Immune Cell Function Assay

Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will not provide coverage for Immune Cell Function Assay. This is considered investigational.

When Policy Topic is covered
n/a

When Policy Topic is not covered
Use of the immune cell function assay to monitor and predict immune function after solid organ transplantation is considered investigational.

Use of the immune cell function assay to monitor and predict immune function after hematopoietic stemcell transplantation is considered investigational.

Use of the immune cell function assay for all other indications is considered investigational.

Description of Procedure or Service

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Careful monitoring of lifelong immunosuppression is required to ensure long-term viability of solid organ allografts without incurring an increased risk of infection. The monitoring of immunosuppression parameters attempts to balance the dual
risks of rejection and infection. It is proposed that individual immune profiles, such as an immune cell function assay, will help assess the immune function of the transplant recipient and individualize immunosuppressive therapy.

The evidence for immune cell function assay in patients who have a solid organ transplant includes numerous studies of the association of assay test values and subsequent rejection or infection, and 1 randomized controlled trial in liver transplant patients. Relevant outcomes are overall survival, test accuracy, other test performance measures, and morbid events (rejection and infection). The ImmuKnow® test shows variable associations with infection and rejection depending on the type of transplant and the context of the study. The predictive characteristics of the test are still uncertain, and do not allow a strong indirect argument of clinical utility. The trial of ImmuKnow® in liver transplant patients showed improvement in overall survival; however, the trial has several shortcomings. Pleximmune™ test results correlated with rejection, but conclusions are uncertain because of extremely limited evidence deriving from a small number of patients described briefly in Food and Drug Administration approval documents. Studies of clinical utility of Pleximmune™ were not identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

The evidence for immune cell function assay in patients who have a hematopoietic stem cell transplant includes studies correlating ImmuKnow® values with subsequent survival. Relevant outcomes are overall survival, test accuracy, other measures of test performance, and morbid events. Small studies show that ImmuKnow® values correlate with long-term survival. This information on predictive capability could not be linked to improved outcomes. No direct studies of clinical utility were identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

Currently, immunosuppression is determined by testing for clinical toxicity (eg, leukopenia, renal failure) and by therapeutic drug monitoring (TDM) when available. However, drug levels are not a surrogate for overall drug distribution or efficacy because pharmacokinetics often differ among individuals due to clinical factors such as underlying diagnosis, age, sex, and race; circulating drug levels may not reflect the drug concentration in relevant tissues; and serum level of an individual immunosuppressant drug may not reflect the cumulative effect of other concomitant immunosuppressants. The main value of TDM is the avoidance of toxic levels and monitoring patient compliance. Further, the appropriate level of immunosuppression may vary from person to person. Individual immune profiles, such as an immune cell function assay, could support clinical decision making and help to manage the risk of infection from excessive immunosuppression and the risk of rejection from inadequate immunosuppression in immunosuppressed patients.

ImmuKnow® measures the concentration of adenosine triphosphate (ATP) in whole blood after a 15- to 18-hour incubation with the mitogenic stimulant, phytohemagglutinin. In cells that respond to stimulation, increased ATP synthesis occurs during incubation. Concurrently, whole blood is incubated in the absence of
stimulant for the purpose of assessing basal ATP activity. CD4+ T lymphocytes are immunoselected from both samples using anti-CD4 monoclonal antibody-coated magnetic particles. After washing the selected CD4+ cells on a magnet tray, a lysis reagent is added to release intracellular ATP. A luminescence reagent added to the released ATP produces light measured by a luminometer, which is proportional to the concentration of ATP. The characterization of the cellular immune response of a specimen is made by comparing the ATP concentration for that specimen with fixed ATP production ranges.

Pleximmune™ measures CD154 expression on T-cytotoxic memory cells in patient’s peripheral blood lymphocytes. CD154 is a marker of inflammatory response. To characterize risk of rejection, the patient’s inflammatory response to (transplant) donor cells is expressed as a fraction of the patient’s inflammatory response to third-party cells. This fraction or ratio is called the Immunoreactivity Index (IR). If the donor-induced response exceeds the response to third-party cells, the individual is at increased risk for rejection. Cells are cultured and then analyzed with fluorochrome-stained antibodies to identify the cells expressing CD154. For posttransplant blood samples, an IR greater than 1.1 indicates increased risk of rejection, and an IR less than 1.1 indicates decreased risk of rejection. For pretransplant samples, the threshold for IR is 1.23.

**REGULATORY STATUS**

In April 2002, ImmuKnow® (Cylex, recently acquired by ViraCor-IBT Laboratories, Lee’s Summit, MO), an immune cell function assay, was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process for detecting cell-mediated immunity (CMI) in an immunosuppressed patient population.

In April 2002, Immune Cell Function Assay (Cylex) was cleared for marketing by FDA through the 510(k) process. FDA determined that this device was substantially equivalent to 2 flow cytometry reagents (“predicate devices”) manufactured by Becton Dickinson, the Tritest CD4 FITC/CD8 PE/CD3 PerCP Reagent and the Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent. These reagents are used to determine CD4+ T-lymphocyte counts in immunocompromised patients. The FDA-indicated use of the Immune Cell Function Assay is for the detection of CMI in an immunosuppressed population. In 2010, a device modification for this assay was cleared for marketing by FDA through the 510(k). There were no changes to the indications or intended use.(1)

In August 2014, Pleximmune™ (Plexision) was approved by FDA through the humanitarian device exemption process.(2) The test is intended for use in the pretransplantation and early and late posttransplantation period in pediatric liver and small bowel transplant patients for the purpose of predicting the risk of transplant rejection within 60 days after transplantation or 60 days after sampling.
Rationale

This evidence review was created in August 2009 and has been updated annually. The most recent literature search was performed for the period through November 10, 2015. This review will first evaluate ImmuKnow®, which has a much larger body of studies, and then evaluate Pleximmune™.

Assessment of a diagnostic technology typically focuses on 3 analyses: (1) analytic validity including test/retest reliability and short term stability; (2) clinical validity including sensitivity, specificity, and positive and negative predictive value in appropriate populations of patients; or validation of risk prediction (3) clinical utility, ie, demonstration that the information from the diagnostic test results in improved health outcomes.

Sensitivity of a test is its ability to detect disease when the disease is present (true positive), and specificity is the ability to detect patients who do not have the disease (true negative). However, these immune cell function assays are generally not meant to diagnose a condition (infection or rejection) that is concurrently present or absent, but to predict future risk of infection or rejection. Thus although many studies evaluate immune function assays using these measures, they are not the ideal method to assess the value of the test, because these measures will be sensitive to the specific context of the study and will vary according to study characteristics (eg, time horizon, baseline risk of outcome). Immune function assays are risk-stratification tools rather than diagnostic tests. Risk stratification can result in improved health outcomes if specific clinical interventions based on test results decrease the risk of a poor health outcome, and the clinical intervention requires the result of the test. In the case of tests such as ImmuKnow®, it is proposed that immunosuppression regimen can be modified based on test results to minimize the risk of infection or rejection. Ideally, clinical trials comparing management of transplant patients with immune function testing versus without immune function testing would provide robust evidence of clinical utility. Lacking such trials, clinical utility might be inferred by a strong chain of indirect evidence that would link evidence on the predictive characteristics of the immune function assay and evidence that the interventions based on test results would produce the desired outcomes.

ImmuKnow®

Analytic Validity of ImmuKnow®

We did not find any studies evaluating the analytic validity of the ImmuKnow® test.

Clinical Validity of ImmuKnow®

Numerous studies have evaluated ImmuKnow® testing in relation to risk of future infection or rejection. In general, these studies have assessed the test using measures for assessing diagnostic tests. They tend to show that test results are correlated with either infection or rejection at specified thresholds, but that diagnostic characteristics tend to show poor sensitivity and poor specificity. This is
to be expected of a test that is not meant to be a diagnostic tool but a risk-stratification tool. Systematic reviews of ImmuKnow® are first summarized, followed by individual studies of transplantation organized by transplant type, and then individual studies of hematopoietic stem cell transplantation.

**Systematic Reviews**

Ling et al (2012) performed a systematic review and meta-analysis of studies published to July 2011 to assess the efficacy of ImmuKnow® for identifying risks of infection and rejection in adult transplant recipients. (3) Nine studies published between 2008 and 2011 met inclusion criteria. Meta-analysis of these 9 studies incorporated 2458 samples from transplant recipients, including 172 samples from patients with infection and 135 samples from patients with rejection. Three studies were of liver transplant recipients, 3 of kidney recipients, and 1 study each of heart, lung, and mixed organ transplant recipients. Pooled estimates of ImmuKnow® performance characteristics for identification of infection risk were: sensitivity of 0.58 (95% confidence interval [CI], 0.52 to 0.64), specificity of 0.69 (95% CI, 0.66 to 0.70), positive likelihood ratio of 2.37 (95% CI, 1.90 to 2.94), negative likelihood ratio of 0.39 (95% CI, 0.16 to 0.70), and diagnostic odds ratio (OR) of 7.41 (95% CI, 3.36 to 16.34). Pooled estimates for ImmuKnow® in identifying risk of rejection were: sensitivity of 0.43 (95% CI, 0.34 to 0.52), specificity of 0.75 (95% CI, 0.72 to 0.78), positive likelihood ratio of 1.30 (95% CI, 0.74 to 2.28), negative likelihood ratio of 0.96 (95% CI, 0.85 to 1.07), and diagnostic OR of 1.19 (95% CI 0.65 to 2.20). Due to significant heterogeneity across studies, review authors conducted subgroup analyses in liver and renal transplant recipients. The liver transplantation group had a pooled sensitivity of 0.85, and the renal transplantation group had a specificity of 0.80, indicating that different types of organ transplanted may be 1 source of observed heterogeneity; however, the positive likelihood ratio of the liver group was low, and the negative likelihood ratio of the renal group was high, suggesting that it may be inappropriate to use the assay result to identify infection risk in either group. Based on the overall findings, the authors suggest that ImmuKnow® does not have sufficient diagnostic accuracy to identify individuals at risk of infection or rejection. In particular, sensitivity is low, and likelihood ratios close to 1.0 indicate that this test does not alter the probability of specified outcomes to a large degree.

Rodrigo et al (2012) conducted a meta-analysis to identify studies (published to March 2012) documenting the use of ImmuKnow® to monitor immune function in adult liver transplant recipients. (4) Five studies analyzed ImmuKnow® performance in infection (total N=651), and 5 in acute rejection (total N=543). Two (of 5) studies also were included in the previously discussed systematic review by Ling et al. Pooled sensitivity, specificity, positive likelihood ratio, diagnostic OR, and mean (SD) area under the summary receiver operating characteristic (ROC) curve for infection were 0.84 (95% CI, 0.78 to 0.88), 0.75 (95% CI, 0.71 to 0.79), 3.3 (95% CI, 2.8 to 4.0), 14.6 (95% CI, 9.6 to 22.3), and 0.824 (0.034), respectively. Pooled estimates for acute rejection were 0.66 (95% CI, 0.55 to 0.75), 0.80 (95% CI, 0.76 to 0.84), 3.4 (95% CI, 2.4 to 4.7), 8.8 (95% CI, 3.1 to 24.8), and 0.835 (0.060), respectively. Heterogeneity was low for
infection and high for acute rejection studies. Based on these findings, ImmuKnow® could be considered a valid tool to assess infection risk in adult liver transplant recipients. However, due to significant heterogeneity across studies, conclusions about prediction of rejection risk with ImmuKnow® are limited.

**Pediatric Transplantation**

Several studies have found no association between adenosine triphosphate (ATP) production as determined by ImmuKnow® and outcomes in pediatric solid organ transplant recipients. Rossano et al (2009) studied 83 pediatric patients (median age, 4.9 years) undergoing heart transplant.(5) ImmuKnow® was performed at routine follow-up visits from 3 months to more than 5 years after transplant. There were 26 episodes of acute rejection, 20 (77%) of which were cell-mediated, and the remainder were humoral rejection. There were 38 infections. No difference in ATP production as measured by ImmuKnow® was detected between patients with or without acute rejection or with or without infection. Further, the manufacturer’s reported risk ranges for rejection (ATP production ≥525 ng/mL) or infection (ATP production ≤225 ng/mL) were not predictive of rejection or infection, respectively. As noted, however, it may be that pediatric patients’ risks for posttransplant infection and rejection correspond to different ATP production. Subsequent retrospective studies by Wong et al,(6) Ryan et al,(7) and Wozniak et al(8) found no association between ATP production and outcomes in pediatric recipients of heart, kidney, or intestinal transplantations, respectively. Ryan et al observed a positive correlation between total peripheral white blood cell (WBC) count and ATP production (r=0.28, p=0.04) and suggested that proportion of activated T cells within submitted samples may provide more useful information.(7)

**Kidney Transplantation**

Torío et al (2011) grouped 227 samples from 116 kidney transplant recipients (mean age, 51.2 years; range, 19-77 years) by clinical course: stable (no infectious syndrome or acute rejection episode 1 month before and after immune cell assay; n=168), infection (fever plus at least 1 positive culture or positive polymerase chain reaction [PCR]; n=24), or rejection (biopsy-proven acute rejection; n=35).(9) Healthy blood donors served as controls (n=108). Immunosuppressive regimens included pretransplant basiliximab (an interleukin-2 receptor inhibitor) or antithymocyte globulin and posttransplant tacrolimus, mycophenolate mofetil, and corticosteroid, or calcineurin inhibitors. Mean (SD) ATP production in the stable group (375.3 [140.1] ng/mL) and in the control group (436.5 [112.0] ng/mL) were higher than in the infection group (180.5 [55.2] ng/mL; p<0.001 for both comparisons). No difference was observed between the rejection group (332.5 [131.7] ng/mL) and the stable group or the control group (p>0.05 for both comparisons).

Two retrospective studies of kidney transplant recipients found statistically significant correlations between ATP production and WBC. In a study of 39 patients at a single center in Japan, Nishikawa et al (2014) reported correlation coefficients (R2) of 0.573 (p=0.03) and 0.510 (p=0.02) for associations between WBC and neutrophil counts, respectively.(10) In this study, ATP levels in 5 patients who
developed viral infections in the early posttransplantation period (<50 days) were within normal limits. Methodologic limitations prevented any conclusion about the association of ATP levels with infections in 8 patients in the late posttransplantation period (>120 days). In a study of 306 patients at a single U.S. center, Sageshima et al (2014) reported a correlation coefficients ($R^2$) of 0.264 (p<0.001) for the association between ATP production and WBC. In this study, mean (SE) ATP production in patients with biopsy proven rejection (389 [56] ng/mL) and borderline/clinical rejection (254 [41] mg/mL) were not statistically higher compared with ATP production in patients without rejection (not reported). Mean (SE) ATP production in patients with opportunistic (349 [48] ng/mL) and other (345 [27] ng/mL) infections were not statistically lower compared with ATP production in patients without infection (not reported).

Reinsmoen et al (2008) studied 126 kidney transplant recipients to determine whether pretransplant immune parameters (ATP production, as well as human leukocyte antigen [HLA] mismatch, HLA-specific antibodies, and interferon-gamma precursor frequencies to donor or third-party cells) were associated with posttransplant early acute rejection, unstable creatinine course, and poor graft outcome. Mean (SD) pretransplant ATP production in recipients who had no clinical reason for a biopsy was significantly lower than those in recipients who had biopsy-proven acute rejection at any posttransplant time point up to 36 months (285.3 [143.2] vs 414.3 [138.5] ng/mL, respectively). Recipients who underwent biopsy but had no diagnosis of acute cellular or antibody-mediated rejection had an intermediate value of 333.7 (156.3) ng/mL. Mean (SD) pretransplant ATP production were also significantly higher for recipients with early (<90 days) unstable creatinine levels, a significant predictor of early acute rejection, than for recipients with stable creatinine values (362.8 [141.2] vs 283.4 [146.4] ng/mL, respectively). Post hoc analysis using a cutoff ATP production of 375 ng/mL revealed that recipients with pretransplant ATP greater than 375 ng/mL were significantly more likely to experience acute rejection (OR=3.67; 95% CI, 1.195 to 11.201). Immune parameters were not used to guide modifications of the immunosuppression protocol. Graft survival and incidence of infection were not reported in this study.

Serban et al (2009) assessed ImmuKnow® results in 76 kidney transplant recipients (mean age, 50 years) receiving antithymocyte globulin induction and maintenance immunosuppression. ATP values were assigned to episodes of infection or rejection only if ImmuKnow® measurement was performed within 30 days preceding the adverse event. Over a median of 10 months of follow-up, there was a statistically significant difference between ATP activity measured in 15 of 18 patients with infection requiring hospitalization (median, □110 ng/mL and 44 stable patients (median, □220 ng/mL; p=0.002). Median ATP value in 9 of 11 patients with rejection (230 ng/mL) did not differ significantly from that observed in stable patients (p not reported). Results of 3 patients whose blood was sampled for ImmuKnow® are unknown. ATP activity did not correlate with the number of CD4+ T cells during the first 5 months posttransplant (r=0.129, p=0.153) but did correlate with the number of neutrophils and total WBCs within the first 3 months posttransplant (r>0.4, p<0.001). Because of substantial myeloid cell
contamination of cells captured by ImmuKnow® in patients with low CD4+ T-cell counts, the authors concluded that cells of the myeloid lineage substantially contributed to the ATP signal measured by ImmuKnow® in these patients. Among 31 patients treated with darbepoetin, median ATP value within the first 2 months posttransplant was approximately 260 ng/mL compared with 160 ng/mL in 38 patients who did not receive darbepoetin (p=0.017). There was no association between ATP values and development of rejection or infection at any time during the entire 10-month follow-up. As suggested by the authors, in darbepoetin-treated patients, increased ATP activity may be due to myeloid cell mobilization induced by darbepoetin rather than T-cell activation and does not justify increased immunosuppression. The relationship between ImmuKnow® results and infections was further analyzed using ROC analysis. Area under the curve (AUC) was 0.736, indicating a fair accuracy of ImmuKnow® results for prediction of infection risk. The ATP cutoff value calculated based on the ROC curve was 165 ng/mL, and corresponding positive and negative predictive values were 0.513 and 0.874, respectively. This cutoff value for increased risk of infection differs from the manufacturer’s cutoff value of 225 ng/mL. However, because of the specific effects of antithymocyte globulin induction, results of this study cannot be extrapolated to transplant recipients receiving no induction therapy or receiving induction agents that do not cause vigorous lymphocyte depletion (eg, alemtuzumab, an anti-CD25 monoclonal antibody).

Zhou et al (2011) grouped 259 Chinese kidney transplant recipients (mean [SD] age, 38.8 [12.3] years) by clinical course: stable (no adverse events 7 days before and after immune cell assay; n=174), infection (clinical and imaging evidence of infection within 7 days before or after assay; n=32), rejection (biopsyproven acute rejection diagnosed within 7 days before or after assay without antirejection therapy; n=16), or methylprednisolone (intravenous methylprednisolone given to treat biopsy-proven acute rejection within 3 days before or after assay; n=33). Posttransplant immunosuppressive regimens included corticosteroids, calcineurin inhibitors, and mycophenolate mofetil. Median ATP production in the infection group (116.4 ng/mL; range, 66.3-169.2) and the methylprednisolone group (182.3 ng/mL; range, 113.6-388.8) were lower than in the stable group (347.7 ng/mL; range, 297.9-411.7; p<0.001 for both comparisons). Median ATP production in the rejection group were higher than in the stable group (615.9 ng/mL; range, 548.8-743.5; p<0.001). ROC analysis was evaluated to determine optimal ATP cutoffs for infection and rejection in this sample. With a cutoff for infection of 238 ng/mL, sensitivity and specificity were 93% and 100%, respectively (AUC=0.991). For rejection, a cutoff of 497 ng/mL maximized sensitivity and specificity at 92% and 94%, respectively (AUC=0.988).

Huskey et al (2011) conducted a single-center, retrospective analysis to assess the predictive ability of ImmuKnow® to identify kidney transplant recipients at risk for opportunistic infection or acute rejection when used in routine clinical management. ImmuKnow® results were categorized according to the manufacturer’s ATP cutoff values and correlated with subsequent infection or rejection occurring within 90 days after the assay. Patients matched for age, sex, and time of testing posttransplant who had neither infection nor rejection served
as controls. Immunosuppressive regimens included prednisone, calcineurin inhibitors, and mycophenolate mofetil. Eighty percent of patients received pretransplant antithymocyte globulin. Standard cytomegalovirus (CMV) and Pneumocystis carinii prophylaxis was administered. Ninety-four ImmuKnow® assays were performed in 85 patients with subsequent opportunistic infection and in matched controls. Mean ATP production did not differ between cases (386 ng/mL) and controls (417 ng/mL; p=0.24). A low ATP production (≤225 ng/mL) was not associated with an increased risk of infection (OR=1.34; 95% CI, 0.64 to 2.82; p=0.43). Forty-seven ImmuKnow® assays were performed in 47 patients with subsequent acute rejection and in matched controls. Mean ATP production did not differ between cases (390 ng/mL) and controls (432 ng/mL; p=0.25). A high ATP production (≥525 ng/mL) was not associated with an increased risk of rejection (OR=1.87; 95% CI, 0.47 to 8.38; p=0.48).

Subsequent studies in kidney transplant recipients have demonstrated no association between ATP production and risk of acute rejection or viral infections using manufacturer-recommended cutoffs for ImmuKnow®(16,17) or have suggested an alternative approach to determining optimal cutoff values.(18,19) In a prospective cohort study of 55 patients followed for 3 years, Libri et al (2014) observed that ATP production was often lower in patients with acute rejection compared with patients without acute rejection, and was often greater in patients with infection compared with patients without infection. Using labelled cutoffs for ImmuKnow®, AUC was 0.44 (95% CI, 0.18 to 0.71) for acute rejection and 0.37 (95% CI, 0.22 to 0.53) for viral or major respiratory tract infections. In a prospective study of 67 patients undergoing kidney transplant, patients with low preoperative ATP production had statistically fewer rejection episodes than those with high preoperative ATP production (p<0.001).(17) The cutoff used for this analysis was 300ng/mL. To optimize ImmuKnow® performance, Quaglia et al (2014)(18) and Wang et al (2014)(19) both proposed assessing change in ATP production over time, rather than single values. In a retrospective study of 118 patients, Quaglia et al reported AUC of 0.632 (95% CI, 0.483 to 0.781) for infection risk using a cutoff of -30 ng/mL for the decrease in ATP production from month 1 to month 3.(18) In a prospective study of 140 patients, Wang et al reported AUC of 0.929 for risk of acute rejection using a cutoff of 172.55 ng/mL for the increase in ATP production from “right before” the rejection episode to the occurrence of rejection.(19)

Heart Transplantation
Two studies have examined ATP production in adult heart transplant recipients. Gupta et al (2008) studied 125 adult heart transplant recipients, most of whom underwent ImmuKnow® testing more than 1 year posttransplant.(20) There was no apparent association between ATP production and rejection (n=3). For 7 patients who developed infection, median ATP production was 267 ng/mL and did not differ statistically from median ATP production in 104 patients who did not develop infection (282 ng/mL). There was a significant correlation between ATP production and white blood cell count but not between ATP production and absolute lymphocyte count, suggesting that nonlymphocytes also may influence ATP response. This idea is supported by a 1994 study of CD4+ T-cell

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responsiveness to 3 stimulants (including PHA) in HIV-positive patients.(21) The authors suggested that assays performed in clinical laboratories should profile immunoregulatory cytokines (eg, interleukin-2), which modulate the complex interplay between cellular and humoral immune mechanisms.

Israeli et al (2010) correlated ImmuKnow® results with clinical status in 50 immunosuppressed heart transplant recipients (median age, 58.5 years).(22) Median ATP value of 280 blood samples collected from patients during clinical quiescence (ie, good clinical status with normal heart function) was 351 ng/mL. ATP values were within the manufacturer’s “moderate” range of immune function (225-525 ng/mL) in 176 (63%) of these samples. Median ATP value of 22 blood samples collected during episodes of biopsy proven acute rejection was 619 ng/mL, a statistically significant difference (p<0.05). Median ATP value of 19 blood samples collected during episodes of fungal or bacterial infection (ie, requiring hospitalization for intravenous antimicrobial therapy) was 129 ng/mL, a statistically significant difference from the value during clinical quiescence (p<0.05). Although these ATP values fall within the manufacturer’s defined ranges for increased risk of infection (≤225 ng/mL) and increased risk of rejection (≥525 ng/mL), blood samples were drawn during the adverse event rather than before.

A retrospective study by Kobashigawa et al (2010) correlated ImmuKnow® results from 296 adult heart transplant recipients (mean [SD] age, 54.6 [12.8] years) with infection or rejection episodes occurring within 1 month of assay.(23) Assays were performed between 2 weeks and 10 years posttransplant (N=864). Infection was diagnosed by the treating physician and resulted in antibiotic therapy. Rejection was defined as any treated episode of cellular or antibody-mediated rejection, with or without hemodynamic compromise. Transplant recipients without infection or rejection served as controls (n=818 assays). All patients received immunosuppression with tacrolimus, mycophenolate mofetil, and corticosteroids, without induction therapy. Oral prednisone bolus and taper was used for asymptomatic rejection, and antithymocyte globulin was used for rejection with hemodynamic compromise. Mean (SD) ATP production was lower in patients with infection (187 [126] ng/mL) compared with controls (280 [126] ng/mL, p<0.001). Ten percent of ATP production less than 200 ng/mL were associated with infection, and 2% of ATP production greater than 200 ng/mL were associated with infection (p<0.001). Mean (SD) ATP production did not differ between patients who developed rejection (327 [175] ng/mL) and controls (p=0.35). The 200 ng/mL cutoff was chosen based on ROC analysis to maximize sensitivity (71%) and specificity (73%; AUC=0.728).

**Liver Transplantation**
Cabrera et al (2009) assessed the ability of ImmuKnow® to differentiate between acute cellular rejection (ACR) and recurrent hepatitis C virus (HCV) infection in 42 adult patients who had HCV-related end-stage liver disease as the indication for liver transplantation.(24) All patients had liver enzyme abnormalities posttransplant and underwent liver biopsy to diagnose both ACR and recurrent HCV. The most sensitive indicator of HCV infection, HCV RNA detection by PCR, was not used to diagnose HCV. ImmuKnow® was performed with blood collected
before biopsy, and biopsy samples were interpreted by histopathologists blinded to ImmuKnow® results. Median ATP value in 12 patients diagnosed with ACR was 283.3 (range, 241.1-423.0), and median ATP value in 15 patients diagnosed with recurrent HCV was 148.0 (range, 33.7-186.0), a statistically significant difference (p<0.001). Median ATP value in 15 patients with mixed biopsy features of both ACR and recurrent HCV, but predominance of neither, was 234.0 (range, 155.3-325.0), a statistically significant difference from both the ACR group (p=0.02) and the recurrent HCV group (p<0.001). Of note, although 100% of patients with recurrent HCV had ATP values within the manufacturer’s range for increased risk of infection (<225 ng/mL), 100% of patients with ACR had ATP values outside of the manufacturer’s cutoff for increased risk of rejection (>525 ng/mL).

To assess ImmuKnow®’s ability to differentiate acute cellular rejection from recurrent HCV infection in patients transplanted for HCV-related liver disease, Hashimoto et al conducted a retrospective review of 54 allograft liver transplant recipients who had concomitant ImmuKnow® results available (mean age, 52 years; range, 40-63).(25) Liver biopsies were performed every 6 months after liver transplantation and when clinically indicated due to elevated liver function tests. Biopsies were read by a pathologist who was blinded to ImmuKnow® results. PCR detection of HCV RNA was not used. Immunosuppressive regimens included basiliximab, calcineurin inhibitors, and mycophenolate mofetil. ImmuKnow® assays were collected before biopsy. Results were divided into 4 groups based on biopsy findings: acute cellular rejection (n=11), recurrent HCV (n=26), normal biopsy (n=12), and overlapping features of both acute cellular rejection and recurrent HCV. Mean (SD) ATP production in acute cellular rejection (365 [130] ng/mL; range, 210-666) was higher compared with normal biopsy (240 [71] ng/mL; range, 142-387; p=0.006). Mean (SD) ATP production in recurrent HCV (152 [100] ng/mL; range, 20-487) was lower than in both acute cellular rejection (p<0.001) and normal biopsy (p=0.019). Mean (SD) ATP production of patients with overlapping features of both acute cellular rejection and recurrent HCV (157 [130] ng/mL; range, 25-355) did not differ statistically from the other groups. Seventy-three percent of patients with acute cellular rejection had ATP production in the manufacturer-defined moderate range. Eighty-eight percent of patients with recurrent HCV had ATP production in the low range (p<0.001). ROC analysis yielded a cutoff level of 220 ng/mL with sensitivity of 89% and specificity of 91% (AUC=0.93; 95% CI, 0.85 to 1.00).

Cheng et al (2011) evaluated the ability of ImmuKnow® to predict recurrence of hepatocellular carcinoma (HCC) in Chinese patients undergoing liver transplantation for HCC.(26) A threshold ATP production of 175 ng/mL was initially determined from 176 assays of 60 patients with HCC (mean [SD] age, 49.8 [8.7] years), 60 (34%) from patients with recurrent HCC posttransplant, and 116 (66%) from stable patients without HCC recurrence, infection, or biopsy-proven rejection. Mean (SD) ATP production in patients with recurrent HCC (137.8 [6.4] ng/mL) were lower compared with those without recurrence (289.2 [133.9] ng/mL, p<0.01). Sensitivity and specificity for the 175 ng/mL threshold value were 83% and 84%, respectively (AUC=0.869). ImmuKnow® was then administered to a second cohort of 92 patients with HCC undergoing liver transplantation (mean
Patients were stratified by high immune response (mean ATP production >175 ng/mL) and low immune response (mean ATP production, ≤175 ng/mL). Seventeen (23%) of 73 patients in the high response group and 16 (84%) of 19 patients in the low response group developed HCC recurrence (p<0.001). Mean (SD) ATP production were 295.3 (85.4) ng/mL and 126.6 (37.9) ng/mL in the high and low immune response groups, respectively (p<0.001). High immune response was associated with recurrence-free survival (OR=7.28; 95% CI, 3.23 to 16.13) but not overall survival (OR=2.20; 95% CI, 0.56 to 8.65). This study also correlated ImmuKnow® results with clinical status (infection or rejection) among a cohort of the original 60 patients with HCC plus 45 additional patients with nonmalignant liver diseases. ImmuKnow® assays were collected during infection (diagnosed by clinical features, positive microbiologic tests, and imaging), biopsy-proven acute or chronic rejection, and stability (defined as good liver function and good general health at least 2 weeks after transplantation, without evidence of infection, rejection, or tumor recurrence). Immunosuppressive regimens were not defined. Rejection episodes were treated with bolus steroids or antithymocyte globulin. Mean (SD) ATP production during infection (145.2 [87.0] ng/mL) and rejection (418.9 [169.5] ng/mL) differed from mean (SD) level during stability (286.6 [143.9] ng/mL, p<0.01 for both comparisons). ROC analysis showed that optimum cutoff for infection was 200 ng/mL with sensitivity of 79% and specificity of 75% (AUC=0.842). Optimum cutoff for rejection was 304 ng/mL with sensitivity of 80% and specificity of 76% (AUC=0.806). Another retrospective study of 87 liver transplant recipients utilized a cutoff level for rejection of 407 ng/mL based on ROC analysis with sensitivity and specificity of 86% and 81%, respectively (AUC=0.869).(27)

**Lung Transplantation**

Bhorade et al (2008) assessed the relationship between low posttransplant ATP production (≤225 ng/mL) and recent infection in 57 immunosuppressed adult lung transplant recipients.(28) ImmuKnow® assays were performed in 143 patients at routine clinic visits when each patient was on a stable dose of tacrolimus. Fifteen patients developed infections (bacterial or fungal pneumonia, CMV infection); 14 of these (93%) had ATP production less than 225 ng/mL at the time of their infections (sensitivity 93%). Among the 42 noninfected patients, 16 (38%) had ATP production less than 225 ng/mL (specificity 62%). Without comparing postinfection ATP production with preinfection ATP production, it is not possible to draw conclusions about whether low ATP production contributed to or resulted from the development of infection. In a 2013 U.S. single-center study on 175 adult lung transplant recipients, Shino et al reported that ImmuKnow® had some predictive ability but was unlikely to be sufficiently accurate for use in clinical care.(29) AUC was relatively low at 0.61. At a cutoff of 525 ng/mL, there was a significant increase in the risk for acute cellular rejection (OR=2.1; 95% CI, 1.1 to 3.8). However, at this cutoff, sensitivity was 35%, with specificity of 82%. When a cutoff of 425 ng/mL was used, sensitivity was 53%, and specificity was 65%.

Husain et al (2009) assessed the correlation of ImmuKnow® results with different types of infections (bacterial, fungal, viral) in 175 adult lung transplant recipients receiving immunosuppression induction with alemtuzumab.(30) Blood samples
were collected prospectively as part of routine surveillance in all patients during 2 to 48 months of follow-up. Periods of stability were defined as no infection occurring 1 month before or after the blood draw. For infectious episodes, only ATP values drawn within 1 month before the episode were analyzed. Median ATP value during stability was 175 ng/mL (25th-75th percentile, 97-306 ng/mL). Significantly lower median ATP values were seen in 13 CMV infections (49 ng/mL, p<0.001), 5 infections with other viruses (1 Epstein-Barr virus, 2 rhinovirus, 1 influenza, and 1 parainfluenza; 70 ng/mL, p<0.05), and 14 bacterial pneumonias (92 ng/mL, p=0.002). Median ATP value in fungal disease (85 ng/mL) did not differ significantly from that in stability (p not reported). Four patients who developed invasive pulmonary aspergillosis all had ATP values less than 50 ng/mL. Generalized estimating logistic regression analysis demonstrated an OR of 2.81 (95% CI, 1.48 to 4.98) for increased risk of infection with ATP values less than 100 ng/mL and an OR of 9 (95% CI not reported) with values less than 50 ng/mL. In comparison, a diagnosis of cystic fibrosis yielded an OR of 2.66 (95% CI, 1.26 to 5.63) and CMV mismatch (donor positive, recipient negative) yielded an OR of 2.97 (95% CI, 1.52 to 5.80). Note that all ImmuKnow® values, both during periods of stability and within the month before infectious episodes, fall below the manufacturer’s cutoff for increased risk of infection (225 ng/mL).

Hematopoietic Stem Cell Transplantation
Two studies examined the association of ImmuKnow® and prognosis in hematopoietic stem cell transplantation (HSCT), one in autologous transplants and one in allogeneic transplants. Manga et al (2010) assessed ATP production in 16 adult patients (mean age, 52 years) with hematologic malignancies (multiple myeloma, B- or T-cell lymphoma, acute myeloid leukemia) undergoing mobilization with granulocyte-colony stimulating factor (G-CSF) with or without granulocyte-macrophage-colony stimulating factor for autologous HSCT. Mean (SD) ATP production on day 5 of G-CSF therapy in 10 patients who survived more than 2 years after mobilization (673 [274] ng/mL) was higher compared with 5 patients who died within 2 years (282 [194] ng/mL; p=0.014). ROC analysis identified a cutoff of 522 ng/mL for predicting patient survival with sensitivity and specificity of 80% and 100%, respectively (AUC=0.880). Gesundheit et al (2010) examined 170 ATP production collected from 40 patients (median age, 34 years; range, 3-64) after engraftment of allogeneic HSCT for various malignant (acute and chronic myeloid leukemia, acute and chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, myelodysplastic syndrome, ovarian, breast, and testicular cancer) and nonmalignant (severe aplastic anemia, thalassemia major, adrenoleukodystrophy) diseases. ImmuKnow® results were categorized “low” or “normal” according to the manufacturer’s ATP cutoff values and correlated with postengraftment clinical course. Overall survival for the immunocompetent (“normal”) group was 83% (10/12 patients) at 13 months of follow-up. Overall survival for the immunocompromised (“low”) group was 12% (3/25 patients) at 12 months of follow-up. Although test results were associated with outcome, it is unclear how such information could be used to improve patient outcomes.
Section Summary: Clinical Validity of ImmuKnow

Across all the studies among various types of patients, ImmuKnow levels are associated with the risk of rejection when levels are high and risk of infection when levels are low. However, the absolute risk and increments of risk are uncertain because of heterogeneity of the studies. The timing of the test in relation to the observed outcomes and whether the test was routine or performed for suspected infection or rejection affect test performance characteristics. In many cases, the threshold values were declared after the study. Validation of the threshold values with external validation samples is needed. It cannot be determined from these studies whether the discrimination of risk is clinically important, and whether there is compelling indirect logic that treatment modifications based on predicted risk would improve patient outcomes.

Clinical Utility of ImmuKnow

Liver Transplantation

The only study that could be identified comparing patients managed with and without immune response assays was a 2015 study by Ravaioli et al.(33) This randomized trial included 202 liver transplant patients. One group was randomized to have ImmuKnow testing at periodic intervals after transplant, and at clinically indicated times after a suspected or confirmed rejection or infection event. In this group, tacrolimus doses where reduced 25% when ImmuKnow values were less than 130, and increased by 25% when ImmuKnow values were greater than 450. In the control group, ImmuKnow testing was performed but not revealed to treating physicians, and tacrolimus was managed according to standard practice. The declared outcomes of the study were survival, infection rate, rejection rate, and graft loss. One-year survival was 95% in the ImmuKnow group and 82% in the control group (p< 0.01). Of the 33 deaths, 11 were caused by infection (distribution of the 11 deaths by treatment group not reported). Patients in the control group were reported to have had higher bacterial and fungal infection rates but the numbers reported in the article included errors and are inconsistent. There were no differences in rejection events between the ImmuKnow group and the control group. Although the study showed a 10% absolute benefit in mortality, there are concerns about study validity. The standard of care monitoring practice is not described. The study was performed at a single center. The control mortality rate may not be representative of modern liver transplant outcomes. The difference in mortality rate seems implausibly large given the known characteristics of ImmuKnow in discriminating risk of infection. Although the study is suggestive of a benefit of monitoring immunosuppression with ImmuKnow in liver transplant patients, many trial shortcomings suggest it needs to be replicated.

Pleximmune™

Analytic Validity of Pleximmune™

U.S. Food and Drug Administration (FDA) documents review precision testing of Pleximmune testing evaluating run-to-run, operator-to-operator, and day-to-day variability.(2) All results met the sponsor’s acceptance criteria for an acceptable
percentage of the coefficient of variation. No data were presented on the variability of test results within individuals over the short term (representing the same clinical state).

Clinical Validity of Pleximmune™
FDA documents describe a clinical validation study of Pleximmune™. (2) Among a sample of 33 pretransplant patients, Pleximmune™ had 57% sensitivity and 89% specificity for identifying rejection.

Among a sample of 64 posttransplant patients, Pleximmune™ had 84% sensitivity and 80% specificity for identifying rejection. Almost no details were provided on study validation. A study by Ashokkumar et al evaluated the association of CD154 expression with rejection among pediatric liver transplant patients. (34) It is difficult to determine if the measure of CD154 expression used in this study is the same as the Pleximmune™ test. Using a different threshold value of IR than the current test, IR was associated with the risk of rejection.

Clinical Utility of Pleximmune™
We did not identify any studies attempting to document clinical utility, in terms of directly showing improved patient outcomes using Pleximmune™, or using indirect chain of logic.

Ongoing and Unpublished Clinical Trials
Some currently unpublished trials that might influence this review are listed in Table 1.

Table 1. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01859832a</td>
<td>Validation of a Novel Diagnostic Tool for the Evaluation of Post Renal Transplant Immunosuppression: The ImmuKnow Assay</td>
<td>19</td>
<td>May 2013 (completed)</td>
</tr>
<tr>
<td>NCT00569842</td>
<td>Investigation of the Cylex® ImmuKnow® Assay</td>
<td>45</td>
<td>Dec 2012 (completed)</td>
</tr>
<tr>
<td>NCT00618267a</td>
<td>Single Center, Pilot Study to Measure ATP Expression in Lymphocytes of MS Patients Undergoing Various Therapies by Means of Using the &quot;ImmuKnow®&quot; Test</td>
<td>100</td>
<td>Dec 2008 (completed)</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.  
a Denotes industry-sponsored or cosponsored trial.

Summary of Evidence
The evidence for immune cell function assay in patients who have a solid organ transplant includes numerous studies of the association of assay test values and subsequent rejection or infection, and 1 randomized controlled trial in liver transplant patients. Relevant outcomes are overall survival, test accuracy, other test performance measures, and morbid events (rejection and infection). The ImmuKnow® test shows variable associations with infection and rejection depending on the type of transplant and the context of the study. The predictive
characteristics of the test are still uncertain, and do not allow a strong indirect argument of clinical utility. The trial of ImmuKnow® in liver transplant patients showed improvement in overall survival; however, the trial has several shortcomings. Pleximmune™ test results correlated with rejection, but conclusions are uncertain because of extremely limited evidence deriving from a small number of patients described briefly in Food and Drug Administration approval documents. Studies of clinical utility of Pleximmune™ were not identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

The evidence for immune cell function assay in patients who have a hematopoietic stem cell transplant includes studies correlating ImmuKnow® values with subsequent survival. Relevant outcomes are overall survival, test accuracy, other measures of test performance, and morbid events. Small studies show that ImmuKnow® values correlate with long-term survival. This information on predictive capability could not be linked to improved outcomes. No direct studies of clinical utility were identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements

Transplantation Society
The International Cytomegalovirus Consensus Group of the Transplantation Society published an international consensus statement on the management of cytomegalovirus in solid organ transplant in 2010.(35) Authors stated that “there are no clinical studies demonstrating that management decisions based on immunologic monitoring affect patient outcomes.” Routine immunologic monitoring was not recommended.

International Society of Heart and Lung Transplantation
Guidelines for the care of heart transplant recipients published in 2010 by the International Society of Heart and Lung Transplantation did not include ImmuKnow®.(36)

American Society of Transplantation
In 2006, the American Society of Transplantation published recommendations for the screening, monitoring, and reporting of infectious complications in immunosuppression trials of organ transplant recipients.(37) These recommendations defined relevant infectious complications to be included in the reporting of immunosuppression trials and recommended specific laboratory monitoring and surveillance methods. The immune cell function assay was not included.

U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National Coverage
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.
Immune Cell Function Assay 2.04.56

References:
Billing Coding/Physician Documentation Information

**Billing Code**

86352  Cellular function assay involving stimulation (eg, mitogen or antigen) and detection of biomarker (eg, ATP)

**ICD-10 Codes**

T86.10- T86.819; T86.890- T86.899

Complications of transplanted organs and tissue code range limited to solid organ transplants

Z94.0- Z94.4; Z94.83; Z94.89; Z94.9

Transplanted organ and tissue status; kidney, heart, lung, heart & lungs, liver, pancreas, other and unspecified codes

**Additional Policy Key Words**

N/A

**Policy Implementation/Update Information**

12/1/15  New Policy; considered investigational.

12/1/16  No policy statement changes.

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