Clinical Policy Bulletin:  
ImmuKnow (Transplantation Immune Cell Function Assay)  

Revised April 2014  
Number: 0773

Policy

Aetna considers the ImmuKnow Assay, also known as the Transplantation Immune Cell Function Assay (Cylex, Inc., Columbia, MD), experimental and investigational for all indications including any of the following because of insufficient evidence of its effectiveness:

- For identification of individuals at risk for rejection prior to kidney, liver, lung, or any other solid organ transplant.
- For management of individuals undergoing allogeneic hematopoietic stem cell transplantation.
- For management of individuals with inflammatory bowel diseases (Crohn's disease and ulcerative colitis).
- For management of organ transplant rejection in individuals undergoing immunosuppressive therapy post solid organ transplant.
- For prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs.
- For prediction of risk infection in individuals with lupus nephritis.

Background

Transplant recipients have an increased risk of infection due to the necessary immunosuppression. Conversely, under-immunosuppression carries the risk of rejection. Biopsy of the transplanted organ can confirm rejection and is sometimes performed before symptoms develop. When organ rejection is suspected, additional tests may be performed prior to organ biopsy. Management of organ transplant rejection by an immune cell function assay to assess the immune function of the transplant recipients and to individualize therapy has been proposed. It has also been investigated as a method of identifying patients at risk for early acute kidney transplant rejection prior to the actual kidney transplant.
In April 2002, the Food and Drug Administration (FDA) cleared for marketing the Cylex Immune Cell Function Assay (Cylex Inc., Columbia, MD) for the detection of cell mediated immune response in populations undergoing immunosuppressive therapy for organ transplant. According to the 510(k) application submitted by the manufacturer to the FDA, the test detects cell-mediated immunity in whole blood after a 15 to 18 hour incubation with a stimulant (i.e., phytohemagglutinin). During incubation, increased adenosine triphosphate (ATP) synthesis occurs within the cells that respond to phytohemagglutinin. Concurrently, whole blood is incubated in the absence of phytohemagglutinin for the purpose of assessing basal ATP activity. Anti-CD4 monoclonal antibody coated magnetic particles are added to immuno-select CD4 cells from both the stimulated and non-stimulated cells. After washing the selected CD4 cells on a magnet tray, a lysis reagent is added to release intracellular ATP. Addition of luminescence reagent (luciferin/luciferase) to the released ATP produces light measured by a luminometer, which is proportional to the concentration of ATP. The concentration of ATP (ng/ml) is calculated from a calibration curve and compared to ATP level ranges to characterize the cellular immune function of the sample.

Kowalski et al (2006) assessed the relative risks of infection and rejection of 504 solid organ transplant recipients (heart, kidney, kidney-pancreas, liver and small bowel) using the ImmuKnow assay. Blood samples were taken from recipients at various times post-transplant and compared with clinical course (stable, rejection, infection). In this analysis, 39 biopsy-proven cellular rejections and 66 diagnosed infections occurred. Odds ratios of infection or rejection were calculated based on measured immune response values. The authors reported that a recipient with an immune response value of 25 ng/ml ATP was 12 times (95 % confidence interval [CI]: 4 to 36) more likely to develop an infection than a recipient with a stronger immune response and that a recipient with an immune response of 700 ng/ml ATP was 30 times (95 % CI: 8 to 112) more likely to develop a cellular rejection than a recipient with a lower immune response value.

Thai et al (2006) compared pancreas recipient clinical states (stable, rejection, infection) with T cell responses using the ImmuKnow assay. Blood samples were taken from pancreas recipients pre-transplant and at approximately 3-month intervals post-transplant for analysis of T cell responses. When possible, T cell responses were also quantified during changes in clinical status (infection or rejection). A range of 100 to 300 ng/ml ATP was found in stable patients (mean 194 +/- 123, n = 51) with good graft function and no infection or rejection. A low T cell response correlated highly with infectious states. Fourteen patients with infections/post-transplant lymphoproliferative disease had a mean ATP of 48 ng/ml. Risk hazard analysis showed that patients with ATP levels less than 100 ng/ml were 4 to 7 times more susceptible to infection compared to stable patients. Four patients with rejection showed a T cell response of 550 ng/ml ATP, which was statistically significant compared to stable patients, although the sampling numbers (n = 9) were too small to be conclusive.

Cadillo-Chavez and colleagues (2006) reviewed the records of 64 kidney transplant patients for associations between ATP levels and immunosuppression type, doses, and levels; creatinine levels; white blood cell count; tissue typing; preformed antibodies; as well as ATP levels on infection and rejection, and
changes in ATP levels with time. Of the 58 patients that had pre-transplant and post-transplant ATP levels tested, the authors reported no association between ATP levels and immunosuppression type, doses, or levels; creatinine levels; white blood cell counts; HLA; and panel-reactive antibody (p > 0.05). However, patients with moderate or high pre-transplant ATP levels had more rejection episodes (8/10) while patients with ATP levels in the low immune response had more infections (6/11) (p < 0.001; relative risk [RR] for rejection = 1.2; RR for infection = 4.4). The mean ATP levels for rejection was 423.3 ng/ml versus 268.45 ng/ml for infection and 277.15 ng/ml for no events (ANOVA, p = 0.0145). Although acute rejections occurred mostly above 300, this was not significant (p = 0.059; RR = 0.9). Infections were more frequent with ATP under 300 (RR = 7.3) and severe infection (e.g., endocarditis, meningitis, peritoneal abscesses, pneumonia, etc) were more frequent under 200 (p < 0.001). When pre-transplant values were compared with post-transplant values at the second week, an increase correlated with rejection (p < 0.001, RR = 15.3), while a decrease did not correlate with the infection (p = 0.845, RR = 1.4). Patients who received anti-rejection treatment had a decrease in their ATP levels at day 5 (p = 0.002).

Batal et al (2008) reported on a retrospective study that found a correlation between decreased immune cell function test results and active BK virus replication, but not with acute rejection, in kidney transplant recipients. ImmuKnow assay measurements were performed on 15 samples from 8 patients with BK viremia, 38 samples from 25 patients with BK viruria, and 243 samples from 148 patients with no BK viruria or viremia. The mean +/- SD amounts of ATP released in these 3 groups were 102.9 +/- 58.6, 227.2 +/- 146.4, and 231.8 +/- 150.8 ng/ml, respectively (p = 0.002, viremia versus all other samples). The investigators reported that, within the viruria group, lower immune cell function assay values were associated with higher urinary viral load (p = 0.037). There was no significant relationship, however, between immune cell function test results and acute transplant rejection. The investigators concluded that prospective studies are needed to determine whether this assay can be used as a screening tool to stratify patients by their ultimate risk of developing BK viremia and BK virus nephropathy.

In a study of kidney transplant recipients, Serban et al (2009) found that, although low ATP levels by ImmuKnow assay identified patients at increased risk for infection, high ATP values failed to correlate with rejection and did not justify increased immunosuppression. The investigators assessed the significance of immune cell function in 76 renal allograft recipients after anti-thymocyte globulin induction and initiation of maintenance immunosuppression. The investigators found that the Immuknow assay yielded paradoxically high ATP values during the first 3 months post-transplantation, despite very low CD4+ counts. The investigators reported that high ATP values were caused by peripheral blood myeloid cells, did not predict rejection, and occurred primarily in transplant recipients who received darbepoietin (p = 0.017). Over the first 5 months post-transplantation, mean ATP activity gradually decreased, whereas CD4+ counts slowly increased. Low ATP values were predictive of infection (p = 0.002). The investigators concluded that ImmuKnow results, therefore need to be interpreted with caution in patients receiving anti-thymocyte induction therapy; although low ATP levels identified patients at increased risk for infection, high ATP values failed to correlate with rejection and did not justify increased immunosuppression.
A study by Bhorade et al (2008) found that the ImmuKnow assay had high sensitivity but poor specificity for infection in lung transplant recipients. The investigators identified the level of functional immunity as measured by the ImmuKnow assay in lung transplant recipients and correlated these values with the dose and trough levels of immunosuppression as well as other clinical parameters in these patients. The investigators assessed the functional immune response in 143 sequential blood samples from 57 lung transplant recipients using the ImmuKnow assay and reported that the average ImmuKnow assay in stable lung transplant recipients was 244 +/- 138 ATP ng/ml and the median level was 236 ATP ng/ml (range 5 to 669 ATP ng/ml), about 703 +/- 695 days after lung transplantation. There was no correlation between ImmuKnow levels and tacrolimus trough levels. Stepwise multiple regression analysis identified African American race as an independent predictor of ImmuKnow assay levels when age, gender and underlying diagnosis were taken into account (p < 0.04). The investigators found that the ImmuKnow assay levels were lower in infected lung transplant recipients compared with non-infected recipients and increased with treatment of these infections. Fifteen infected lung transplant recipients had a lower ImmuKnow level at the time of their infections as compared with stable lung transplant recipients (111 +/- 83 versus 283 +/- 143 ATP ng/ml, respectively, p = 0.0001). Sixteen of the remaining 42 patients had low ImmuKnow assay values (less than 225 ATP ng/ml), but did not have active infection. The investigators found that, while the sensitivity for infection of an ATP value of less than 225 ng/ml was 93 % in this study, the specificity was only 38 %. In addition, the utility of ATP measurements was not assessed, as only 2 recipients in the patient sample had rejection. The authors concluded, "It remains unclear whether the ImmuKnow assay reflects over-immunosuppressed individuals at risk of infection or bone marrow suppression by infectious agents. Further investigation will determine the role of the ImmuKnow assay in tailoring immunosuppression in lung transplant recipients."

Husain et al (2009) reported the correlation between Cylex ImmuKnow (ng/ml ATP) values and various infectious syndromes in a large prospective cohort of lung transplant recipients. These investigators followed-up 175 lung transplants that developed 129 infectious episodes. Multiple logistic regression analysis was performed; generalized estimating equations were used to determine the odds ratio (OR) for infections. The median ImmuKnow values in cytomegalovirus disease (49.3 ng/ml ATP), viral infection (70 ng/ml ATP), and bacterial pneumonia (92 ng/ml ATP) were significantly different from stable state (174.8 ng/ml ATP). The median ImmuKnow values of fungal disease (85 ng/ml ATP) and tracheobronchitis (123 ng/ml ATP) had a tendency to be lower than stable state (p = 0.10), whereas patients with fungal colonization had comparable ImmuKnow values (167 versus 174.8 ng/ml ATP). Of the patients colonized with fungus who subsequently developed fungal disease within 100 days, the median value of ImmuKnow was significantly lower than in those who did not develop fungal disease (22.5 versus 183.5 ng/ml ATP; p < 0.0001). Generalized estimating equation regression analysis showed ImmuKnow values less than or equal to 100 ng/ml ATP to be an independent predictor of infections (OR of 2.81). The authors concluded that Cylex ImmuKnow assay monitoring has the potential to identify the patients at risk of developing infection and those colonized with fungus that are at risk of developing disease.
Another study, published in abstract form, demonstrated a very poor correlation between histologically proven rejection and the ImmuKnow assay, with 87% of the allograft rejection episodes occurring in the setting of a low to moderate ATP level (Huang et al, 2007).

Cabrera et al (2009) used the ImmuKnow assay to help assess the etiology of abnormal liver function test results in liver transplant recipients. Blood samples for the immune functional assay were taken from 42 recipients prospectively at various times post-transplant and compared with clinical and histologic findings. In patients whose liver biopsy showed evidence of cellular rejection, the immune response was noted to be very high, whereas in those with active recurrence of hepatitis C, the immune response was found to be very low. This finding was found to be statistically significant (p < 0.0001). In those patients in whom there was no predominant histologic features suggesting 1 entity over the other, the immune response was higher than in those with aggressive hepatitis C but lower than in those with cellular rejection. The authors concluded that these data show the potential utility of the ImmuKnow assay as a means of distinguishing hepatitis C from cellular rejection and its potential usefulness as a marker for outlining the progression of hepatitis C.

Macedo et al (2009) investigated the impact of Epstein-Barr virus (EBV) load on T-cell immunity from pediatric transplantation recipients, using clinically applicable tests for improved assessment of T-cell immune competence. A total of 35 asymptomatic pediatric thoracic transplantation patients were categorized into 3 groups according to their EBV load levels: (i) undetectable viral load (UVL), (ii) chronic low viral load (LVL) and (iii) chronic high viral load (HVL). Global and EBV-specific T-cell immunity were assessed by ATP release using Cylex Immuknow and T Cell Memory assays. Patients with UVL exhibited normal ATP release to Concanavalin A (ConA) and phytohemagglutinin (PHA; 190 +/- 86 ng/ml, 328 +/- 163 ng/ml) and detectable EBV-specific (37 +/- 34 ng/ml) ATP responses. Patients with LVL displayed significantly stronger responses to ConA (373 +/- 174 ng/ml), PHA (498 +/- 196 ng/ml) and EBV (152 +/- 179 ng/ml), when compared with UVL or to HVL patients (ConA 185 +/- 114 ng/ml, PHA 318 +/- 173 ng/ml, and EBV 33 +/- 42 ng/ml). Moreover, patients with HVL displayed significant inverse correlation between CD4+ T-cell ATP levels and EBV loads. The authors concluded that evaluation of global and EBV-specific T-cell immunity provides a rapid assessment of patients' immune competence. However, it is still unclear if selective over-suppressed ATP release by CD4+ T cells reflects HVL patients at risk of post-transplant lymphoproliferative disease. They stated that further longitudinal studies will determine the importance of Immuknow test in identifying asymptomatic HVL patients vulnerable to EBV complications.

Rossano et al (2009) tested the hypothesis that the Cylex ImmuKnow cell function assay (CICFA) is a clinically useful test in pediatric heart transplant patients. All children undergoing heart transplantation at the study center (1989 to 2006) for whom CICFA levels were obtained were reviewed. The association of CICFA levels with episodes of acute rejection (AR) and significant infections was determined. Among 83 patients (34 girls, 41%), 367 CICFA levels were obtained (median of 4.0; interquartile range [IQR], 2.0 to 6.0 per patient). There were 26 episodes of AR in 17 patients (20%) and 38 infections in 34 patients (41%).
CICFA levels were similar among patients with AR at the time of the CICFA measurement (median of 325 [IQR, 163 to 480] ATP ng/ml) versus patients without AR (median of 330 [IQR, 227 to 441] ATP ng/ml; p = 0.36). CICFA levels were similar among patients with infections within 1 month of CICFA measurement (median of 295 [IQR, 216 to 366] ATP ng/ml) and those without infections (median of 330 [IQR, 226 to 453] ATP ng/ml; p = 0.24). The authors concluded that the CICFA is not predictive of AR or significant infections in pediatric heart transplant patients. On the basis of the available evidence, these investigators stated that this assay can not be recommended as part of the routine management of pediatric heart transplant patients.

Gupta et al (2008) reported that the ImmuKnow assay had limited clinical utility as an adjunct to routine clinical evaluation in assessing risk of infection or rejection in heart transplant recipients. The authors performed a retrospective review of the clinical course of all adult cardiac transplant recipients who underwent an ImmuKnow assay at University of Texas Southwestern Medical Center between January 2004 and September 2007. The authors reported that 111 patients were free of significant rejection or infection at the time of the first ImmuKnow assay. Most patients (92%) were more than 1 year post-transplant. Over the next 157 +/- 41 (mean +/- SD) days, 2 patients had 3 episodes of rejection requiring therapy and 7 patients had 8 infections requiring therapy. The ImmuKnow responses ranged from 17 to 894 ng/ml. No correlation was observed between the baseline ImmuKnow response and subsequent risk of either infection or rejection within 6 months. Lower white blood cell count and African American ethnicity were correlated with a lower ImmuKnow response. The authors concluded that the Cylex assay had limited utility as an adjunct to routine clinical evaluation in assessing risk of infection or rejection in cardiac transplant recipients.

Gesundheit et al (2010) stated that following allogeneic hematopoietic stem cell transplantation (alloHSCT), immunosuppressed patients are susceptible to opportunistic infections, and uncontrolled function of the graft can result in graft-versus-host disease (GVHD). Accurate immune monitoring may help early detection and treatment of these severe complications. Between October 2005 and November 2007, a total of 170 blood samples were collected from 40 patients after alloHSCT in the Hadassah Hebrew University Medical Center and from 13 healthy controls. These researchers utilized the Cylex ImmuKnow assay for CD4 ATP levels to compare known clinically immunocompromised versus immunocompetent patients after alloHSCT. They also compared the reconstitution of white blood cell (WBC) count to the ImmuKnow results and clinical status. The patients’ clinical course correlated with the stratification of immune response established by the ImmuKnow assay for solid organ transplantation (immunocompetent versus immunocompromised), and this often differed from their WBC count. The authors concluded that the Cylex ImmuKnow assay should be evaluated prospectively in clinical trials.

The American Society of Transplantation (AST) does not mention the use of the Cylex Immune Cell Function assay in its recommendations for the screening, monitoring and reporting of infections complications in the evaluation of recipients of organ transplantation (AST, 2006). Chon and Brennan (2009) commented that there is no consensus on the utility of the Immuknow assay in renal transplant rejection other than in the research setting. Martinu et al (2009) commented that
"the data in lung transplantation are scarce and not very promising to date", and that "the ImmuKnow assay does not seem to have the potential to differentiate between infection and rejection in lung transplant recipients and, until more data becomes available, should not be used clinically in this patient population."

Bennett et al (2010) noted that infection of a transplanted kidney with the polyomavirus, BK, is associated with poor allograft survival. In an attempt to prevent this transplant complication, these investigators studied 144 consecutive transplant recipients for the presence of BK infection with plasma and urine polymer chain reaction (PCR) testing at 1, 2, 3, 6 and 12 months. Viruria alone was followed by serial studies. If plasma PCR became positive at greater than 2.6 log copies, mycophenolate was reduced until there was no detectable plasma viral load. Urine PCR was positive in 34 (24 %), while plasma PCR turned positive in 22 cases (15 %). No patients developed viremia with less than 6.8 log copies in the urine. Viremia resolved within 3 months or less in 20 of 22 patients after reduction of immunosuppression. Surveillance biopsies at 2 and 6 months revealed no BK nephropathy. Eight patients had acute rejection during reduced immunosuppression; however, all of these reversed with pulse steroids. Patient and graft survival at 1 year was 99 % and 98 %, respectively. Use of the cell-mediated immunity assay (ImmuKnow) was not useful in identifying infected patients.

Kobashigawa and colleagues (2010) examined the utility of ImmuKnow in heart transplant recipients. Between November 2005 and July 2008, a total of 296 heart transplant recipients had a total of 864 immun monitoring (IM) assays performed at 2 weeks to 10 years post-transplant and were correlated with infection and rejection events that occurred within 1 month after IM testing. All patients received standard triple-drug immunosuppressive therapy with tacrolimus, mycophenolate mofetil and corticosteroids, without induction therapy. There were 38 infectious episodes and 8 rejection episodes. The average IM score was significantly lower during infection than steady state (187 versus 280 ng ATP/ml, p < 0.001). The average IM score was not significantly different during rejection when compared with steady state (327 versus 280 ng ATP/ml, p = 0.35). Interestingly, 3 of 8 rejection episodes were antibody-mediated rejections and had hemodynamic compromise and, for these, the mean IM score was significantly higher than for steady-state patients (491 versus 280 ng ATP/ml, p < 0.001). The authors concluded that ImmuKnow appears to predict infectious risk in heart transplant patients. The association between high IM scores and rejection risk is inconclusive due to the small number of rejection episodes. They stated that further studies with larger sample sizes for rejection episodes are needed.

Torio et al (2011) stated that the Cylex ImmuKnow assay provides a rapid assessment of global immune function in immunocompromised patients by measuring the global immune responses of CD4 T cells from a whole-blood sample. It may help to monitor the immune status of immunosuppressed transplant patients. However, earlier studies have shown that there is no consensus on the utility of the ImmuKnow assay in renal transplant rejection. T-cell activation was determined by measuring an increase of intracellular ATP (iATP) from CD4 cells in 227 samples from 116 kidney transplant patients. The results were analyzed regarding patient clinical status, namely, rejection, infection, or stability. In addition, these researchers measured the immunologic response of
108 healthy control subjects. There were 24 infectious and 36 rejection episodes.
Intracellular ATP concentrations differed significantly between stable and infected
patients (180.5 +/- 55.2 versus 375.3 +/- 140.1 ng/ml; p < 0.001) and between
infected patients and control subjects (180.5 +/- 55.2 versus 436.5 +/- 112 ng/ml; p
< 0.001). No correlation was observed between patients suffering an acute
rejection episode with this response. The authors concluded that these findings
confirmed that the ImmuKnow assay identified transplant patients at risk for
infection. It may provide information to guide immunosuppressive therapy, but the
assay did not seem to have the potential to differentiate subjects experiencing
rejection.

De Paolis et al (2011) evaluated the value of ImmuKnow (IK), a new tool to
measure the net state of immune function among renal transplant recipients, in
correlation with clinical and laboratory data among unselected renal transplant
recipients. A total of 49 recipients of mean age of 51 years were enrolled and
followed for 1 year after transplantation. All subjects received the same
immunosuppressive strategy with basiliximab induction and tacrolimus,
mycophenolate mofetil and steroid maintenance therapy. Samples for IK were
collected before transplantation as well as at 7, 14, 21 and 42 days and after 3, 6,
and 12 months. There were 54 samples with IK less than 225 ng/ml, 201 samples
with normal IK values, and 135 samples with greater than 525 ng/ml. These
investigators divided recipients into 3 groups with respect to their basal IK values:
Group 1 (Gr1; IK less than 225 ng/ml); Group 2 (Gr2; normal values of IK between
226 and 524 ng/ml); and Group 3 (Gr3; IK greater than 525 ng/ml). At 1 year,
these investigators observed a significant difference among IK values at the start
and the end of the study: Gr1 versus Gr2, p < 0.0001; Gr2 versus Gr3, p < 0.06
and Gr 1 versus Gr 3, p < 0.01). They observed reduced IK values to predict an
increased risk of infection, particularly with cytomegalovirus (CMV) replication
while higher IK value did not correlate with an increased risk of acute rejection
episodes. Reduction of serum creatine levels occurred within 1 year in all groups
(p < 0.005), but there was a significant difference between Gr 2 versus Grs 1 and
3 (p < 0.0001 and p < 0.0005, respectively). There findings suggested that more
stable IK values were associated with clinical quiescence and laboratory stability.
The authors concluded that this preliminary analysis showed a beneficial capacity
of this assay to represent the global depression of the immune system. They
noted that reduced IK values, as a sign of excessive immunosuppressive therapy,
were associated with an increased risk of infection. They did not confirm the
predictive value of higher IK values for an increased risk of an acute rejection
episode.

Huskey et al (2011) retrospectively analyzed 1,330 ImmuKnow assay values in
583 renal transplant recipients at a single center from 2004 to 2009 and correlated
these values with episodes of opportunistic infections (OI) and acute rejection (AR)
in the subsequent 90 days. Assay values were compared with a control
population matched for age, gender, and time post-transplantation. In patients
with OI (n = 94), there were no differences in prior mean assay values compared
with matched controls (386 versus 417 ng/ml, p = 0.24). In 47 patients with AR,
again no differences were detected in prior assay results (390 versus 432 ng/ml, p
= 0.25) when compared with controls. "Low" values (less than or equal to 225
ng/ml) lacked sensitivity and specificity as a predictive test for subsequent OI, as
did "strong" (greater than or equal to 525 ng/ml) values as a predictive test for
subsequent AR. The authors concluded that these findings fail to show an association between single time point ImmuKnow assay values and the subsequent development of an adverse event in the subsequent 90 days. The optimal use of the ImmuKnow assay in kidney transplantation has yet to be determined.

Cheng et al (2011) determine the utility of the ImmuKnow assay in assessing the risk of infection, rejection, and tumor recurrence in liver transplant recipients. Immune function, as determined by the ImmuKnow assay, was used to monitor the global immune status in 342 whole blood samples from 105 liver transplant recipients. The association between ATP value and post-transplant tumor recurrence was evaluated in 60 hepatocellular carcinoma (HCC) patients. The ATP value in predicting tumor recurrence in other independent cohort of 92 recipients with HCC was analyzed prospectively. The mean ATP values of liver transplant recipients with infection (145.2 +/- 87.0 ng/ml) or acute rejection (418.9 +/- 169.5 ng/ml) were different from those with stable state (286.6 +/- 143.9 ng/ml, p < 0.05). In recipients with HCC who developed recurrent tumors, the values were significantly lower than those without recurrence (137.8 +/- 66.4 versus 289 +/- 133.9 ng/ml, p < 0.01); the optimal threshold value to predict post-transplant tumor recurrence was 175 ng/ml. Comparing with the patients in lower immune group (ATP less than or equal to 175 ng/ml), patients in the higher immune group (ATP greater than 175 ng/ml) experienced significantly better disease-free survival (p < 0.01). Multi-variate Cox regression analysis showed the ATP value was an independent predictor of HCC recurrence. The authors concluded that the ImmuKnow assay has the potential to evaluate the risk of infection and rejection in liver transplantation and to predict post-transplant tumor recurrence in recipients with HCC.

Rodrigo and colleagues (2012) conducted a systematic literature review to identify studies documenting the use of ImmuKnow to monitor immune function in liver transplant recipients until March 2012. Study quality was assessed by using the Quality Assessment of Diagnostic Accuracy Studies 2 score. These investigators identified 5 studies to analyze ImmuKnow performance in infection and 5 in acute rejection. Pooled sensitivity, specificity, positive likelihood ratio, diagnostic odds ratio and area under a summary receiver-operating characteristic curve were 83.8 % (95 % CI: 78.5 % to 88.3 %), 75.3 % (95 % CI: 70.9 % to 79.4 %), 3.3 (95 % CI: 2.8 to 4.0), 14.6 (95 % CI: 9.6 to 22.3) and 0.824 +/- 0.034 for infection and 65.6 % (95 % CI: 55.0 % to 75.1 %), 80.4 % (95 % CI: 76.4 % to 83.9 %), 3.4 (95 % CI: 2.4 to 4.7), 8.8 (95 % CI: 3.1 to 24.8) and 0.835 +/- 0.060 for acute rejection. Heterogeneity was low for infection and high for acute rejection studies. The authors concluded that ImmuKnow test is a valid tool to know the risk of further infection in adult liver transplant recipients. Moreover, they stated that significant heterogeneity across studies precludes concluding that ImmuKnow identifies liver transplant patients at risk for rejection.

Shino et al (2012) hypothesized that the ImmuKnow assay can be used to assess the immune function of lung transplant recipients and identify those at risk of developing acute cellular rejection and respiratory infection. Lung transplant recipients at University of California Los Angeles between January 1, 2006 and December 31, 2009 received a bronchoscopy with bronchoalveolar lavage, transbronchial biopsy and ImmuKnow values drawn at regular intervals as well as
during episodes of clinical deterioration. The recipient's clinical condition at each time-point was classified as healthy, acute cellular rejection, or respiratory infection. Mixed-effects models were used to compare the ATP levels among these groups, and odds ratios for rejection and infection were calculated. The mean ATP level was 431 +/- 189 ng/ml for the rejection group versus 377 +/- 187 ng/ml for the healthy group (p = 0.10). A recipient with an ATP level greater than 525 ng/ml was 2.1 times more likely to have acute cellular rejection (95 % CI: 1.1 to 3.8). Similarly, the mean ATP level was 323 +/- 169 ng/ml for the infection group versus 377 +/- 187 ng/ml for the healthy group (p = 0.03). A recipient with an ATP level less than 225 ng/ml was 1.9 times more likely to have respiratory infection (95 % CI: 1.1 to 3.3). However, the test was associated with poor performance characteristics. It had low sensitivity, specificity with an area under the receiver operating characteristic curve of only 0.61 to diagnose rejection and 0.59 to diagnose infection. The authors concluded that the ImmuKnow assay appears to have some ability to assess the overall immune function of lung transplant recipients. However, this study does not support its use as a reliable predictor of episodes of acute cellular rejection or respiratory infection.

In a meta-analysis, Ling et al (2012) evaluated the effectiveness of the Cylex ImmuKnow cell function assay (CICFA) in identifying risks of infection and rejection post-transplantation. After a careful review of eligible studies, sensitivity, specificity, and other measures of the accuracy of CICFA were pooled. Summary receiver operating characteristic curves were used to represent the overall test performance. A total of 9 studies met the inclusion criteria. The pooled estimates for CICFA in identification of infection risk were poor, with a sensitivity of 0.58 (95 % CI: 0.52 to 0.64), a specificity of 0.69 (95 % CI: 0.66 to 0.70), a positive likelihood ratio of 2.37 (95 % CI: 1.90 to 2.94), a negative likelihood ratio of 0.39 (95 % CI: 0.16 to 0.70), and a diagnostic odds ratio of 7.41 (95 % CI: 3.36 to 16.34). The pooled estimates for CICFA in identifying risk of rejection were also fairly poor with a sensitivity of 0.43 (95 % CI: 0.34 to 0.52), a specificity of 0.75 (95 % CI: 0.72 to 0.78), a positive likelihood ratio of 1.30 (95 % CI: 0.74 to 2.28), a negative likelihood ratio of 0.96 (95 % CI: 0.85 to 1.07), and a diagnostic odds ratio of 1.19 (95 % CI: 0.65 to 2.20). The authors concluded that current evidence suggests that CICFA is not able to identify individuals at risk of infection or rejection. They stated that additional studies are still needed to clarify the usefulness of this test for identifying risks of infection and rejection in transplant recipients.

Akimoto et al (2013) examined the ability of the ImmuKnow (assay to predict the risk of infection in rheumatoid arthritis (RA) patients receiving synthetic or biological disease-modifying anti-rheumatic drugs (DMARDs). The amount of ATP produced by CD4+ cells in response to phytohemagglutinin was measured in whole blood from 117 RA patients without infection versus 17 RA patients with infection, and compared with results in 75 healthy controls. The mean ATP level was significantly lower in patients with infection compared to both healthy controls (p < 0.0005) and patients without infection (p = 0.040). Also, the mean ATP level in patients without infection was significantly lower than that in healthy controls (p = 0.012). There was no correlation between the ATP level and the Disease Activity Score in 28 joints. The authors concluded that the ImmuKnow assay results may be effective in identifying RA patients at increased risk of infection, but
the results showed no correlation with RA activity. They stated that larger studies are needed to establish the clinical advantages of this assay in RA treatment.

There is insufficient evidence of the effectiveness of the ImmuKnow assay in the management of organ transplant rejection in individuals undergoing immunosuppressive therapy post solid organ transplant and for the identification of individual risk for rejection prior to kidney or any other solid organ transplant. Prospective clinical outcome studies are needed to determine its role in the management of solid organ or stem cell transplant recipients.

Lopez-Hoyos et al (2013) stated that ImmuKnow is an in-vitro diagnosis method that uses patient samples of whole blood polyclonally stimulated with phytohemagglutinin. It also measures ATP production by CD4+ T cells. The test aims to offer an objective and overall measurement of each individual's cellular immune response. The assay was designed with the idea of individually monitoring the immunosuppression administered to transplant patients. At the same time, it aims to help achieve a balance as a way of avoiding immunosuppression excess and the associated adverse effects (infections, cancer, etc.) or an immunosuppression defect and the subsequent risk of allograft rejection. The majority of studies that have evaluated its clinical usefulness display great diversity in terms of patient recruitment, the immunosuppressant treatment received, the clinical variables analyzed and, above all, the time between performing ImmuKnow and the evaluated clinical event. The most consistent data showed that this assay on CD4+ T cell functioning is useful for predicting the risk of infection in renal transplant patients. However, its use as a rejection risk indicator is unclear. Lastly, given the great variability of immune response amongst individuals and that of existing publications, it can be deduced that the isolated ImmuKnow value does not have diagnostic capacity and only individual serial monitoring could provide definitive assistance in clinical decision making and immunosuppressant treatment changes. Moreover, the authors stated that other aspects of ImmuKnow application in the clinical routine, such as assay cycles, require randomized prospective studies for more comprehensive information.

Israeli et al (2013) noted that currently there is no standardized non-invasive diagnostic tool for the evaluation of immunological complications such as GVHD and for managing the cellular immune function of the transplant recipient. The ImmuKnow assay for cellular immune function monitoring has been incorporated successfully into the clinical follow-up routine of solid organ transplant recipients. These researchers examined the relevance and potential contribution of immune monitoring using the assay in the setting of HCT. They found that ImmuKnow-level measurement can distinguish between states of immune function quiescence and between events of acute GVHD. ImmuKnow levels were significantly higher in patients going through GVHD than the levels measured for the same patients during immunological stability. Moreover, they demonstrated a patient case where longitudinal monitoring using the ImmuKnow assay provided a trustworthy depiction of the patient's cellular immune function post-HCT. The authors concluded that they provided evidence for the potential contribution of the ImmuKnow assay for longitudinal individualized cellular immune function monitoring of patients following HCT. Moreover, they stated that further studies are needed to establish the optimal practice for utilizing the assay for this purpose.
Brandhorst et al (2013) stated that Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBDs), which are characterized by dysfunctional regulation of the immune system. A number of immune modifying drugs are used to treat CD and UC. Therapy is adjusted largely on the bases of subjective reports of disease activity and non-specific laboratory tests. Identification of a single or combination of immune markers of disease activity could be useful to select and monitor therapeutic responses. However, to-date no reliable quantitative associations between IBD activity and laboratory measures of immune function have been identified. These investigators evaluated the usefulness of ImmuKnow as a surrogate marker of IBD activity. Adult IBD patients with either CD (n = 55, 27 males, mean, SD age = 38.5, 11.5 years) or UC (n = 45, 24 males, mean, SD age = 41.7, 15.4 years) were enrolled. Patients both in clinical remission and with active disease provided responses to structured, validated questionnaires (CDAI and HBI for CD patients and SCCAI for UC patients) used to monitor IBD activity. Whole blood and plasma samples were collected to quantify various markers of disease status including routine cell counts and differentials (CBCs), C-reactive protein (CRP), and albumin (Alb), as well as CD4(+) immune response (ImmuKnow, n = 98). Results were compared between all IBD patients as well as between CD and UC subgroups. There was a good correlation between the results of CDAI and HBI scores (r = 0.811, p < 0.01, Spearman-Rho), but HBI scores correlated slightly better (r = 0.575, p < 0.001) than the CDAI's (r = 0.449, p = 0.001) with CD patients' reported perception of their general condition. Furthermore, CDAI and HBI scores categorized 12/55 versus 36/55 of CD patients respectively as having active disease; SCCAI scores indicated that 25/45 of UC patients had active disease. ImmuKnow results (in ng/ml of ATP) were increased in 74/98 IBD subjects (greater than or equal to 525 ng/ml, but were influenced by the use of systemic corticosteroids (SCS) and infliximab. There were weak but statistically significant Spearman-Rho correlations between Alb concentrations and both CDAI (r = 0.413, p = 0.002) and HBI (r = 0.325, p = 0.017) scores as well as between CRP values and HBI scores (r = 0.331, p = 0.016). Correlations between CRP and both CDAI and SCCAI scores and between Alb and SCCAI scores were not significant and there were no significant positive associations between any of the 3 clinical scores and ImmuKnow results. The authors concluded that CD4(+) immune responses (ImmuKnow results) were significantly elevated in IBD patients whether or not they were in clinical remission, but were influenced by treatment. There were some significant correlations between the clinical scores and CRP or Alb, but not with the CD4(+) results. Both other clinical scoring systems, other measures of immune function, and CD4(+) immune response changes over time should be examined to see if this or other laboratory measures of immune response are predictive of actual disease activity or symptoms in CD or UC patients.

Wong et al (2014) stated that the ImmuKnow ICFA reports ex-vivo CD4 lymphocyte activation to quantify immunosuppression. Limited organ and age- specific data exist for pediatric heart transplant recipients. These investigators examined their normative values and ICFA's association with rejection/infection. A total of 380 ICFAs from 58 heart transplant recipients (6.5/recipient) were studied retrospectively. The median age at the time of their first ICFA was 5.3 yrs (IQR 2.4 to 12.1 yrs). ICFA levels during immunologic stability (n = 311) were a median of 305 (IQR: 172 to 483) and mean of 353 (S.D. ± 224) ng ATP/ml. ICFA levels
trended lower with advancing age. ICFA levels during immunologic stability increased over time from transplant after the first 6 months but were not correlated with calcineurin inhibitor levels or the type used. There was no association between ICFA values during stability and rejection (median of 368 ATP ng/ml; IQR 153 to 527) or infection (median of 293 ATP ng/ml; IQR 198 to 432). In contrast to the manufacturer’s suggested ranges, the immunologic stable ranges in pediatric cardiac recipients were very different. The authors concluded that ICFA values during immunologic stability are related to time from transplant in pediatric heart recipients; ICFA’s ability to discriminate rejection or infection from immunologic stability was not demonstrated.

Ryan et al (2014) noted that management of pediatric renal transplant patients involves multi-factorial monitoring modalities to ensure allograft survival and prevent opportunistic infection secondary to immunosuppression. An ICFA, which utilizes CD4 T-cell production of ATP to assess immune system status, has been used to monitor transplant recipients and predict susceptibility of patients to rejection or infection. However, the validity of this assay to reflect immune status remains unanswered. In a 2-yr retrospective study that included 31 pediatric renal transplant recipients, 42 patient blood samples were analyzed for immune cell function levels, creatinine, WBC (white blood cell) count, immunosuppressive drug levels, and viremia, concurrent with renal biopsy. T-cell ATP production as assessed by ICFA levels did not correlate with allograft rejection or with the presence or absence of viremia. ICFA levels did not correlate with serum creatinine or immunosuppressive drug levels, but did correlate with WBC count. The authors concluded that ICFA is unreliable in its ability to reflect immune system status in pediatric renal transplantation; further investigation is needed to develop methods that will accurately predict susceptibility of pediatric renal transplant recipients to allograft rejection and infection.

An UpToDate review on “Investigational methods in the diagnosis of acute renal allograft rejection” (Chon and Brennan, 2104) states that “The ImmuKnow assay is a US Food and Drug Administration (FDA)-approved test intended to estimate the net state of immune system in immunocompromised patients. It measures the ability of CD4 cells to respond to mitogenic stimulation by phytohemagglutinin-L in-vitro by quantifying the amount of adenosine triphosphate (ATP) produced and released from these cells following stimulation. At the present time, there is no consensus on the utility of these tests, other than in the research setting”.

Liu and colleagues (2014) stated that it is uncertain whether ImmuKnow can predict the risk of infection in lupus nephritis (LN) patients receiving immunosuppressive therapy. The ImmuKnow Immune Cell Function Assay was applied to measure the activity of CD4+ T cells, as a marker of global immune-competence. The correlation between changes in T cell activation and the relative risk of over-immunosuppression as well as infection was studied. The amount of ATP produced by CD4+ T cells in response to PHA was measured for 74 LN patients without infection, 22 LN patients with severe infection (i.e., required hospitalization), and 28 healthy controls. No correlation was found between the ATP level and systemic lupus erythematosus (SLE) activity. The mean ATP level was significantly lower in LN patients with infection than that in healthy controls (p < 0.01) and non-infected LN patients (p < 0.01). The mean ATP level in non-infected LN patients was not significantly different compared to healthy controls. A
cut-off ATP value of 300 ng/ml predicted infection in LN patients with a specificity of 77% and a sensitivity of 77%. Multi-variable partial correlation coefficient between the ATP assay and severe infection was $r = -0.040$, $p < 0.001$; CRP was $r = 0.962$, $p < 0.001$. The authors concluded that the ImmuKnow assay may be effective in identifying an increased risk of infection in LN patients but is not correlated with SLE activity. Combined CRP value will increase the diagnostic rate of severe infection in SLE. Moreover, they stated that larger studies are needed to establish clinical advantages of this assay in SLE treatment.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes not covered for indications listed in the CPB:

86352

Other CPT codes related to the CPB:

38240  Hematopoietic progenitor cell (HPC); allogeneic transplantation per donor

ICD-9 codes not covered for indications listed in the CPB:

238.77  Post-transplant lymphoproliferative disorder
279.50 - 279.53  Graft-versus-host disease
555.0 - 555.9  Regional enteritis [Crohn’s disease]
556.0 - 556.9  Ulcerative colitis
996.80 - 996.84, 996.86 - 996.89  Complications of transplanted organ
V42.0 - V42.2, V42.6 - V42.7  Organ replaced by transplant
V42.83 - V42.89  Other specified organ replaced by transplant
V49.83  Awaiting organ transplant status
V58.44  Aftercare following organ transplant

Other ICD-9 codes related to the CPB:

135  Sarcoidosis [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]
<table>
<thead>
<tr>
<th>Code</th>
<th>Condition</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>277.0-277.09</td>
<td>Cystic fibrosis [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>287.31</td>
<td>Immune thrombocytopenic purpura [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>358.00-358.01</td>
<td>Myasthenia gravis [not covered for anti-rheumatic drug conditions]</td>
<td></td>
</tr>
<tr>
<td>415.0</td>
<td>Acute cor pulmonale [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>416.0</td>
<td>Primary pulmonary hypertension [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>416.8</td>
<td>Other chronic pulmonary heart disease [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>491.21</td>
<td>Obstructive chronic bronchitis with (acute) exacerbation [COPD] [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>492.0-492.8</td>
<td>Emphysema [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>494.0-494.1</td>
<td>Bronchiectasis [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>516.34</td>
<td>Respiratory bronchiolitis interstitial lung disease [not covered for identification of individuals at risk for rejection prior to liver or lung transplant and prediction of infection risk in individuals receiving disease-modifying anti-rheumatic drugs]</td>
<td></td>
</tr>
<tr>
<td>573.0-573.9</td>
<td>Other disorders of liver [not covered for identification of individuals at risk for rejection prior to liver or lung transplant</td>
<td></td>
</tr>
</tbody>
</table>
The above policy is based on the following references:


