient over time, to establish a trend, are essential in determining the clinical significance of these quantitative PCR results. Additional validation of the clinical significance of quantitative PCR results, by correlation with other clinical, morphological, and immunophenotypic information, is needed and is an area of active clinical research in both presenting labs, as well as many other AMP labs participating in the workshop.

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Infectious Diseases Session

Infectious Disease Section Chair Dr. Stephen Dumler (Johns Hopkins Medical Institutions) and Chair-elect Dr.

Brian Dawson (Mayo Clinic) assembled an exciting and informative program for this year's annual meeting. The Infectious Diseases Plenary Session dealt with two topics with broad appeal to the AMP membership. Dr. Clare Fraser provided an excellent overview of comparative microbial genomics and shared some of her experiences in this field at The Institute for Genomic Research (Rockville, MD). She showed how comparative genomics has contributed to our understanding of bacterial species diversity and challenged our conventional wisdom regarding bacterial evolution, taxonomy, and virulence. She also highlighted how the knowledge derived from comparative genomics can be used in epidemiology, prediction of clinical outcome, and detection of genetically modified agents of biowarfare. Dr. Michael Gale from the University of Texas Southwestern Medical Center talk focused on the topic of the response of hepatitis C virus (HCV) infection to α -interferon therapy. He presented a comprehensive overview of antiviral actions of interferon and the studies that his group and others have performed to define polymorphisms in the NS5A region of the HCV genome that play a role in determining sensitivity to α -interferon. His group demonstrated that the NS5A region contains a PKR-binding domain that binds to, and inhibits PKR, a double-stranded RNA-activated protein kinase that is an important interferon induced anti-viral protein.

The Infectious Diseases Section organized three workshops this year. The first workshop focused on the use of molecular methods for detection and quantitation of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in bone marrow and solid organ transplant recipients. Richard Scheuermann from the University of Texas Southwestern Medical Center described their experience developing quantitative PCR assays for all of the human herpes viruses and how their CMV assay is used to diagnose and manage CMV disease in organ transplant recipients and HIV-1 infected patients. Dr. Richard Hodinka from the Children's Hospital of Philadelphia reviewed EBV-associated clinical illnesses and malignancies, and the laboratory methods available to diagnose and manage patients with these. The major focus of his presentations was on the use of quantitative PCR for EBV DNA in peripheral blood lymphocytes for diagnosis of EBV associated post-transplant lymphoproliferative disorders. Following the speakers' presentations a lively discussion of the various methods used, sample types, and cut-off values, an interpretation of test results ensued.

The second workshop focused on the use of 16S ribosomal RNA sequencing for identification of bacterial pathogens and was presented by Dr. Jeanne Jordan from Magee-Women's Research Institute, and Mr. Michael Waddington from Accugenix (Newark, DE). In this workshop the various approaches to identification of pathogens, the rationale for using 16S rRNA as a target for sequence analysis, the different approaches to 16S rRNA gene sequencing, and the commercially available options for 16S rRNA sequence analysis were reviewed.

The third workshop was a joint workshop with the Solid Tumor Section on the role of human papilloma virus (HPV) testing in the diagnosis and management of cervical neoplasia. Dr. Sharon Wilczynski from City of Hope National Medical Center discussed the role of HPV in cancers other than cervical cancer. Dr. Mark Stoler, from the

University of Virginia Health Sciences Center reviewed the role of HPV in cervical cancer and the results of a major clinical trial of a DNA hybridization test for HPV as a means to triage women with atypical cytological findings on Pap smear to colposcopy. The data from this trial clearly demonstrates a role for reflex testing of all specimens with atypical squamous epithelial cells of unknown significance found on Pap smears. Dr. Elizabeth Unger from the Centers for Disease Control and Prevention discussed the performance characteristics of the DNA hybridization test for HPV and whether these performance characteristics were appropriate for a cervical cancer screening test. The discussion following the presentations was spirited and focused mainly on the eventual role for HPV testing relative to Pap smears in screening women for cervical cancer. This workshop represented the AMP meeting at its best. It brought together participants with very different backgrounds and perspectives, united by their interest in advancing diagnostics through the application of molecular methods.

Other infectious disease related topics at the annual meeting included a session on molecular testing for *Bordetella pertussis* presented by Qi Wei from the Children's Hospital of Denver as part of the Medical Technology Selected Technical Topics and a talk on the agents of bioterrorism presented by Dr. Ron McGlennen from the University of Minnesota as part of the Special Topics Plenary Session.

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Solid Tumors Session

The Solid Tumors Plenary Session was presented by John N. Weinstein, M.D., Ph.D. from the National Cancer Institute and Charles M. Perou, M.D. from the University of North Carolina, Chapel Hill. Dr. Weinstein discussed the integration of the many "-omics" disciplines such as genomics, proteomics, and pharmacogenetics, with an emphasis on the synergy between traditional hypothesis driven research and the more expansive "-omics" studies. A brief review of methods used in expression analysis studies and a useful NCI website (<http://discover.nci.nih.gov>) were also discussed in Dr. Weinstein's presentation. Dr. Perou continued the session on gene expression analysis in his presentation on molecular-based subclasses of in breast tumors. Using cDNA microarrays analyzed by hierarchical clustering, primary breast carcinomas could be grouped based on distinctive expression profiles that reflect intrinsic properties of the tumors or that are highly correlated with patient outcome.

The first presenter of the Solid Tumors Workshop was Adekunle M. Adesina, M.D., Ph.D. of the University of Oklahoma Health Sciences Center. Dr. Adesina presented an overview of brain tumors and associated syndromes. Among the topics covered were the genetic differences between primary and secondary glioblastoma multiforme, genetically distinct subgroups of anaplastic oligodendroglioma, and specific gene alterations in medulloblastoma. De-

borah A. Dillon, M.D. of Yale New Haven Medical Center gave the second presentation on early diagnosis of breast cancer. By using the sensitive Mutational Load Distribution Assay (MLDA), p53 mutations can be identified in 40 to 50% of breast tumors, including ductal carcinoma *in situ*. The final presenter of the Workshop was Jessa B. Jones of John Hopkins University, who gave a nice overview on homoplasmic polymorphisms in mitochondrial DNA as potential tumor markers. She reported her studies on mitochondrial DNA polymorphisms that are uniformly found in pancreatic tumors but are not normal pancreatic tissue.

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Special Topics Session

In Special Topics, the final session of the meeting, we heard two speakers in diverse areas. Dr. Ron McGlen-

nen of the Fairview University Medical Center, gave us a glimpse into bioterrorist opportunities more subtle than those we usually consider. Stemming from his involvement in ****XXX****, Dr. McGlennen gave us several examples of agents targeting livestock or crops which would produce insidious yet major effects on the population and economy of a country. In an inspiring final talk of the meeting, Dr. Brian Druker from Oregon Health Sciences University related his work on STI571, a tyrosine kinase inhibitor used for treatment of chronic myelogenous leukemia and certain other tumors. Dr. Druker presented the science behind the development of this revolutionary treatment, discussing current treatment protocols, and investigation into the mechanism of resistance to STI571 observed in some patients.

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