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Transplantomics and Biomarkers in Organ Transplantation: A Report From the First International Conference

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> "At no other time has the need for a robust, bidirectional information flow between basic and translational scientists been so necessary"
>
> Elias Zerhouni, Director of the NIH, NEJM (1)

The First International Conference on Transplantomics and Biomarkers in Organ Transplantation was held in San Francisco on February 24 to 26, 2010. Hosted by The Transplantation Society and co-hosted by the Institute for Immunology, Transplantation and Infection (ITI) at Stanford University, the conference brought together myriad disciplines in transplantation research including genomics, transcriptomics, proteomics, metabolomics, informatics, next-generation sequencing technologies, and imaging and clinical transplantation. This report highlights the goals and key topics discussed at this inaugural meeting.

Goals of Transplantomics

In opening the conference, Jeremy Chapman highlighted the advancements of transplantation biology over the past 60 years, along with two main challenges that lie ahead. First, rates of long-term graft survival are not different now than they were 30 years ago. Second, although mortality rates of those with transplants are reduced compared with those on dialysis, they are still significantly higher than the normal population. To address these challenges, he proposed that this new decade of research may be the "era of individualized therapy" driven by the technologies of genomics, metabolomics, and proteomics.

Individualized therapy was the focus of the conference, with the prominent topic being the search for both diagnostic and predictive biomarkers for allograft dysfunction that could be used for personalized treatment of patients. A variety of other themes were also highlighted, including, but not limited to, the use of protocol and for-cause biopsies for microarray and histologic analysis, the use of noninvasive methods for biomarker discovery, the pitfalls of relying solely on the gold-standard criteria for biopsy classification, low-cost and time-efficient diagnostic and analysis tools, defining and identifying causative versus correlative biomarkers, and compiling/harnessing information in the public domain.

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Conference Chair Minnie Sarwal and Co-Chairs Atul Butte and Mark Davis, all from Stanford University in Palo Alto, California, highlighted the meeting as a way to bridge the gap between basic research and medical science to help to unify and advance the nascent field of high-throughput genome-wide technologies in transplant medicine. The Chairs coined a new word for this field of study, calling it “Transplantomics.” It is clear that to go from bench to bedside, the current silos of data from individual laboratories need to give way to a more collaborative and progressive environment. To break down these walls, the conference featured a wide breadth of topics in transplantation biology and biomarker research.

Genomic and Proteomic Technologies

The main themes of the opening day related to the advancement of genomic and proteomic analyses, use of genomic and noninvasive methods for biomarker discovery, human immunology, importance of sample and data collection, and the breaking down of intellectual barriers. An important point, raised by Mark Davis, Director of the ITI, was the limitation of animal models and the importance of appreciating human-specific immunology if we are to speed the translation of basic immunology into clinical practice. This highlighted the need for a systems biology approach to analyze the human immune system, without a priori bias of knowledge or simplistic application of animal model data, which may be only minimally relevant in human immunity. More than 400,000 publicly available microarray data sets and an increasing number of proteomic and peptidomic data points can be used to rethink and evaluate molecular signatures of disease. Clustering publicly available data could provide a revolutionary approach for new biomarker discovery, leading to existing drugs being used to treat seemingly “unrelated” diseases and new biomarker discovery, without a single new experiment (2).

Biomarkers for Acute Transplant Injury

New molecular and biomarker discovery efforts for acute transplant rejection (AR) were discussed in relation to the graft, peripheral blood, and urine. Microarrays of dysfunctional kidney biopsies were shown to uncover profound transcriptional differences between antibody-mediated rejection (AMR) and T-cell–mediated rejection, with possibly different outcomes (3). These data highlighted the ongoing importance of pathology as an important means to stratify treatment choices for AR.

A novel approach of cross-platform microarray data mining analysis, followed by quantitative polymerase chain reaction (PCR) validation, was shown to provide an exquisitely sensitive and specific five gene sets used to predict AR with a more than 90% probability 3 months before biopsy-proven rejection (4) (Sarwal et al., unpublished data, 2011) or an increase in the serum creatinine. Using a subset of these same data, new software programming has provided a means to deconvolute the peripheral blood transcriptome and assign the primary signal for graft rejection to the trafficking monocytes. These data, published soon after this meeting in *Nature Methods*, were presented from a biostatistical viewpoint by Dr. Robert Tibshirani from Stanford University, who echoed the theme of collaborative research by stating that the new code for deconvolution analysis would be publicly available as a free software application called cell-specific significance analysis of microarray (csSAM) in the current freely available statistical program, written by the same group (5). To facilitate the application of these refined gene sets for clinical care at the bedside, Ron Davis from Stanford University led discussions focused on a reductionist approach for genomic studies and showed how collaborations between biologists and engineers are able to turn theoretical ideas into practical and inexpensive devices. Examples

include microchips and magnetic devices that allow for isolation of various cell types from the blood, converting biologic information into a molecular barcode.

A more focused approach of selected gene analysis in urine in AR, using the kidney as an in vivo “flow cytometer,” were reviewed, as a means to diagnose AR from a combination of cytotoxic, regulatory, and cell-specific molecules (6). The summation of the first series of talks focusing on the application of high-throughput “omics” in organ transplantation thus highlighted the importance of novel discovery, without bias of prior knowledge, providing new insights and biomarkers for the processes governing AR in the organ and the periphery. This theme was subsequently revisited in a review of the application of these similar technologies to understand the responses of the normal immune system in health and infection, which is an important theme of study in the Human Immune Monitoring Core in ITI at Stanford University (http://iti.stanford.edu/), led by Dr. Mark Davis. The parallels between tumor and allograft rejection were also later highlighted, as were the common signatures of organ rejection, across different tissue sources (7).

Biomarkers for Chronic Transplant Injury

Understanding the pathogenesis of chronic allograft dysfunction, with a focus on kidney transplantation (8, 9), yields the challenge of discriminating genes associated with chronic histologic damage confounded by concomitant acute inflammation that may predate the appearance of histologic lesions evaluated by needle biopsy. Studying gene expression changes associated with chronic damage in the absence of rejection allowed for 6-month protocol biopsies to predict progression of interstitial fibrosis/tubular atrophy (IF/TA) at 24 months. Interestingly, the sensitivity of the arrays to detect genes from infiltrating cells was a better predictor than graft histology. Discussions followed on the utility of noninvasive diagnostic and prognostic biomarkers (from blood and urine) of fibrosis progression in liver transplantation (10). The next generation imaging tools for visualizing injury pathways in vivo brought into focus novel tools that can detect and monitor small number of cells in vivo to visualize engraftment and expansion through the use of molecular markers linked to cellular metabolism in stem-cell and transplantation biology (11).

Predicting Graft Risk by Transplantomics

Unpublished work was discussed from the Wellcome Trust funded UK consortium on how genetic variations between donor and recipient genomes determine early and late renal allograft dysfunction. This large undertaking, currently with approximately 600 people and expanding to 10,000 renal transplant donor-recipient pairs, is working on finding predictive single-nucleotide polymorphisms (SNPs) of rejection, end-stage kidney failure, and long-term transplant outcomes (http://www.wtccc.org.uk/). Similarly, early results were presented from the Deterioration of Kidney Allograft Function study, a multicenter, prospective observational study to detect SNPs associated with transplant outcomes in AR and chronic graft dysfunction on a test cohort of 1000 patients and a validation cohort of 3000. One finding was that certain SNPs, often found in Blacks, were associated with a reduction in tacrolimus trough concentration (Israni et al., unpublished data).

The application of microRNAs as novel predictive biomarkers of T-cell–mediated AR was presented (12) with the hypothesis that differences in microRNAs in AR may relate to relative proportions of graft infiltrating immune cells and changes in resident parenchymal cells (13). Transplant vasculopathy was predicted by modeling allograft gene expression with machine learning algorithms (Mannon et al., unpublished data). On a subset of 1000 biopsies from more than 150 patients, real-time PCR was used to validate the gene targets of interest. The data were incorporated into a Bayesian Model, a method used to explore gene network relationships, in which critical relationships between allograft pathology and gene

expression signatures could be identified. A Genome Canada supported consortium based in Vancouver presented data from the PROOF Center of Excellence, where there is ongoing systemized utilization of genomics, proteomics, and metabolomics platforms in an attempt to identify diagnostic biomarkers of AR and chronic rejection in heart and kidney allografts. Interestingly, they found genes involved in cell migration and cytoskeletal remodeling, in addition to classic biomarkers in antigen processing, immune response, so forth. The next planned steps for all biomarker discoveries are to set up clinical trials to prospectively validate and qualify these biomarkers for clinical use.

The Genomics of Tolerance

The genomics of transplant tolerance was addressed with a focus on liver tolerance mechanisms because up to 20% of liver transplant patients no longer need immunosuppressant therapy. Harnessing means to identify and monitor this condition, which would help to reduce immunosuppression safely in many patients. Using both microarrays and real time PCR to search for biomarkers of tolerance in peripheral blood, gene signatures were shown that tracked with patients who developed spontaneous tolerance (14). These biomarkers are currently being validated in a prospective withdrawal study supported through the European Union RISET consortium (www.risetfp6.org). A deeper understanding of an individual's immunologic and inflammatory distinctiveness may in the near future be harnessed to improve clinical therapies tailored to individual patient needs.

Humoral Immunity

Cross talk between alloimmunity and autoimmunity in organ transplantation brought into focus the potential role of antibodies to self-antigens or autoantigens in alloimmune injury such as bronchiolitis obliterans (15). There was discussion on the crosslinking of major histocompatibility complex molecules on endothelial cells and the generation of phosphorylated proteins as a means to follow AMR in cardiac transplants, even when the tissues did not stain for C4d (16). The problems relating to the use of C4d-positive staining as the gold-standard diagnostic marker of AMR were considered, and the need for better assessment for and definition of AMR was agreed. Based on recent publications using protein array technology (17), strong reactivity to self-antigens was demonstrated in kidney transplants compared with those on dialysis, along with stronger reactivity in those with transplant glomerulopathy compared with stable kidney function. It was clear that assessment of the formation of antibodies after transplantation, not only to graft but also to self-antigens, was a potential pathogenic mechanism of chronic antibody-mediated injury.

Proteins and Metabolites in Transplantation

Moving down from the antibodyome, the advantages of studying the proteome, peptidome, and metabolome were highlighted, especially when it is possible to link them to the genome and clinical information in the study of transplant responses. A point of great interest was a discussion of population proteomics analysis of 3000 patients using a new platform for discovery and validation of candidate biomarkers at the Pacific Northwest National Laboratory (http://pnl.gov/).

The power of metabolomics in monitoring organ transplants was also elucidated. Immediate metabolic changes in the body can be detected noninvasively, quickly, and inexpensively. Combinatorial metabolomics methods allow for approximately 3500 compounds to be detected and quantified in the blood. Currently, four metabolome databases cover human metabolomes, toxins, drugs, and small molecule pathways. (http://www.hmdb.ca/, http://t3db.org/, http://smpdb.ca/, and http://www.drugbank.ca/). As part of the Deterioration of Kidney Allograft Function study, urine metabolomes of patients with IF/TA, IF/TA with

inflammation, and transplant glomerulopathy using ^{1}H-NMR spectroscopy reveal highly sensitive and specific “classifiers” (Rush et al., unpublished data).

The importance of identification and nomenclature of human leukocyte antigen alleles was elucidated, as an exemplar of the problems that will face other areas of extreme molecular diversity. Currently, more than 4300 alleles have been recognized, and methods of finding and defining novel alleles were discussed, along with the new nomenclature system implemented April 1, 2010 (http://www.ashi-hla.org/docs/newsletter/ASHI_Quarterly/34_1_2010/hla_nomenclature.pdf).

Personalizing Care for the Transplant Patient

On the second day of the conference, the first session opened with a discussion of one of the endpoints of Transplantomics, namely personalizing individual care. The review of personalized medicine addressed how to follow a generalized process in biomarker discovery for personalized medicine: collecting and storing samples for data analysis; compiling and understanding the mass of data from samples; and translating the research findings into clinical practice. There was discussion about clinical trials conducted by the National Institutes of Health including the creation of the Immune Tolerance Network (http://www.immunetolerance.org/) to assist in the conduct of clinical trials in transplant tolerance, whereby myriad biologic samples from many transplant patients are being conducted to store samples in a centralized biorepository. Currently, more than 30,000 sample aliquots from more than 750 patients are available for study. Linking clinical data to sample data allows clinicians and scientists to break the barriers in better understanding allograft dysfunction and thus reach the endpoint of customizing immunosuppression.

Through facilities, such as those available at the Broad Institute, tools for integrative analyses are being provided (http://www.broadinstitute.org/), which can be powerful adjuncts for new discoveries for disease causatives (18). Free access to data analysis and management software, such as GenePattern and Integrative Genomics Viewer at the Broad Institute, aim to make these integrative tools available to all scientists and clinicians, not just those in the genomic analysis field (http://www.broadinstitute.org/cancer/software/genepattern/ and http://www.broadinstitute.org/igv). Careful consideration of how to take biomarkers from bench to bedside was presented by the Federal Drug Administration. There was an eloquent outline of the qualification process that researchers should take to define a biomarker for specific clinical use, which includes a consultation and advice stage followed by a review stage. Importantly, researchers need to define a “context of use” for the biomarker: a specific clinical definition of what the biomarker is useful for and for whom.

Novel Applications

The meeting concluded by considering novel applications of omics. The Stanford group highlighted how the use of csSAM accurately analyzed gene expression from heterogenous samples to estimate cell-type-specific gene expressions from whole blood (5). To test the method, five cell types were analyzed from blood of AR versus stable kidney transplant patients. The importance of monocytes, which was observed by csSAM, was obscured by the high background noise in traditional SAM analysis.

A novel positron emission tomography (PET) probe can now be used in vivo to measure T-cell activation by molecular imaging. The PET probe is phosphorylated and trapped by an enzyme in a DNA pathway required for T-cell proliferation. With the unique approach of the University of California, Los Angeles, group (19), the target of a PET imaging tool has now become a drug target in which they are screening for small molecule inhibitors.

Finally, the use of nanoparticles for augmenting mucosal immunity raised the novel concept of studying "vaults," again by a UCLA group (20). Vaults are hollow, self-assembling cell particles made up of 96 copies of major vault protein and are able to target and mature dendritic cells, which are central for initiating immune responses. Encapsulating an immunogenic protein from *Chlamydia* within the vault nanocapsules protected mice from *Chlamydia* and prevented tissue inflammation. Next steps include creating immunovaluts that package cytokines to help dampen immune responses in inflammation and organ rejection.

Conclusions

The First International Conference on Transplantomics and Biomarkers in Organ Transplantation brought together researchers, technologists, bioinformaticians, clinicians, industry, and regulators from around the world with the ultimate goal of advancing the field of transplantation. Concern about age-old strategies in diagnosing allograft dysfunction has led to the use of the genome, proteome, and metabolome, in concert with clinical data, for omics approaches in predicting and diagnosing disease.

The challenge is now to apply these fantastic advances in technology to clinical applications. Without unified models of community and collaboration between laboratories, hospitals, and institutions, the wealth of data cannot be properly used. Fortunately cross-collaborative projects, highlighted in talks by Mark Davis, Minnie Sarwal, Graham Lord, Allan Kirk, Robert Tibshirani, Ajay Israni, Bruce McManus, and others, are well on their way to harnessing these data. Attendees suggested expanding the discussion to include long-term graft survival and ethnical issues in transplantomics. Because of the resounding success of this first conference, a new annual meeting was voted in by the attendees. Transplantomics will now be an annual meeting run by the Transplantation Society, alternating its location between being within and outside the United States every alternate year, and focused on the evolution of high-throughput technological advances in biomedicine and their translation to clinical practice in organ transplantation. Transplantomics 2011 is being held in Barcelona March 14 and 15, 2011 (www.tts.org).

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