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CELL-MEDIATED IMMUNITY AND ANTI-HLA ANTIBODY TESTS PROVIDE INSIGHT TO IMMUNE RESPONSE IN RENAL TRANSPLANT PATIENTS

**The ImmuKnow[®] and anti-HLA antibody assays both contribute to
characterization of renal transplant patients being weaned off immunosuppression**

COLUMBIA, MD – Data developed by Cylex in conjunction with the University of Pittsburgh Medical Center, and presented today at the American Transplantation Congress in Toronto, Canada, suggest that cell-mediated immunity and anti-HLA antibody assays may be independent measures of different facets of the immune response (at least in renal transplant patients).

“Cell-mediated immunity, as measured with Cylex’s ImmuKnow test, may indicate the effectiveness of augmented immunosuppression and the risk of viral infections,” said Brad Stewart, president of Cylex. “By contrast, levels of anti-HLA antibodies may determine the risk for antibody-mediated rejection. In renal transplant patients who are being weaned from immunosuppression, it seems that both assays may contribute independently to characterize these patients.”

Researchers at the University of Pittsburgh Medical Center have independently shown that immune function, assessed using the ImmuKnow assay for cell-mediated immunity, may correlate with decreased immunosuppression in a cohort of renal transplant recipients enrolled in a protocol of immunosuppression minimization using depletion induction and monotherapy.* They have also determined humoral sensitization by assessing the circulating anti-HLA antibodies.† The current study (“Relationship between immune function and humoral sensitization in renal transplant recipients;” see abstract no. 974) was designed to assess the relationship between immune function and the level of humoral sensitization in serially matched samples.

The ImmuKnow assay of cell-mediated immunity is used to measure the degree of immune activation of CD4+ T-cell function. Circulating anti-HLA antibodies were determined by ELISA and expressed as



percent ELISA-PRA for anti-HLA class I and II antibodies. Immune function was determined by using the ImmuKnow assay and ELISA-PRA in 290 samples from 39 patients receiving renal transplantation. Samples were collected pre-treatment through 2,160 days post-treatment (mean 289 ± 428 SD). For 33 patients, sequential analysis (of between 4 and 14 samples per patient) also correlated with clinical outcomes and responses to various therapies.

There was no correlation between the cell mediated immunity (measured in nanograms of ATP per milliliter) and percent ELISA-PRA for class I or class II ($R^2 < 0.1$). However, the median ATP concentration of samples that exhibited ELISA-PRA class II $< 20\%$ ($n = 203$) was significantly higher than the median ATP concentration of samples ($n = 87$) with ELISA-PRA $> 20\%$ (216 vs 138; $p = 0.0002$). A similar pattern, but less significant ($p = 0.07$) was observed for ELISA-PRA class I.

Three different patterns were evident among the renal transplant patients:

- Those in whom the immune function values were in a stable zone (100-300 ng ATP/mL) with or without anti-HLA antibodies
- Those who exhibited diminished levels of immune function (< 100 ng ATP/mL) following treatment for mixed forms of rejection (steroid bolus + plasmapheresis/IVIG + MMF) with or without a change in ELISA-PRA
- Those who displayed low immune function (< 100 ng ATP/mL) in association with viral infections such as BK or CMV.

*Tan HP, Donaldson J, Basu A, et al. 411 Living donor kidney transplants using alemtuzumab pre-conditioning and tacrolimus monotherapy: 5 year experience. Abstract no. 137 presented at the American Transplant Congress, Toronto, Canada, May 30, 2008.

†Tan HP, Donaldson J, Basu A, et al. Rejection characteristics of 200 living donor kidney transplants using alemtuzumab induction and tacrolimus monotherapy: 3 year follow-up. Abstract no. 221 presented at the American Transplant Congress, Toronto, Canada, May 30, 2008.

About ImmuKnow[®]

ImmuKnow is an immune cell function assay that can detect cell-mediated immunity (CMI) in adult patient populations undergoing immunosuppressive therapy for organ transplantation, by measuring the concentration of adenosine triphosphate (ATP) released from CD4 cells following cell stimulation.



The ImmuKnow test is a qualitative assay and does not directly quantify the level of immunosuppression. Results of ImmuKnow assays should be used in conjunction with clinical presentation, medical history, and other clinical indicators when assessing the immune status of any individual patient. The use of the ImmuKnow assay as described in this study has not been approved or cleared by the FDA. The company may use data from this or similar studies to support a future FDA marketing application for a similar indication.

About Cylex, Incorporated

Cylex™ is a privately held global life sciences company that is the leader in the development and manufacture of *in vitro* diagnostic products intended to illuminate immunity. ImmuKnow is the *in vitro* diagnostic utilized to detect cell-mediated immunity (CMI) in patient populations undergoing immunosuppressive therapy, and is increasingly being adopted at organ transplant centers throughout the US and abroad. The Company's patented technology provides an innovative platform allowing clinical researchers to simply and reproducibly measure immune cell function for the development of new diagnostics, biomarkers, and companion assays. The company is based in Columbia, MD.