Biometric Research Branch
Annual Report 2005

Background

The Biometric Research Branch is the biostatistical and biomathematical component of the DCTD. During 2005 it consisted of 13 permanent doctoral level research investigators supplemented by post-doctoral research fellows and guest researchers. The staff have doctoral degrees and expertise in biostatistics, biomathematics, computational biology and computer science. The philosophy of the BRB is to have the staff combine two functions – (i) collaboration and consultation with DCTD scientific administrators and NCI intramural investigators; (ii) conduct of self initiated research on topics important to cancer research and to the collaborative investigations. Combining these functions has enabled the BRB to recruit and retain a very high quality research staff, to provide the highest quality collaborative and consulting staff to DCTD and NCI scientists, and to accomplish research in the areas of statistical, mathematical, and computational sciences that are motivated and informed by real and important problems of current cancer research. The BRB does not have a grant, cooperative agreement or contract portfolio and does not sponsor or fund extramural research.

Staff of the BRB have specific areas of responsibility for collaborative and consulting activities. Collaborations with the Cancer Therapy Evaluation Program are primarily handled by Drs. Edward Korn, Larry Rubinstein, Boris Freidlin and Sally Hunsberger. These collaborative activities include statistical review of all CTEP sponsored clinical trials, service on Data Safety Monitoring Committees of the cooperative oncology groups, and participation in the design of clinical trials for the development and evaluation of investigational drugs. Collaborations with the Cancer Diagnosis Program are handled by Drs. Lisa McShane and Kevin Dobbin. Their activities include providing guidance in statistical matters related to CDP initiatives, attending retreats, monthly science sessions, and biweekly staff meetings, reviewing statistical aspects of research proposals submitted for special exception, accelerated review, or supplements, reviewing R21/R33 grants for appropriateness of milestones, providing statistical reviews of all cooperative group correlative science protocols, providing statistical reviews of requests for specimens submitted to the Inter-Group Breast Correlative Science Review Committee, statistical review of applications for tissue from NCI funded tissue resources, and providing statistical expertise to scientific administrators in the monitoring and development of important NCI initiatives such as tissue resources and the Program for the Assessment of Clinical Cancer Tests (PACCT), and Strategic Partnering to Evaluate Cancer Signatures (SPECS).

Collaborations with the Biomedical Imaging Program are handled by Dr. Lori Dodd and collaborations with the Developmental Therapeutics Program are handled by Dr. Larry Rubinstein. These collaborations include an extensive and diverse mix of activities

including the design and analysis of major DCTD studies, protocol design and review, providing statistical advice to extramural investigators, and service on data monitoring committees. The BRB also provides collaboration to investigators of the NCI Center for Cancer Research in the areas of statistical genomics and biostatistics. Drs. Paul Albert, Joanna Shih and George Wright participate in these activities. Dr. Richard Simon is the branch chief overseeing the activities of the branch and leading the efforts in computational and systems biology. Dr. Yingdong Zhao and Myong-Hee Sung participate in the computational and systems biology activities.

The following paragraphs provide a summary of the major initiatives and scientific advances in the BRB research activities during 2005. This report will not repeat description of extramural initiatives or scientific advances that BRB staff participated in that were sponsored by DCTD programs.

Clinical Trials

Developing methods for designing and analyzing trials of cytostatic agents
Many new anticancer agents being developed are not cytotoxic, and therefore may not cause tumors to shrink appreciably. However, these agents may still offer significant clinical benefit to patients by delaying the progression of disease. Since standard phase I/II/III clinical trial development of agents depend on their ability to show activity in phase II trials by tumor shrinkage, new approaches are needed. In our work, we discuss and evaluate many approaches, including those that have been mentioned in the literature.

Korn EL, Rubinstein LV, Hunsberger SA, Pluda JM, Eisenhauer E and Arbuck SG (2005). Clinical trial design for cytostatic agents and agents directed at novel molecular targets. In Strategies for Discovery and Clinical Testing of Novel Anticancer Agents (eds., A.A. Adjei and J. Buolamwini), Elsevier.

Hunsberger S, Rubinstein LV, Dancey J, Korn EL Dose escalation trial designs based on a molecularly targeted endpoint. Statistics in Medicine 24 (14): 2171-2181 JUL 30 2005.

Chemoprevention of esophageal squamous cancer

In collaboration with investigators from the Center for Cancer Research (NCI), Division of Cancer Prevention (NCI), and Chinese Academy of Medical Sciences, a randomized factorial trail of two chemoprevention agents was tested for their ability to slow the rate of progression (or increase the rate of regression) of esophogeal dysplasias. Dr. Korn of BRB is the study statistician for this trial. Results suggest that further study of selenomethionine as a potential esophageal squamous cancer chemoprevention agent appears warranted.

Limburg PJ, Wei W, Ahnen DJ, Qiao Y, Hawk ET, Wang G, Giffen CA, Wang G, Roth MJ, Lu N, Korn EL, Ma Y, Cadwell KL, Dong Z, Taylor PR, and Dawsey SM (2005) Randomized, placebo-

controlled, esophageal squamous cell cancer chemoprevention trial of selenomethionine and celecoxib. Gastroenterology 129, 863-873.

Surrogate endpoints

In many clinical trials it would be useful to have a surrogate endpoint that could be measured earlier or less invasively than the definitive endpoint. This work describes statistical methods for using the surrogate and definitive endpoint results from a series of previously completed trials to assess whether the surrogate endpoint could be used for a future trial. Examples are given assessing whether objective response or complete surgical response can be used as a surrogate for survival in chemotherapy trial of ovarian cancer, and whether change in CD4 cell counts can be used as a surrogate for time to development of AIDS for drug trials in HIV positive patients.

Korn EL, Albert PS and McShane LM (2005) Assessing surrogates as trial endpoints using mixed models (with discussion). Statistics in Medicine 24,163-190.

Early release of interim data in randomized clinical trials

Standard data monitoring procedures for clinical trials only allow release of interim efficacy results at the end of the trial or earlier if the results have crossed a data monitoring boundary. We suggest specific clinical situations in which it might be preferable to release interim efficacy results even though no boundary has been crossed. The situations are such that the interim release of data will not interfere with the final analysis of the trial but will potentially offer a significant benefit to the public.

Korn EL, Hunsberger S, Freidlin B, Smith MA, and Abrams JS (2005) Preliminary data release for randomized clinical trials of noninferiority: a new proposal. Journal of Clinical Oncology, 23, 5831-5836.

Multiple comparisons and clinical trials

Multiple comparisons issues arise in clinical trials with subgroup analysis, multiple variables, interim monitoring, and data-driven choice of hypotheses. It has been suggested that a non-standard type of analysis of clinical trial data ("likelihood-based methods") can eliminate the problems with multiple comparisons. We examine this proposition in detail and find it to be lacking.

Korn EL and Freidlin B (in press) The likelihood as statistical evidence in multiple comparisons in clinical trials: no free lunch. Biometrical Journal.

Sample size calculations for trials with historical controls

In the 1980's, Simon and his colleagues showed that it was incorrect to ignore the variability of the historical control data when performing sample size calculations for

trials using historical controls. In the present paper, we show how these widely used methods from the 1980's can be improved upon.

Korn EL and Freidlin B (in press) Conditional power calculations for clinical trials with historical controls. Statistics in Medicine.

Rubinstein LV, Korn EL, Freidlin B, Hunsberger S, Ivy SP, Smith MA. Design issues of randomized phase II trials and a proposal for phase II screening trials**.** Journal Of Clinical Oncology 23 (28): 7199-7206 OCT 1 2005.

Evaluation of randomized discontinuation design

We evaluated two kinds of randomized designs for the early development of target-based cytostatic agents: randomized discontinuation and upfront randomization designs. Results: The randomized discontinuation design is not as efficient as upfront randomization if treatment has a fixed effect on tumor growth rate or if treatment benefit is restricted to slower-growing tumors. On the other hand, the randomized discontinuation design can be advantageous under a model where only a subset of patients, those expressing the molecular target, is sensitive to the agent. To achieve efficiency, the design parameters must be carefully structured to provide adequate enrichment of the randomly assigned patients. Conclusion: With careful planning, the randomized discontinuation designs can be useful in some settings in the early development of targeted agents where a reliable assay to select patients expressing the target is not available.

Freidlin B, Simon R. (2005). An evaluation of randomized discontinuation design. Journal of Clinical Oncology 23:5094-5098.

Evaluating treatment effects in the presence of competing risks

Competing risks are often encountered in clinical research (a cancer patient may experience local failure, distant failure or die without recurrence). In comparing treatments use of endpoints based on the type of failure directly related to the treatment mechanism of action allows one to focus on the aspect of the disease targeted by treatment. We evaluate statistical methodology commonly used for testing failure specific treatment effects. We demonstrate that the cause-specific log-rank test is superior to the cumulative incidence based approach.

Freidlin B, Korn EL. (2005). Testing treatment effects in the presence of competing risks. Statistics in Medicine 24:1703-1712.

We developed Early Stopping Guidelines for Slow Accruing Trials. The guidelines are used to monitor accrual to CTEP cooperative group phase III trials. This allows early identification of the trials that are likely to fail to reach their objectives. The guidelines were developed and validated using CTEP data base containing 239 phase III Cooperative Group trials.

Dr. Rubinstein of BRB developed the statistical section for the CTEP phase 0 trial template, and developed the statistical design for the initial CCR phase 0 trial, involving a new PARP inhibitor, in collaboration with CTEP and CCR staff. The phase 0 trial is an innovative concept used by DCTD and CCR to accelerate the introduction of promising new agents into the clinic in small proof of principle studies.

Analyses were conducted with Dr. Seiichiro Yamamoto (chief statistician of the Japanese National Cancer Center) involving toxicity and efficacy of phase I drugs tested over the past decade under CTEP sponsorship. The collaboration involved staff from CTEP and the NIH Clinical Bioethics Department.

Horstmann, E., McCabe, M.S., Grochow, L., Yamamoto, S., Rubinstein, L., Budd, T., Shoemaker, D., Emanuel, E.J., Grady, C., Risks and benefits of phase 1 oncology research: Evaluating response rates and toxicities 1991-2002, New England Journal of Medicine 352:895-904, 2005).

The accelerated titration design is a novel design for phase I trials developed by BRB statisticians in collaboration with CTEP investigators. It permits more rapid dose escalation as well as dose titration within individual patients. Drs. Freidlin and Rubinstein, in collaboration with CTEP, have conducted a review of the use of accelerated titration phase 1 designs, and its performance in actual trials.

Dancey, J., Freidlin, B., Rubinstein, L.V., Accelerated titration designs. In Chevret, S. (ed.) *Statistical Methods for Dose-Finding Studies*, Wiley Press, in press, 2006.

Longitudinal Data Analysis:

Dr. Albert and Hunsberger have continued a productive research program in developing new methods for the analysis of longitudinal data. Most of this work has been motivated by problems in analyzing repeated biomarker measurements over time, particularly with semi-continuous data, that is, data are data which are either continuous or are zero. For example, tumor volume is a good example of a semi-continuous variable. Albert and Shen (2005) propose new methodology for analyzing longitudinal data with serial correlation. The methodology was applied to data from an acupuncture clinical trial examining the effect of acupuncture on reducing nausea resulting from breast cancer treatment.

Albert, P.S. and Shen, J. Modeling longitudinal semi-continuous emesis volume data with serial correlation in an acupuncture clinical trial. Royal Statistical Socieity-Series C (Applied Statistics) 54, 707-720, 2005.

Albert, P.S. On the interpretation of marginal inference with a mixture model for clustered semi-continuous data. Biometrics 61, 879-880, 2005.

Albert, P.S. and Hunsberger, S. On analyzing circadian rhythm data using non-linear mixed models with harmonic terms. Biometrics 61, 1115-1122, 2005.

Albert, P.S. and Follmann, D.A. Random effects and latent process approaches for longitudinal binary data with missingness: with applications to the analysis of opiate clinical trial data. To appear in Statistical Methods in Medical Research, 2005.

Co-Development of Diagnostics and Therapeutics: Pharmacogenomic Targeting and Personalization of Treatment

During 2004 we published two papers that demonstrated the vast improvement in efficiency of randomized phase III trials that can be achieved from using a biomarker or genomic classifier to select patients likely to respond to the new treatment. In many cases, however, such classifiers are not available at the start of phase III trials. During 2005 we published a new phase III design that addresses this limitation. The design does not limit entry based on a biomarker but requires that tumor specimens be collected at the time of entry. At the end of the trial outcomes for all patients on the new treatment are compared to those for all patients on the control. If the difference is significant at a level of 0.04 or better, results are taken to support approval of the new drug with a broad labeling indication. If not, then the specimens from the first half of patients randomized are used to develop a classifier of which patients appear to benefit from the new regimen. That classifier is then applied to the second half of the randomized patients and those predicted to be sensitive to the new treatment are identified. If the outcomes for patients in that subset on the new treatment are significantly better than for the control patients in the subset and if the significance level is 0.01 or less, then the data is taken to support approval with a narrowed labeling indication for the new treatment. The design is structured to encourage commercial sponsors to invest in the development of pharmacogenomic signatures without endangering their opportunity to obtain regulatory approval with a broad labeling indication if results warrant. We published this new design in Clinical Cancer Research during 2005. It has received considerable favorable notice at the FDA and I have been asked to speak at several FDA-DIA meetings. The FDA and industry are particularly interested in the simple way we have been able to transform the traditionally unreliable practice of subset analysis into a structured test of a single subset hypothesis. This simple idea is already being generalized from the context in which we presented it and applied to pivotal studies.

Freidlin B and Simon R. Adaptive signature design. An adaptive clinical trial design for generating and prospectively testing a gene expression signature for sensitive patients. Clinical Cancer Research 11:7872-78, 2005.

There is substantial mis-understanding of the meaning of biomarkers for treatment selection and on the standards that are appropriate for the validation of such biomarkers. We have tried to clarify these issues so that the field can utilize genomic technologies to rapidly identify, validate and translate into clinical practice the use of treatment selection markers. We have collaborated with FDA and industry scientists in numerous meetings on this topic. In order to facilitate the development of treatment selection markers in the context of new drug development, Dr. Richard Simon of BRB has established formal Pharmacogenomic agreements with Johnson & Johnson Pharmaceutical Research &

Development, and with Centicor.

Simon R. Roadmap for developing and validating therapeutically relevant genomic classifiers. Journal of Clinical Oncology 23:7332-41, 2005.

Simon R and Wang SJ. Use of genomic signatures in therapeutics development. The Pharmacogenomics Journal (In Press).

Trepicchio WL, Essayan D, Hall ST, Schechter G, Tezak Z, Wang SJ, Weinrich D and Simon R. Designing prospective clinical pharmacogenomic trials. Effeftive use of genomic biomarkers for use in clinical decision making. The Pharmacogenomics Journal (In Press).

Simon R. Validation of pharmacogenomic biomarker classifiers for treatment selection. Disease Markers (In Press).

Simon R. A checklist for evaluating reports of expression profiling for treatment selection. Clinical Advances in Hematology and Oncology (In Press).

Simon R. Guidelines for the design of clinical studies for development and validation of therapeutically relevant biomarkers and biomarker based classification systems. In *Biomarkers in Breast Cancer: Molecular Diagnostics for Predicting and Monitoring Therapeutic Effect*, Hayes DF and Gasparini G (eds). Humana Press, 2005.

Simon R. DNA microarrays for diagnostic and prognostic prediction. Encyclopedia of Medical Genomics & Proteomics (J Fuchs, M Podda eds), Marcel Dekker, NY (In Press).

Simon R. Development and validation of therapeutically relevant multi-gene biomarker classifiers. Journal of the National Cancer Institute 97:866-7, 2005.

Simon R. An agenda for clinical trials: Clinical trials in the genomic era. Clinical Trials 1:468-70, 2004.

Drug Discovery & Pre-clinical Development

Dr. Rubinstein reviewed the reproducibility of the results of the NCI human tumor 60 cell line screen, in collaboration with DTP staff. This review was utilized by the external committee that reviewed the performance of the screening system.

Dr. Simon led a collaboration involving CTEP and DTP investigators to discover and develop specific inhibitors of the protein product of the mutatant BRAF gene. A single point mutation was discovered in over 60% of human melanoma tumors. It occurs in the phosphorylation loop of the BRAF gene and exists in very early lesions. The DCTD committee consisting of Dr. Simon, Dr. John Wright of CTEP, Dr. Robert Shoemaker and Dr. Joe Tomaszewski of DTP met with companies to identify promising drug candidates for inhibiting this very promising target, and determining how DCTD could expedite development of such inhibitors. This project represents an outgrowth of the thesis that failure to identify key oncogenic mutaions has been the rate-limiting step in developing effective cancer therapeutics and the important role of multidisciplinary collaboration in identifying and taking advantage of key oncogenic mutations.

Simon R. Bioinformatics in cancer therapeutics – hype or hope? Nature Clinical Practice Oncology 2:223, 2005.

Simon R. Targets for treatment success. Nature Clinical Practice Oncology 3:1, 2006.

Molecular Diagnosis

Dr. Lisa McShane of BRB engaged in the following activities supporting the Cancer Diagnosis Program:

1. Cooperative Breast Cancer Tissue Resource (CBCTR)
- Conduct two pathologist concordance studies, analyze data, and produce report of results.
- Design tissue microarrays and oversee construction (see below).
- Develop detailed case selection criteria (in collaboration with contractor IMS) for applications that have been approved to receive specimens.
- Lead monthly data manager conference calls to clarify issues that arise in case selection and data field interpretation, and monitor progress in filling applications.
- NCI statistical representative to the Research Evaluation Panel (REP): duties include reviewing many letters of intent and applications each year from investigators applying to use CBCTR specimens.

2. Tissue microarrays (TMAs)
- CBCTR 2nd generation progression TMA – Design and analyze study to evaluate TMA quality.
- Colon cancer and rectal cancer progression and prognostic TMAs – Design studies to evaluate TMA quality.
- Matched colon cancer-polyp TMA – Oversee case selection and develop clinical database.
- Effects of storage conditions on tissue microarray sections – Monitor study in progress.
- CBCTR prognostic TMA – Design TMA, develop detailed case selection criteria. Review of cases for eligibility currently in progress.

3. Member of the Strategy Group of PACCT (Program for the Assessment of Clinical Cancer Tests) and the Breast and Prostate Working Group Subcommittees. We developed a major intergroup protocol for TAILORx (PACCT-1) trial of OncotypeDx classifier in node negative, receptor positive breast cancer patients approved by CTEP and sent to CIRB. Goal is to launch trial by end of 2005.

4. Member of DataMart Steering Committee – Joint effort between NCI and Cooperative Groups to establish a data repository of clinical trials data (including marker data) in

order to allow more contemporaneous and frequent analyses of pooled breast cancer clinical trial research data.

Dr. Kevin Dobbin of BRB led a collaborative study involving four Director's Challenge groups resulting in the first major published study of the comparability of gene expression microarray data produced at different laboratories. The study was an important validation that different laboratories using a common protocol can get consistent results, as well as providing guidance for future large microarray studies involving multiple laboratories.

Dobbin, K., Beer, D.G., Meyerson, M., Yeatman, T., Gerald, W., Jacobson, J., Conley, B., Buetow, K., Heiskanen, M., Simon, R., Minna, J., Girard, L., Misek, D., Taylor, J., Hanash, S., Naoki, K., Hayes, D. N., Ladd-Acosta, C., Enkemann, S., Viale, A., Giordano, T. (2005) Inter-laboratory comparability study of cancer gene expression analysis using oligonucleotide microarrays. Clinical Cancer Research, 11: 565-72.

Dr. Dobbin of BRB collaborated with four extramural groups in the NCI's Cooperative Prostate Cancer Tissue Resource to compare biological characteristics of prostate tumors in men with low diagnostic PSA blood levels versus those with higher levels. The study identified a subgroup of patients in which low diagnostic PSA levels were associated with less aggressive tumors.

Datta, M.W., Dhir, R., Dobbin, K., Melamed, J., Becich, M.J., Orenstein, J.M., Kajdacsy-Balla, A.A., Bosland, M.C., Patel, A., Macias, V., Berman, J.J., and the Cooperative Prostate Cancer Tissue Resource (2005) Prostate cancer in patients with pre-diagnostic serum PSA values less than 4.0 ng/dl: Results from the Cooperative Prostate Cancer Tissue Resource . Journal of Urology, 173: 1546-1551.
Dobbin, K. and Simon, R. (2005) Experimental design [Specialist Review]. In: *Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics*. John Wiley and Sons, New York

Dr. Dobbin designed four prostate cancer and one melanoma tissue microarrays for use by the cancer biomarker research community.

Development of guidelines for cancer biomarker studies:

Despite years of research and hundreds of reports on tumor markers in oncology, the number of markers that have emerged as clinically useful is pitifully small. Often initially reported studies of a marker show great promise, but subsequent studies on the same or related markers yield inconsistent conclusions or stand in direct contradiction to the promising results. A variety of methodologic problems have been cited to explain these discrepancies. Many tumor marker studies have not been reported in a rigorous fashion, and published articles often lack sufficient information to allow adequate assessment of the quality of the study or the generalizability of study results. The development of guidelines for the reporting of tumor marker studies was a major recommendation of the National Cancer Institute-European Organisation for Research and Treatment of Cancer (NCI-EORTC) First International Meeting on Cancer Diagnostics in 2000. Dr. McShane of BRB collaborated with CDP staff and extramural statisticians to develop publication guidelines to provide relevant information about the study design, pre-planned

hypotheses, patient and specimen characteristics, assay methods, and statistical analysis methods. The goal of these guidelines is to encourage transparent and complete reporting so that the relevant information will be available to others to help them to judge the usefulness of the data and understand the context in which the conclusions apply.

McShane, L.M., D.G. Altman, W. Sauerbrei. Identification of clinically useful cancer prognostic markers: What are we missing? Journal of the National Cancer Institute, 97(14): 1023-1025, 2005.

McShane, L.M., D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark for the Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. REporting recommendations for tumor MARKer prognostic studies (REMARK).

Manuscript published in the following journals:

British Journal of Cancer, 93(4): 387-391, 2005.
European Journal of Cancer, 41: 1690-1696, 2005.
Journal of Clinical Oncology, 23(36): 9067-9072, 2005.
Journal of the National Cancer Institute, 97(16): 1180-1184, 2005.
Nature Clinical Practice Oncology, 2(8): 416-422, 2005.

Re-publication or linkage to REMARK guidelines in the following journals:

Nature Clinical Practice Gastroenterology, 2(8): A416-A422, 2005.
Nature Clinical Practice Urology, 2(8): A416-A422, 2005.
Breast Cancer Research and Treatment (in press).

McShane, L.M., D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark. Response to Popat and Houlston Re: 'REporting recommendations for tumor MARKer prognostic studies (REMARK)'. Journal of the National Cancer Institute, 97(24): 1855-1856, 2005.

McShane, L.M., D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark. Response to Katz and Kattan Re: 'REporting recommendations for tumor MARKer prognostic studies (REMARK)'

Methodology Development in Computational Cancer Biology and Statistical Genomics

Re-sampling Methods for Estimating Prediction Accuracy With High Dimensional Data

Genomic assays such as DNA expression microarrays are often used to develop predictors of patient outcome. These applications are characterized, however, by the fact that the number of candidate predictors is orders of magnitude greater than the number of cases. Consequently, standard statistical methods often do not work. One of the most common errors in developing genomic classifiers is the mis-use of cross-validation for estimating prediction accuracy, as we pointed out in a highly cited publication in 2003. During 2004-2005, Dr. Simon in collaboration with Dr. Ruth Pfeiffer of the Biostatiscs Branch in the Division of Cancer Genetics & Epidemiology and a post-doctoral fellow who they co-mentored, Dr. Annette Molinaro conducted research comparing a wide range of re-sampling methods for estimating prediction accuracy with high dimensional

data. The results were striking in that they demonstrated that leave-one-out cross validation is much superior to split-sample validation or repeated split-sample validation, in contradiction to much of current conventional wisdom. Dr. Wenyu Jiang, a current post-doctoral fellow in BRB and Dr. Simon have continued this research in conducting a study evaluating a wide variety of bootstrap resampling methods for the "p>n" setting. We found that many of the claims in the biostatistical literature concerning bootstrap methods are not applicable to p>n problems, and that many of the methods are either highly biased or highly variable. We developed a new adjusted bootstrap method that appears to be superior to previously reported methods. Dr. Sudhir Varma, a BRB post-doctoral fellow and Dr. Richard Simon have conducted research on valid and invalid methods of optimizing classifier tuning parameters using re-sampling methods.

Molinaro A, Simon R, Pfeiffer R. Prediction error estimation. A comparison of re-sampling methods. Bioinformatics 21:3301-07, 2005.

Jiang W, Simon R. A comparison of bootstrap methods and an adjusted bootstrap for estimating prediction error in microarray analysis. Submitted for publication.

Varma S, Simon R. Bias in error estimation when using cross-validation for model selection. Submitted for publication.

Dr. Alain Dupuy, a guest researcher from France and Dr. Richard Simon have reviewed all publications on whole genome expression profiling of cancers that used patient outcome. We wrote a critical review of this body of work and developed guidelines for use by authors, journal reviewers, and readers.

Dupuy A, Simon R. Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. Submitted for publication.

Pooling is often perceived as an efficient approach for microarray studies comparing gene expression between two classes because it may decrease the number of expensive microarray hybridizations required through reduction of the biological variability. Here we describe a microarray experiment using MCF-7 breast cancer cell line studied under two different experimental conditions for which the same number of independent pools as the number of individual samples was hybridized on Affymetrix GeneChips. Dr. McShane and collaborators showed the unexpected result that the number of probe sets found differentially expressed between treated and untreated cells when three individual samples per treatment class were hybridized on the GeneChips was about 3 times greater than that found using 3 independent pools per treatment class. Also, probe set-specific variability in pools was greater than that in individuals for more than 60% of the cases. Pooling samples in microarray experiments where the biological variability is expected to be small might not be helpful and could even decrease one's ability to identify differentially expressed genes.

Lusa, L., V. Cappelletti, M. Gariboldi, C. Ferrario, L. DeCecco, J.F. Reid, S. Toffanin, G. Gallus, L.M. McShane, M.G. Diadone, M.A. Pierotti. Caution regarding the utility of pooling samples in microarray experiments with cell lines. Submitted to BioTechniques.

Drs. Kevin Dobbin and Richard Simon have developed methods for planning sample size for studies whose objective is to identify genes that are differentially expressed among phenotypic or genotypic classes of tissue. They have considered how sample size depends on the microarray hybridization design utilized with dual label arrays, and considered a wide range of designs including the common reference design, balanced block design and loop design. They have also developed a method for sample size planning of clinical studies whose objective is to develop a predictor of outcome or predictor of phenotypic/genotypic class based on whole genome expression profiling.

Dobbin, K. and Simon, R. (2005) Sample size determination in microarray experiments for class comparison and prognostic classification. *Biostatistics*, **6**: 27-38.

Dobbin K and Simon R. Sample size planning for developing classifiers using high dimensional DNA expression arrays, Submitted for publication.

Dobbin and Simon studied the role of dye bias not removed by standard normalization methods. They corrected commonly held misconceptions about the implication of dye bias for the design of dual label microarray studies and also corrected a statistical modeling flaw that had appeared in the literature that had led to erroneous conclusions about how to design and analyze microarray experiments.

Dobbin, K.K., Kawasaki, E.S., Petersen, D.W., and Simon, R.M. (2005) Characterizing dye bias in microarray experiments. *Bioinformatics*, **21**: 2430-2437.

Dobbin, K.K., Shih, J.H. and Simon, R.M. (2005) Comment on`Evaluation of the gene-specific dye bias in cDNA microarray experiments'. *Bioinformatics*: **21**, 2803-2804.

Dobbin K and Simon R.Experimental design in expression profiling. Encyclopedia of Genetics, Proteomics and Bioinformatics, L Jorde editor, Wiley, 2005.

The goal of many gene-expression microarray profiling clinical studies is to develop a multivariate classifier to predict patient disease outcome from a gene expression profile measured on some biological specimen from the patient. Techniques such as cross-validation or bootstrapping can be used in this setting to assess predictive power, and if applied correctly, can result in a less biased estimate of predictive accuracy of a classifier. However, some investigators have attempted to apply standard statistical inference procedures to assess the statistical significance of associations between true and cross-validated predicted outcomes. We demonstrate in this paper that naïve application of standard statistical inference procedures to these measures of association can result in greatly inflated testing type I error rates and confidence intervals with poor coverage probabilities. Our results suggest that some of the claims of exceptional prognostic classifier performance that have been reported in prominent biomedical journals in the past few years should be interpreted with great caution.

L. Lusa, L.M. McShane, M.D. Radmacher, J.H. Shih, G.W. Wright, and R. Simon. Appropriateness of some resampling-based inference procedures for assessing performance of prognostic classifiers derived from microarray data. Revision under review with *Statistics in Medicine*.

Drs. Zhao, Li, and Simon developed a mixture model based normalization method that adaptively identifies non-differentially expressed genes and thereby substantially improves normalization for dual-labeled arrays in settings where the assumptions of global normalization are problematic.

Zhao, Y., Li, MC, and Simon R. An adaptive method for cDNA microarray normalization. *BMC Bioinformatics* 6:28, 2005.

Mathematical modeling of cancer oncogenesis

Dr. Xinan Zhang, a BRB post-doctoral fellow and Dr. Richard Simon used SEER age-incidence data to try to determine the number of rate limiting events in breast cancer oncogenesis. We developed a model that incorporated the age dependent dynamics of breast epithelium and clonal expansion of intermediate cells without the full complement of mutations required for an invasive tumor. We found that it was unlikely there are more than three rate limiting events in breast cancer oncogenesis occurring at a rate characteristic of point mutations in normal mammalian cells. Although breast tumors typically contain more mutations, our interpretation is that the initial set of 2-3 mutational events de-stabilize the genome and put in place a process that almost inevitably leads to an invasive tumor. We analyzed similar age-incidence data for breast cancer in BRCA1 and BRCA2 mutation carriers and found results consistent with our initial conclusions and we are currently analyzing data on contralateral breast tumors utilizing a similar model. Dr. Myong-Hee Sung and Dr. Simon have extended this modeling work to colon cancer. We have developed a model to attempt to further elucidate the sequence of genetic changes that occur during oncogenesis and to identify the mechanisms most likely to be the targets of the rate limiting oncogenic events.

Zhang X and Simon R. Estimating the number of rate-limiting genomic changes for human breast cancer. Breast Cancer Research & Treatment 91:121-124, 2005.

Simon R and Zhang X. On the nature of carcinogenic events in patients carrying germline BRCA1 and BRCA2 mutations. Submitted for Publication.

Sung MH and Simon R. Modeling tumorigenesis based on specific types of pathway de-regulation. Submitted for publication.

Development of Bioinformatic Tools – BRB-ArrayTools

Dr. Richard Simon has continued to develop BRB-ArrayTools software. This is state-of-the-art statistical software for the analysis of gene expression data and is designed to be utilized by non-statisticians. It has been extremely successful and well received. It now has 5125 registered users in 1026 laboratories in 62 countries, with over 90 new registrations per month. It is a successful experiment in using software to empower

biomedical scientists to take advantage of DNA microarray software. Dr. Simon continues to add state-of-the-art analysis tools to the software and features to enhance the training of biomedical scientists in statistical bioinformatics. He received the NIH Director's Award in 2005 for this work. The software is programmed and maintained under a contract with SRA International and the Emmes Corporation. The software is available at http://linus.nci.nih.gov/brb

Drs. Yingdong Zhao and Richard Simon have developed a data archive of publicly available gene expression datasets and corresponding clinical data for published human cancer gene expression profiling studies. The data is stored as BRBArrayTool project folders. This makes it easy for BRB-ArrayTools users to make their data publicly available, and it enables other clinical and biological investigators to easily download and start analyzing published data utilizing the most statistically powerful methods available. The archive currently contains data from 24 major studies of human cancer and is available at http://linus.nci.nih.gov/~brb/DataArchive.html

The BRB maintains a website at http://linus.nci.nih.gov/brb that contains other software, such as software for the generation of optimal and minimax two-stage phase II clinical trial designs, and software for managing dose administration for patients on accelerated titration design phase I designs. The website also contains technical reports and Powerpoint presentations of talks given by BRB staff. The Technical Reports and Powerpoint presentation sections are particularly rich in statistical genomics material and is accessed approximately 500 times per month.

Statistical Genomics Collaborative Research

Collaborations with The NCI Center for Cancer Research, NCI

Evaluation of two phosphorylation sites improves the prognostic significance of Akt activation in NSCLC tumors. Collaboration of Dr. Joanna Shih of BRB with Jin Jen (Laboratory of Population Genetics) and Phillip A. Dennis (Cancer Therapeutics Branch)

Akt is a serine/threonine kinase that has been implicated in lung tumorigenesis and lung cancer therapeutic resistance. Full activation of Akt requires two phosphorylation events, but only one site of phosphorylation (S473) has thus far been evaluated in clinical NSCLC specimens, which has resulted in conflicting results regarding the prognostic significance of Akt activation in NSCLC. In this study, we sought to determine whether evaluation of Akt phosphorylation at T308 would improve prognostic accuracy. To achieve this goal, phospho-specific antibodies against T308 and S473 were validated and used in an immunohistochemical analysis of tissue microarray slides containing NSCLC specimens (n=300) and surrounding lung tissue specimens (n=100). We observed that phosphorylation of either S473 or T308 was positive in most NSCSLC specimens, but was rarely detected in surrounding normal tissues. When Akt activation was defined by using both sites of phosphorylation, Akt activation was specific for NSCLC tumors vs.

surrounding tissue, was higher in adenocarcinoma than in squamous cell carcinoma, and was associated with shorter overall survival for all stages of disease. In multivariate analyses, increased phosphorylation of T308 alone was a poor prognostic factor for stage I patients or for tumors <5 cm. These results suggest that monitoring phosphorylation of Akt at T308 improves the assessment of Akt activation, and show that Akt activation is a poor prognostic factor for all stages of NSCLC.

Junji Tsurutani, Junya Fukuoka, Hiroko Tsurutani, Joanna H. Shih, Stephen M. Hewitt, Jin Jen, and Phillip A. Dennis (2005). Evaluation of two phosphorylation sites improves the prognostic significance of Akt activation in NSCLC tumors. Journal of Clinical Oncology, Dec 5; [Epub ahead of print]

Ingenuity Network Assisted Transcription Profiling: Identification of a new pharmacological mechanism for MK886. Collaboration of Dr. Joanna Shih of BRB with Anatoly L. Mayburd (Cell and Cancer Biology Branch)

MK886 is known to inhibit 5-lipoxygenase activating protein ALOX5AP (FLAP) and demonstrates anti-tumor activity in multiple human cell lines. The broad anti-tumor therapeutic window reported *in vivo* for MK886 in rodents supports further consideration of this structural class. Better understanding of the drug's mode of action is important for application in humans to take place. Affymetrix microarray study was conducted to explore MK886 pharmacological mechanism. Ingenuity Pathway Analysis software was applied to validate the results at the transcriptional level by putting them in the context of an experimental proteomic network. Genes most affected by MK886 included actin B and focal adhesion components. A subsequent NCI-60 panel study, RT-PCR validation followed by confocal microscopy and Western blotting also pointed to actin B down-regulation, F-actin loss and disorganization of the transcription machinery. In agreement with these observations, MK886 was found to enhance the effect of UV radiation in H720 lung cancer cell line. In light of the modification of cytoskeleton and cell motility by lipid Phosphoinositide Kinase-3 (PI3K) products, MK886 interaction with actin B might be biologically important. The low toxicity of MK886 *in-vivo* was modeled and explained by binding and transport by dietary lipids. The rate of lipid absorbance is generally higher for tumors, suggesting a promise of a targeted liposome-based delivery system for this drug. These results suggest a novel anti-tumor pharmacological mechanism.

Anatoly L. Mayburd, Alfredo Martínez, Daniel Sackett, Huaitian Liu, Joanna Shih, Jordy Tauler, Ingalill Avis, James L. Mulshine (2005). Ingenuity Network Assisted ranscription Profiling: Identification of a new pharmacological mechanism for MK886. To appear, Clinical Cancer Research.

Desmoglein 3 as a prognostic indicator in lung cancer. Collaboration of Dr. Joanna Shih of BRB with Jin Jen (Laboratory of Population Genetics)

Desmoglein 3 is a 130-KD desmosomal transmembrane glycoprotein, which belongs to the cadherin super family of calcium-dependent adhesion molecules. Desmoglein 3 is known as pemphigus vulgaris antigen, in which the patient of pemphigus vulgaris has a target antigen of the Desmoglein 3 causing blisters of the skin. Our cDNA expression

profile demonstrated that Desmoglein 3 was highly expressed in squamous cell carcinoma of the lung but not detected in normal lung or pulmonary adenocarcinoma. We investigated the clinical significance of Desmoglein 3 in lung cancer using tissue microarray technique.

Fukuoka J., Dracheva T., Shih, J.H., Hewitt S.M., Travis, W.D., and Jen J. (2005). Desmoglein 3 as a prognostic indicator for pulmonary carcinoid tumors. Submitted to Cancer Research.

Cross-species comparisons of Mouse Mammary Tumor Models and human breast Cancer by expression profiling: identification of luminal and basal phenotypes and a conserved gene signature discriminating ER+ and ER- tumors. Collaboration of Dr. Joanna Shih of BRB with Dr. Jeff Green, Laboratory of cell regulation and carcinogenesis.

Defining molecular similarities and differences between genetically engineered mouse models of mammary cancer and human breast cancer is critical to identifying models most relevant to human disease that can be appropriately used for preclinical studies. Expression profiling of mammary tumors from relevant transgenic mice and mice with targeted genetic mutations reveals that the mouse tumors can be clustered with human tumors into three major groups: 1) a luminal human ER-positive/mouse ER-negative sub-group (MMTV- her2/neu, -myc, ras, and PyMT); 2) a basal human ER-negative/mouse ER-negative sub-group (including all models with impaired p53 and BRCA1 function); and 3) a luminal human ER-positive/mouse ER-positive group (Wap-cre;$p53^{fl/fl}$). Combining array data from both mouse and human tumors also led to the development of the most robust gene expression predictor of ER status for tumors from both species, demonstrating that expression information from appropriate mouse models may improve classifiers for human cancer and identify functionally conserved genes between species important for defining crucial aspects of cancer biology.

Aleksandra M. Michalowska, Ting-Hu Qiu, Claudine Kavanaugh, Eva Lee, Daniel Medina, John I. Powell, Joseph R. Nevins, Joanna H. Shih, and Jeffrey E. Green (2005). A novel integrative analysis of mouse and human mammary cancer gene- expression profiles identifies conserved luminal and basal phenotypes and genetic networks defined by ERα status. Submitted to Proceedings of National Academy of Sciences.

Histological staining method preparatory to laser capture microdissection significantly affects detection of mRNAs in microarray hybridization. Collaboration of Dr. Joanna Shih of BRB with Dr. Fredrick Mushanski of the Laboratory of Genetics.

Gene expression profiling (GEP) by microarray analysis of cells enriched by laser capture microdissection (LCM) faces several technical challenges. Frozen sections yield higher quality RNA than paraffin-imbedded sections, but staining methods used for histological identification of cells of interest in the frozen sections could still damage the mRNA in the cells. To study the contribution of staining methods to degradation of results from GEP of LCM samples, we subjected pellets of the mouse plasmacytoma cell line TEPC1165 to direct RNA extraction and to parallel frozen sectioning for LCM and subsequent RNA extraction. We used microarray hybridization analysis to compare GEP

of RNA from cell pellets with that of RNA from frozen sections that had been stained with hematoxylin and eosin (H&E), Nissl Stain (NS), and for immunofluorescence (IF) as well as with the plasma cell-revealing methyl green pyronin (MGP) stain. All RNAs were amplified with two rounds of T7-based in vitro transcription and analyzed by twocolor expression analysis on 10-K cDNA microarrays. The MGP-stained samples showed the least introduction of mRNA loss, followed by H&E and immunofluorescence. Nissl staining was significantly more detrimental to GEP, presumably owing to an aqueous step in which RNA may have been damaged by endogenous or exogenous RNAases. This study demonstrates that RNA damage can occur during the staining steps preparatory to laser capture microdissection, with the consequence of loss of representation of certain genes in microarray hybridization analysis. Inclusion of RNAase inhibitor in aqueous staining solutions appears to be important in protecting RNA from loss of gene transcript.

Hongyang Wang, James D Owens, Joanna H. Shih, Ming-Chung Li, Robert F. Bonner, and J. Frederic Mushinski (2005). Histological Staining Method Preparatory to Laser Capture Microdissection Significantly Affects Detection of mRNAs in Microarray Hybridization. Submitted to BMC Genomics.

Colorectal Cancer Oncogenesis

Colorectal carcinomas develop through sequential stages of increasing morphological dysplasia. While the correlation of tumor phenotype with associated genomic alterations has been firmly established, the correlation with global gene and protein expression profiles is sketchy. Drs. L McShane and E Korn of BRB with collaborators have now analyzed tissue samples from 36 patients, including the complete mucosa-adenoma-carcinoma sequence from eight patients, to identify sequential alterations of the genome, transcriptome, and proteome that define the transformation of normal epithelium and the progression from adenomas to invasive disease. Comparative genomic hybridization (CGH) revealed patterns of stage specific, recurrent genomic imbalances. Gene expression analysis was performed on 9K cDNA arrays and identified 58 genes differentially expressed between normal mucosa and adenoma, 116 genes between adenoma and carcinoma, and 158 genes between primary carcinoma and liver metastasis ($p < 0.001$). Mean expression levels of 616 genes increased constantly from normal mucosa via adenoma and carcinoma to metastasis, whereas 1100 genes showed decreased mean expression levels. The differentially expressed genes are involved in a total of 20 canonical pathways which are affected in more than one stage comparison. Parallel analysis of our samples by CGH and expression profiling revealed a direct correlation of chromosomal copy number changes with chromosome-specific average gene expression levels. Protein profiling was analyzed by two-dimensional gel electrophoresis and subsequent mass spectrometry. While there was no direct match of differentially expressed proteins and genes, the majority of them belonged to identical pathways or networks, including, e.g., death receptor-, ERK/MAPK-, IL-6-, integrin-, and VEGF-signaling. In conclusion, increasing genomic instability and a recurrent pattern of chromosomal aberrations as well as specific gene and protein expression patterns correlate with distinct stages of colorectal cancer progression. Chromosomal aneuploidies exert a direct effect on average expression levels of the genes residing on

the aneuploid chromosomes, thereby contributing to a massive deregulation of the cellular transcriptome. The identification of novel genes and proteins might deliver relevant molecular targets for diagnostics and therapeutic interventions.

Habermann, J.K., U. Paulsen, U. Roblick, M.B. Upender, L.M. McShane, E.L. Korn, D. Wangsa, S. Kruger, H.-P. Bruch, G. Auer, and T. Ried. Stage-specific alterations of the genome, transcriptome, and proteome during colorectal carcinogenesis. Manuscript to be submitted shortly.

Statistical Genomics Collaborative Research With The International Leukemia/Lymphoma Molecular Profiling Project

Drs. George Wright and Richard Simon collaborate with the Staudt Laboratory of CCR in the International Lymphoma Classification Project, with Dr. Wright serving as principal statistician. These studies utilize whole genome technologies to characterize non-Hodgkins lymphomas and to develop new classifications that are prognostic and facilitate appropriate treatment selection for patients.

Data from DLBCL patients on Retuximab reviewed every 3 months, indicated that a sizable proportion of the cases had late onset neutropenia. Using a Bayesian model, the incidence of late onset neutropenia among the population was estimated, taking into account that patients may have had undiagnosed neutropenia between points of review. (Dunleavy K. et al. Blood 2005).

Gene copy number was analyzed utilizing whole genome CGH arrays for DLBCL samples, identifying copy number abnormalities that were related to lymphoma subtype, survival, and indirect changes in gene expression. (Bea S. et al. Blood 2005)

Tumor samples that were phenotypically mantle Cell, but lacked Cyclin D1 expression were analyzed. The gene expression profiles for these samples were found to strongly resemble the gene expression of standard mantle cell lymphoma, but indicated activation of Cyclin D2 and D3, thus raising the possibility of an alternative oncogenic pathway. (Fu K et al. Blood 2005)

Expression of BCL2 at the gene and protein level was evaluated for each subtype of DLBCL tumor as it relates to survival. A paper relating to this has been accepted and the Journal of Clinical Oncology.

Developed a gene expression signature diagnostic of Burkitt's lymphoma. A paper relating to this has been re-submitted for publication to the New England Journal, and is awaiting a response.

Analyzed array CGH data from multiple platforms in order to determine which platform would best suit the needs of the Molecular profiling project. Investigated algorithms to identify regions of copy number change from the raw array data. In the coming year a large number of tumor samples will be profiled using array CGH.

Dunleavy K., Hakim F., Kim HK, Janik JE, Grant N, Nakayama T, White T, Wright G, Kwak L, Gress R, Tosato G. Wilson W. B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostais. Blood 106 (3): 795-802 (2005)

Bea S, Zettl A, Wright G, Salaverria I, Jehn P, Moreno V, Burek C, Ott G, Puig X, Yang LM, Lopez-Guillermo A, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Gascoyne RD, Connors JM, Grogan TM, Braziel R, Fisher RI, Smeland EB, Kvaloy S, Holte H, Delabie J, Simon R, Powell J, Wilson WH, Jaffe ES, Montserrat E, Muller-Hermelink HK, Staudt LM, Campo E, Rosenwald A. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. Blood 106 (9): 3183-3190 (2005)

Fu K, Weisenburger DD, Greiner TC, Dave S**,** Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R, De Jong D, Jaffe ES, Wilson WH, Delabie J, Ott G, Dave BJ, Sanger WG, Smith LM, Rimsza L, Braziel RM, Muller-Hermelink HK, Campo E, Gascoyne RD, Staudt LM, Chan WC Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood 106 (13): (2005)

Collaboration of Dr. L Dodd and L McShane on nasopharyngeal oncogenesis with Allen Hildesheim of the NCI Division of Cancer Genetics & Epidemiology

Dodd, L., S. Sengupta, I. Chen, J. denBoon, Y. Cheng, M. Chen, W. Westra, M. Newton, B. Mittl, L. McShane, C. Chen, W. Sugden, P. Ahlquist, and A. Hildesheim.Evaluation of DNA expression levels in tissue obtained from normal and cancerous nasopharyngeal tissue suggests a role of host factors involved in nitrosamine activation and DNA repair in the etiology of nasopharyngeal carcinoma (NPC). Manuscript to be submitted shortly.

Collaborations of Dr. Sudhir Varma, a BRB post-doctoral fellow and Dr. Richard Simon with Dr.Tomas Ried's laboratory in the NCI Center for Cancer Research to find gene signatures of rectal cancer pathogenesis. We have also been developing new methods for combining data from different genomic assays to discover links between chromosomal loss and gain and changes in gene expression.

Ghadimi BM, Grade M, Difillippantonio MJ, Varma S, Simon R, Montagna C, Fuszesi L, Langer C, Becker H, Liersch T, Ried T. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas in preoperative chemoradiotherapy. Journal of Clinical Oncology 23:1826-38, 2005.

Grade M, Ghadimi M, Varma S, Simon R, Wangsa D, Stapleton L, Liersch T, Becker H, Ried T, Difilippantonio MJ, Selective utilization of the Wnt/β-catenin signaling pathway and aneuploidy dependent massive deregulation of the cellular transcriptome in human rectal carcinomas. Cancer Research 66, No 1, 2006.

Collaborations of Dr. R Simon of BRB with Dr. Sandra Swain's laboratory on expression profiling of breast tumors for patients receiving molecularly targeted therapy.

Yang SX, Simon RM, Tan AR, Nguyen D and Swain SM. Gene expression patterns and profile changes pre and post erlotinib treatment in patients with metastatic breast cancer. Clinical Cancer Research 11:6226-32, 2005.

Yang SX, Simon RM, Wedam S, Nguyen D, Modrusan Z, Smith V, deSauvage F, and Swain SM. Response in gene expression profile to Bevacizumab treatment in patients with inflammatory and locally advanced breast cancer. Submitted for publication.

Immunoinformatics

The following publications result from collaborations of Dr. Yingdong Zhao and Dr. Richard Simon of BRB with Dr. Roland Martin's laboratory in Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, NIH. Papers by Sospedra et al. demonstrate for the first time that the promiscuous restriction or redundant antigen presenting function is observed with all DR and DQ molecules in a disease-associated HLA-class II type. Our data also suggest that repeated infections with common pathogenic and even nonpathogenic viruses could expand T cells specific for conserved protein domains that are able to cross-react with tissue-derived and ubiquitous autoantigens. The paper by Markovic-Plese et al. demonstrates that TCR degeneracy is a general phenomenon, present even in the clone with a high degree of TCR specificity for an acute infectious agent. The paper by Venturini et al. reports the findings that an HIV-1-specific human T helper clone can react efficiently with peptides from other pathogens and imply that cellular immune responses identified as being specific for one human pathogen (HIV-1) could arise from exposure to other pathogens.

Sospedra, M., Muraro, P., Stefanova, I., Zhao, Y., Chung, K., Li, Y., Hamashin, C. Simon, R., Mariuzza, Clemencia, P. and Martin R. Promiscurous HLA restriction contributes to degenerate specificity of autoreactive CD4+ T cells. *J. Immunology, In press.*

Sospedra, M., Zhao, Y., Hausen, H., Muraro, P.A., Villiers, E.M., Pinilla, C., Martin, R. Arginine-Enriched protein domains from the non-pathogenic Torque Teno Virus (TTV) and other common viruses trigger autoreactive T Cells in Multiple Sclerosis. *PLoS Pathogens* 2005 Dec 16;1(4):e41

Markovic-Plese, S., Hemmer, B., Zhao, Y., Simon, R., Pinilla, C., and Martin R. High level of cross-reactivity in influenza virus hemagglutinin-specific CD4+ T-cell response: Implications for the initiation of autoimmune response in multiple sclerosis. *J Neuroimmunol.* 169(1-2):31-8, 2005.

Venturini, S., Allicotti, G., Zhao, Y., Simon, R., Burton, DR, Pinilla, C., and Poignard, P. Identification of peptides from human pathogens able to cross-activate an HIV-1-gag specific CD4+ T cell clone. Eur J Immunol. 2006 Jan;36(1):27-36

Zhao, Y., Sung, M.H., and Simon, R. Artificial intelligence methods for predicting T-cell epitopes. In *Immunoinformatics: predicting Immunogenicity in silico.* Methods in Molecular Biology. Flower D.R. (eds) Humana Press, Totawa NJ, *In press*.

Dr. Zhao of BRB has also collaborated with Dr. Francesco Marincola's laboratory in Department of Transfusion Medicine of NIH. The paper by Nambiar et al. describes a novel application of a software HLAMatchmaker to determine platelet compatibility in 16 alloimmunized patients with aplastic anemia refractory to random donor platelet transfusions. The paper by Basil et al. strove to identify cancer-specific markers for the molecular detection of a broad range of cancer types using cDNA microarray technology, which could be used broadly to increase the sensitivity and accuracy of cancer staging and early detection of loco-regional or systemic recurrence.

Nambiar, A., Duquesnoy, R., Adams, S., Zhao, Y., Oblitas, J., Leitman, S., Stroncek, D., Marincola, F. HLAMatchmaker-driven analysis of responses to HLA-typed platelet transfusions in alloimmunized thrombocytopenic patients. Blood. 2005 Nov 3

Basil, C.F., Zhao, Y., Zavaglia, K., Jin, P., Panelli, M. C., Voiculescu, S., Mandruzzato, S., Lee, H. M., Seliger, B., Freedman, R. S., Taylor, P. R., Hu, N., Zanovello, P., Marincola, F. M., and Wang, E. Common Cancer Biomarkers for Colon, Melanoma, Ovarian and Esophageal Tumors. *In press at* Cancer Research

Other Biostatistical Research

Smoothing-based approaches for estimating the risk of a disease by quantile-categories of a predictor variable. Work by Dr. Paul Albert of BRB was motivated from collaborative work on the Polyp prevention trial.

Borkowf, C.B. and Albert, P.S. Efficient estimation of risk of a disease by quantile-categories of a predictor variable using generalized additive models. Statistics in Medicine 24, 623-645, 2005.

In case-control studies of genetic epidemiology, participating subjects (probands) are often interviewed to collect detailed data about disease history and age-at-onset information in their family members. Genotype data are typically collected for the probands, but not for their relatives. In this article, Dr. Shih and collaborators consider a combined approach of case-control analysis of data from the probands and kin-cohort analysis of family history data of the relatives. Assuming a marginally specified multivariate survival model for joint risk of disease among family members, we describe methods for estimating relative-risk, absolute-risk and familial aggregation. We also describe a variation of the methodology that can be used for kin-cohort analysis of the family history data from a sample of only genotyped cases. We illustrate the application of the proposed methodologies for estimation of risk of breast cancer from BRCA1/2 mutations using data from the Washington Ashkenazi Study.

Chatterjee, N., Zeynep, K., Shih, J.H. and Gail, M. (2005). Case-control and case-only designs with genotype and family history data: Estimating relative-risk, familial aggregation and absolute risk. Biometrics, Online publication date: 20-Oct-2005

Genomic control for association studies under various genetic models

Case-control studies are commonly used to study whether a candidate allele and a disease are associated. However. spurious association can arise due to population substructure or cryptic relatedness. A novel genomic control (GC) approach had been developed to adjust for this spurious association under additive genetic model. Dr. Freidlin of BRB and collaborators expand this approach to recessive and dominant genetic models.

Zheng G, Freidlin B, Li ZH, Gastwirth GL. (2005) Genomic control for association studies under various genetic models. Biometrics 61, 187-193

The case-cohort design is an efficient and economical design to study risk factors for infrequent disease in a large cohort. It involves the collection of covariate data from all failures ascertained throughout the entire cohort, and from the members of a random subcohort selected at the onset of follow-up. In the literature, the case-cohort design has been extensively studied, but was exclusively considered for univariate failure time data. In this paper, Dr. Shih of BRB develops case-cohort designs adapted to multivariate failure time data. An estimation procedure with the independence working model approach is used to estimate the regression parameters in the marginal proportional hazards model (Cox, 1972), where the correlation structure between individuals within a cluster is left unspecified. Statistical properties of the proposed estimators are developed. The performance of the proposed estimators and comparisons of statistical efficiencies are investigated with simulation studies. A data example from Translating Research Into Action for Diabetes (TRIAD) study is used to illustrate the proposed methodology.

Lu, S., Shih, J.H. (2005). Case-cohort designs and analysis of clustered failure time data. Accepted, Biometrics.

Dr. Joanna Shih of BRB considered the problem of estimating covariate effects in the marginal Cox proportional hazard model and multi-level associations for child mortality data collected from a vitamin A supplementation trial in Nepal (Nepal Nutrition Intervention Project-Sarlahi, or NNIPS), where the data are clustered within households and villages. For this purpose, a class of multivariate survival models that can be represented by a functional of marginal survival functions and accounts for hierarchical structure of clustering is exploited. Based on this class of models, an estimation strategy involving a within-cluster resampling procedure is proposed. The asymptotic theory for the proposed estimators is established, and the simulation study shows that the estimates are consistent. The analysis of the NNIPS study data shows that the association of mortality is much greater within households than within villages.

Shih, J.H., Lu, S. (2005). Analysis of failure time data with multi-level clustering, with application to the child vitamin A supplementation trial in Nepal. Revision submitted to Biometrics.

In 2004 Dr. Lori Dodd and Dr. Paul Albert of BRB published a paper on potential problems when one estimated diagnostic error of binary tests without a gold standard using latent class modeling approaches (a common practice in many biological studies). We showed that these modeling approaches are sensitive to the dependence structure between tests, yet it is nearly impossible to distinguish between competing models with only a few experimental tests. We have a follow-up paper which examines the robustness of the estimation procedures when, in a fraction of cases, we observe the gold standard test. We propose semi-latent modeling approaches for this problem and show that, even with a small percentage of gold standard information, estimates of diagnostic error are insensitive to the assumed dependence structure between tests. This work (Albert and

Dodd, 2005) is being revised for JASA-applications. Another paper which examines an imputation approach for estimating diagnostic error with partial gold standard evaluation (Albert, 2005) has been submitted to Biometrics.

Albert, P.S. and Dodd, L. A cautionary note on the robustness of latent class models for estimating diagnostic error without a gold standard. Biometrics 60, 427-435, 2004.

Albert, P.S. and Dodd, L. On estimating diagnostic accuracy from studies with multiple raters and partial gold standard evaluation. In revision at The Journal of the American Statistical Association, 2005.

Albert, P.S. An imputation approach for estimating diagnostic accuracy from partially verified designs. Submitted to Biometrics, 2005.

Albert, P.S. Misclassification Models. Encyclopedia of Biostatistics, 2nd Edition. Editors: Peter Armitage and Theodore Colton, Wiley Press, 2005.

Collaborative Clinical Research with the Center for Cancer Research Intramural Program

Dr Albert collaborates with a number of CCR Branches and Laboratories including the Radiation Oncology, Neuro-Oncology, Metabolism, Urologic Oncology, and Basic Sciences laboratory. He helps investigators design their studies as well as write protocols which are then submitted to the PRMC of which he is a member. Collaborations which resulted in publications during 2005 include:

Collaborations on the Polyp Prevention Trial

Hartman, T.J., Albert, P.S., Snyder, K., et al. The association of calcium and vitamin D with risk of colorectal adenomas. Journal of Nutrition 135, 252-259, 2005.

Cantwell, M.M, Forman, M.R., Albert, P.S., et al. No association between fatty acid intake and adenomatous polyp recurrence in the Polyp Prevention Trial. Cancer Epidemiology Biomarkers and Prevention 14, 2059-60, 2005.

Lanza, E., Hartman, T.L., Albert, P.S., et al. The association of dried bean intake and colorectal adenoma recurrence. Revised for The American Journal of Nutrition.

Hartman, T.J., Yu, B., Albert, P.S., et al. Does non-steroidal anti-infammatory drug use modify the effect of a low-fat, high-fiber diet on recurrence of colorectal adenomas? Cancer Prevention Epidemiology and Biomarkers 10, 2359-2365, 2005.

Collaborations on the Women's Alcohol Study (WAS). WAS was a cross-over study examining the effect of alcohol on hormones associated with cancer.

Lavigne, J.A., Baer, D.J., Wimbrow, H.H., Albert, P.S., et al. Alcohol-induced changes in insulin like growth factor-1 and insulin-like growth factor binding protein-3 in postmenopausal women. American Journal of Clinical Nutrition 81, 503-507, 2005.

Roth, M.J., Paltoo, D.N., Albert, P.S. et al. Common leptin receptor polymorphisms do not modify the effect of alcohol ingestion on serum leptin levels in a controlled feeding and alcohol ingestion study. Caner Epidemiology, Biomarkers, and Prevention. 14, 1576-1578, 2005.

Wei, W., Abnet, C.C., Lu, N., Roth, M.J., Dye, B.A., Dong, Z.W., Taylor, P.R., Albert, P.S., et al. Risk factors for esophageal squamous dysplasia in adult inhabitants of a high-risk region of cancer. Gut 54, 759-763, 2005.

Radiation Oncology Collaborative Studies

Muanza, T., Albert, P.S., Smith S., et al. Comparing measures of acute bowel toxicity in patients with prostate cancer treated with external beam radiation therapy. International Journal of Radiation Oncology, Biology, and Physics 62, 1316-1321, 2005.

Brooks, J.P., Albert, P.S., Wilder, R.B., et al. Long-term salvage radiotherapy (SRT) outcome after radical prostatectomy (RP) and relapse predictors. Journal of Urology 174, 2204-2208, 2005.

Brooks, J.P., Danforth, D., Albert, P.S., et al. Early ipsilateral breast tumor recurrences after breast conservation affects survival: An analysis of the NCI randomized trial. International Journal of Radiation Oncology, Biology, and Physics 62, 785-789, 2005.

Brooks, J.P., Albert, P.S., O'Connell, et al. Lymphovascular invasion (LVI) in prostate cancer: prognostic significance in patients treated with radiotherapy after radical prostectomy (RP). In Press at Cancer, 2005.

Brown, M.W., Ning, H. Arora, B., Albert, P.S. et al. A dosimetric analysis of dose escalation using two intensity modulated radiation therapy techniques in local advanced unresectable pancreatic carcinoma. Submitted to International Journal of Radiation Oncology, Biology, and Physics.

Druzgal, C.H., Chen, Z., Yeh, N.Y., Thomas, G, Ondrey, F.G., Duffey, D.C., Vilela, R., Ende, K., McCullagh, L., Rudy, S.F., Muir, C., Hersher, L.L., Albert, P.S., Van Waes, C. A pilot study of longitudinal serum cytokine and angiogenesis factor levels as markers of therapeutic response and survival in patients with head and neck squamous cell carcinoma. Head and Neck 27, 771-784, 2005.

Collaborations with Urologic Oncology Branch

Corbin, N.S., Glenn, G., Albert, P.S., et al. Clinical and genetic delination of von Hippe-Lindau Syndrome. Submitted, 2005.