2.04.56	Immune Cell Function Assay		
Original Policy Date:	July 2, 2010	**Effective Date:**	March 1, 2022
Section:	2.0 Medicine	**Page:**	Page 1 of 22

Policy Statement

Use of immune cell function assay testing to monitor and predict immune function is considered **investigational** in **either** of the following:

I. After solid organ transplantation
II. After hematopoietic cell transplantation

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

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

The following CPT code is specific to this type of testing:

- **86352**: Cellular function assay involving stimulation (e.g., mitogen or antigen) and detection of biomarker (e.g., ATP)

Effective January 1, 2022, there is a new code that represents Pleximmune by Plexision. It is intended for use as an aid in the evaluation of the risk of acute cellular rejection in patients less than 21 years old with liver or small bowel transplantation. The algorithm underlying the assay produces a numeric score -- the Immunoreactivity Index ("IR"). For post-transplant samples, an IR > 1.1 indicates increased risk of transplant rejection.

- **81560:** Transplantation medicine (allograft rejection, pediatric liver and small bowel), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score

Description

Careful monitoring of lifelong immunosuppression is required to ensure the 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.

Related Policies

- N/A

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

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Regulatory Status

In April 2002, ImmuKnow® (Cylex, acquired by ViraCor-IBT Laboratories), an immune cell function assay, was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process (K013169). The FDA indicated use of ImmuKnow® is for the detection of a cell-mediated immune response in populations undergoing immunosuppressive therapy for an organ transplant.

In April 2002, Immune Cell Function Assay (Cylex) was cleared for marketing by the FDA through the 510(k) process. The FDA indicated use of the Immune Cell Function Assay is for the detection of a cell-mediated immune response in an immunosuppressed population. In 2010, a device modification for this assay was cleared for marketing by FDA through the 510(k) (K101911). There were no changes to the indications or intended use.[1,]

In August 2014, Pleximmune™ (Plexision) was approved by the 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

Background
Immunosuppression for Transplant
In current clinical practice, levels of immunosuppression in patients being managed after a solid organ transplant or hematopoietic cell transplantation are determined by testing for clinical toxicity (e.g., leukopenia, renal failure) and by therapeutic drug monitoring 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 therapeutic drug monitoring is the avoidance of toxicity. 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.

Treatment
Several commercially available tests of immune cell function have been developed to support clinical decision making.

ImmuKnow measures the concentration of adenosine triphosphate (ATP) in whole blood after a 15- to 18-hour incubation with phytohemagglutinin (a mitogenic stimulant). Cells that respond to stimulation show increased ATP synthesis during incubation. Concurrently, whole blood is incubated in the absence of stimulants for the purpose of assessing basal ATP activity. CD4-positive T lymphocytes are immunoselected from both samples using anti-CD4 monoclonal antibody-coated magnetic particles. After washing the selected CD4-positive 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 the risk of rejection, the patient's inflammatory response to transplant donor cells is expressed as a fraction

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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 an increased risk of rejection, and an IR less than 1.1 indicates a decreased risk of rejection. For pretransplant samples, the threshold for IR is 1.23.

Literature Review
Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Immune Cell Function Testing
The immune cell function assays are generally not meant to diagnose a condition (infection or rejection) that is concurrently present or absent; instead, the assays are designed to predict future risk of infection or rejection. Thus, although many studies have evaluated 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 (e.g., time horizon, baseline risk of outcome). Risk-stratification can result in improved health outcomes if specific clinical interventions are based on the test results and also decrease the risk of a poor health outcome.

In the case of immune cell function tests, it is proposed that the immunosuppression regimen can be modified based on test results to minimize the risk of infection or rejection. Ideally, clinical trials comparing the management of transplant patients with or without immune function testing would provide robust evidence of clinical utility. Lacking such trials, the clinical utility might be inferred by a strong chain of 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.

Clinical Context and Test Purpose
The purpose of immune cell function assay testing in patients who have received solid organ or hematopoietic cell transplant (HCT) is to inform treatment and management decisions with immunosuppressive therapy.

The question addressed in this evidence review is: Does immune cell function assay testing improve the net health outcome in individuals who have received solid organ transplant or HCTs?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals who have a solid organ transplant or an HCT.

Interventions
The test being considered is immune cell function testing with ImmuKnow or Pleximmune.

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Comparators
The following practices are currently being used to manage solid organ transplant and HCTs: standard monitoring of immunosuppression for those who have solid organ transplants and standard of care for those with HCTs.

Outcomes
The general outcomes of interest are acute and chronic rejection episodes, graft dysfunction, graft survival, morbidity associated with graft dysfunction and overall survival (OS) posttransplant.

Acute rejection following any transplant typically occurs within weeks, with the highest risk during the first 3 months, and rarely occurs years after transplant. Chronic rejection typically develops years after transplant.

Study Selection Criteria
For the evaluation of clinical validity of immune cell function testing, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

ImmuKnow Test for Solid Organ Transplants
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Numerous studies have evaluated ImmuKnow testing in relation to the risk of future infection or rejection. In general, these studies have assessed the test using measures for assessing diagnostic tests. The studies tend to show that test results correlate 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 as a diagnostic tool but as a risk-stratification tool. Systematic reviews of ImmuKnow are first summarized, followed by individual studies of solid organ transplantation, organized by transplant type .

Systematic Reviews
Ling et al (2012) performed a 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 each of heart, lung, and mixed organ transplant recipients. Pooled estimates of ImmuKnow performance characteristics for identifying infection risk were: sensitivity of 58% (95% confidence interval [CI], 52% to 64%), specificity of 69% (95% CI, 66% to 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 for identifying risk of rejection were: sensitivity of 43% (95% CI, 34% to 52%), specificity of 75% (95% CI, 72% to 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, reviewers conducted subgroup analyses in the liver and renal transplant recipients. The liver transplantation group had a pooled sensitivity of 85%, and the renal transplantation group had a specificity of 80%, indicating that different types of organs transplanted may be a source of observed heterogeneity; however, the positive likelihood ratio of the liver group was low, and the negative likelihood ratio of the

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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, reviewers suggested 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 systematic review and 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 (651 patients) and 5 in acute rejection (543 patients). Two (of 5) studies also were included in the previously discussed systematic review by Ling et al (2012). Pooled sensitivity, specificity, positive likelihood ratio, diagnostic OR, and mean (standard deviation [SD]) area under the summary receiver operating characteristic (ROC) curve for infection were 84% (95% CI, 78% to 88%), 75% (95% CI, 71% to 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 66% (95% CI, 55% to 75%), 80% (95% CI, 76% to 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. These findings suggested that 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 the prediction of rejection risk with ImmuKnow are limited.

Pediatric Transplants

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 testing 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. The studies noted, however, that pediatric patients' risks for posttransplant infection and rejection may correspond to different ATP production levels. Subsequent retrospective studies by Wong et al (2014),[6] Ryan et al (2014),[7] and Wozniak et al (2014)[8] found no association between ATP production and outcomes in pediatric recipients of heart, kidney, or intestinal transplants, respectively. Ryan et al (2014) observed a positive correlation between total peripheral white blood cell (WBC) count and ATP production (r=0.28, p=.04) and suggested that the proportion of activated T cells within submitted samples may provide more useful information.[7]

Liu et al (2019) found a correlation between low ATP levels and infection following a living-donor liver transplantation in pediatric patients.[9] The retrospective analysis evaluated 66 patients from a single center in China. The patients were divided into 2 groups: those who were diagnosed with an infection post-transplant (n=28) and those who did not develop an infection (n=38). ImmuKnow testing was performed pre-transplant and at 1 to 4 weeks, 2 months, and 3 months post-transplant. The mean pre-transplant ATP level in the overall cohort was 302.5±195.7 ng/mL. The post-transplant ATP levels were significantly lower in the infection group (188.6±93.5 ng/mL) compared to the non-infection group (424.4±198.1 ng/mL; p<.05). An ROC curve was generated to determine a reference ATP level for the diagnosis of infection. At an ATP level of 200.5 ng/mL in patients diagnosed with an infection, the sensitivity and specificity were 89.5% and 64.3%, respectively; the area under the curve (AUC) was 0.866.

Similar results were found in a prospective cohort study conducted by Xue et al (2021).[10] The prospective analysis evaluated 216 pediatric patients (mean age, 7 months; range, 3 to 36 months) undergoing liver transplantation from 2 medical centers in China. Among the patients, 97.7% (n=211) underwent living donor transplant and the other patients underwent deceased

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donor transplant. ImmuKnow testing was performed a maximum of 5 days pre-transplantation and weekly from weeks 1 to 4 post-transplantation and once at 8 weeks, 12 weeks, and 24 weeks post-transplantation. Testing was also performed if an episode of infection or rejection occurred. Patients were categorized based on clinical status of stable (clinical, experimental, and imaging examinations without infection or rejection; n=44), infection (signs, symptoms, and imaging consistent for infection and a positive polymerase chain reaction; n=160), and rejection (biopsy-proven acute rejection or elevated liver function tests consistent with rejection; n=12). Immunosuppression regimens included tacrolimus and corticosteroids with or without mycophenolate mofetil. The median pre-transplant ATP level in the full cohort was 193 ng/mL. The median post-transplant ATP levels were significantly lower in the infection group than those in the stable group (137 ng/mL vs. 269 ng/mL, respectively; p<.0001). There was no significant difference between the rejection and stable groups in ATP levels. An ROC curve was generated to determine a reference ATP level for the diagnosis of infection. At an ATP level of 152 ng/mL in patients diagnosed with an infection, the sensitivity and specificity were 57.3% and 95.5%, respectively; the AUC was 0.784 (95% CI, 0.72 to 0.848; p<.0001).

Kidney Transplants

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 (R^2) of 0.573 (p=.03) and 0.510 (p=.02) for associations between WBC and neutrophil counts, respectively.[11] 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 between ATP levels and 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 coefficient (R^2) of 0.264 (p<.001) for the association between ATP production and WBC.[12] In this study, mean (standard error) ATP levels in patients with biopsy-proven rejection (389 [56] ng/mL) and borderline/clinical rejection (254 [41] mg/mL) were not statistically higher than ATP levels in patients without rejection (not reported). Mean (standard error) ATP levels in patients with opportunistic (349 [48] ng/mL) and other (345 [27] ng/mL) infections were not statistically lower than ATP levels in patients without infection (not reported).

Torío et al (2011) grouped 227 samples from 116 kidney transplant recipients (mean age, 51.2 years; range, 19 to 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; n=24), or rejection (biopsy-proven acute rejection; n=35).[13] 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<.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>.05 for both comparisons).

Zhou et al (2011) grouped 259 Chinese kidney transplant recipients (mean age, 38.8 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 (biopsy-proven 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).[14] Posttransplant immunosuppressive regimens included corticosteroids, calcineurin inhibitors, and mycophenolate mofetil. Median ATP production in the infection group (116.4 ng/mL; range, 66.3 to 169.2 ng/mL) and the methylprednisolone group (182.3 ng/mL; range, 113.6 to 388.8 ng/mL) was lower than in the stable group (347.7 ng/mL; range, 297.9 to 411.7 ng/mL; p<.001 for both comparisons). Median ATP production in the rejection group was higher than in the stable group

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(615.9 ng/mL; range, 548.8 to 743.5 ng/mL; p<.001). An ROC curve was evaluated to determine optimal ATP cutoffs for infection and rejection in this sample. With a cutoff for infection of 238 ng/mL, the sensitivity and specificity were 93% and 100%, respectively (AUC, 0.991). For rejection, a cutoff of 497 ng/mL maximized the 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.[15] ImmuKnow results were categorized by the manufacturer's ATP cutoff values and correlated with infection or rejection occurring within 90 days after the assay. Patients were selected who had neither infection nor rejection as controls; patients were then matched according to age, sex, and time of testing posttransplant. Immunosuppressive regimens included prednisone, calcineurin inhibitors, and mycophenolate mofetil. Of the total patient population, 80% of the patients received pretransplant antithymocyte globulin. Standard cytomegalovirus and *Pneumocystis jirovecii* prophylaxis were 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=.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=.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=.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=.48).

Reinsmoen et al (2008) studied 126 kidney transplant recipients to determine whether pretransplant immune parameters (ATP production, human leukocyte antigen mismatch, human leukocyte antigen-specific antibodies, and interferon-gamma precursor frequencies to donor or third-party cells) are associated with posttransplant early acute rejection, unstable creatinine course, and poor graft outcome.[16] Mean (SD) pretransplant ATP production in recipients who had no clinical reason for a biopsy was significantly lower (285.3 [143.2] ng/mL) than those in recipients who had biopsy-proven acute rejection at any posttransplant time point up to 36 months (414.3 [138.5] ng/mL). Recipients who underwent biopsy but had no diagnosis of acute cellular rejection (ACR) or antibody-mediated rejection had an intermediate value of 333.7 (156.3) ng/mL. Mean (SD) pretransplant ATP production was also significantly higher for recipients with early (<90 days) unstable creatinine levels (362.8 [141.2] ng/mL), a significant predictor of early acute rejection, than for recipients with stable creatinine values (283.4 [146.4] ng/mL). 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 for 76 kidney transplant recipients (mean age, 50 years) receiving antithymocyte globulin induction and maintenance immunosuppression.[17] ATP levels 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 an infection requiring hospitalization (median, »110 ng/mL) and 44 stable patients (median, »220 ng/mL; p=.002). Median ATP production for 9 of 11 patients with rejection (230 ng/mL) did not differ significantly from that observed in stable patients (p-value not reported). Results of 3 patients whose blood was sampled for ImmuKnow are unknown. ATP activity did not correlate with the number of CD4-positive T-cells during the first 5 months posttransplant (r=0.129; p=.153) but did correlate with the number of neutrophils and total WBCs within the first 3 months posttransplant (r>0.4; p<.001). Because of substantial myeloid cell contamination of cells captured by ImmuKnow in patients with low CD4-positive T-cell counts, authors concluded that cells of the myeloid lineage substantially contributed to the ATP signal measured by ImmuKnow

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in these patients. Among 31 patients treated with darbepoetin, median ATP production 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=.017). There was no association between ATP production 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 might be due to myeloid cell mobilization induced by darbepoetin rather than T-cell activation and does not justify increased immunosuppression. The relation between ImmuKnow results and infections was further analyzed using ROC curve analysis. The AUC was 0.736, indicating a fair accuracy of ImmuKnow results for predicting infection risk. The ATP cutoff 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 for increased risk of infection differs from the manufacturer's cutoff of 225 ng/mL. However, because of the specific effects of antithymocyte globulin induction, the results of this study cannot be extrapolated to transplant recipients not receiving induction therapy or receiving induction agents that do not cause vigorous lymphocyte depletion (e.g., alemtuzumab, an anti-CD25 monoclonal antibody).

Subsequent studies in kidney transplant recipients have failed to demonstrate an association between ATP production and the risk of acute rejection. Studies of that nature have also failed to demonstrate an association between ATP production and viral infections using manufacturer-recommended cutoffs for ImmuKnow.[18,19] Moreover, not a single kidney study has suggested an alternative approach to determining optimal cutoff values.[20,21] In a prospective cohort study of 55 patients followed for 3 years, Libri et al (2013) observed that ATP production was often lower in patients with acute rejection than in patients without acute rejection, and was often greater in patients with infection than in patients without infection. Using labeled cutoffs for ImmuKnow, the 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 2014 prospective study of 67 patients undergoing a kidney transplant, patients with low preoperative ATP production had statistically fewer rejection episodes than those with high preoperative ATP production (p<.001).[19] The cutoff used for this analysis was 300 ng/mL. To optimize ImmuKnow performance, Quaglia et al (2014)[20] and Wang et al (2014)[21] both proposed assessing change in ATP production over time, rather than single values. In a retrospective study of 118 patients, Quaglia et al (2014) reported an 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.[20] In a prospective study of 140 patients, Wang et al (2014) reported an 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.[21]

Heart Transplants

Four studies have examined ATP production in adult heart transplant recipients. Weston et al (2020) evaluated use of ImmuKnow in heart transplant recipients with severe systemic infections.[22] Patients were followed at the time of scheduled biopsy and weekly with the ImmuKnow assay if diagnosed with a systemic infection. On detection of a systemic infection, maintenance immunosuppression, typically mycophenolate mofetil or azathioprine, was withdrawn and tacrolimus dose was reduced by 50%. Weekly ImmuKnow levels informed further dose reductions of tacrolimus, but the procedure for these reductions was not reported. Maintenance immunosuppression was restarted once the infection was cleared and ImmuKnow levels increased to greater than 225 ng/mL. Thirteen patients had severe systemic infections accounting for 16 total infectious episodes. At the time of the infection, the mean ImmuKnow level was 109 ± 49 ng/mL (from 311 ± 118 ng/mL prior to the diagnosis) and increased to 315 ± 135 ng/mL after the infection cleared (p<.01). The ImmuKnow level during the infection also correlated with the underlying infectious microorganism. Infections caused by a virus, a fungus, or a bacteria had mean ImmuKnow levels of 75 ng/mL, 95.07 ng/mL, and 123.4 ng/mL, respectively. Patients without infections or non-severe systemic infections served as a control group (n=67). The control group had a mean ImmuKnow level of 294 ± 167 ng/mL. There were 8 episodes of moderate rejection and 6 episodes of severe rejection out of a total of 435 endomyocardial biopsies and 7 episodes of infection in the control group. The mean ImmuKnow

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level in patients with rejection was 368.7 ng/mL and with infection was 183.3 ng/mL. The study was limited by its single center design and lack of statistical comparisons between patients with severe infections and the control group.

Israeli et al (2010) correlated ImmuKnow results with clinical status in 50 immunosuppressed heart transplant recipients (median age, 58.5 years).[23] Median ATP production for 280 blood samples collected from patients during clinical quiescence (i.e., good clinical status with normal heart function) was 351 ng/mL. ATP levels were within the manufacturer's "moderate" range of an immune function (225 to 525 ng/mL) in 176 (63%) of these samples. Median ATP production for 22 blood samples collected during episodes of biopsy-proven acute rejection was 619 ng/mL, a statistically significant difference ($p<.05$). Median ATP production for 19 blood samples collected during episodes of fungal or bacterial infection (i.e., requiring hospitalization for intravenous antimicrobial therapy) was 129 ng/mL, a statistically significant difference from the production during clinical quiescence ($p<.05$). Although these ATP levels fell 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, making it uncertain whether the ImmuKnow results were predictive of the adverse event.

A retrospective study by Kobashigawa et al (2010) correlated ImmuKnow results from 296 adult heart transplant recipients (mean age, 54.6 years) with infection or rejection episodes occurring within 1 month of the assay.[24] 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) than in controls (280 [126] ng/mL, $p<.001$). 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 ($p<.001$). Mean (SD) ATP production levels did not differ between patients who developed rejection (327 ng/mL) and controls (280 ng/mL; $p=.35$). The 200 ng/mL cutoff was chosen based on ROC curve analysis to maximize sensitivity (71%) and specificity (73%; AUC, 0.728). Although limited by its retrospective design, this study suggested that ImmuKnow might be associated with the prediction of infection, not with transplant rejection, in heart transplant patients.

Gupta et al (2008) studied 125 adult heart transplant recipients, most of whom underwent ImmuKnow testing more than 1-year posttransplant.[25] There was no apparent association between ATP production and rejection (n=3). For 7 patients who developed an infection, median ATP production was 267 ng/mL and did not differ statistically from median ATP production in 104 patients who did not develop an infection (282 ng/mL). There was a significant correlation between ATP production and WBC count but not between ATP production and absolute lymphocyte count; this would suggest that nonlymphocytes may be able to influence ATP response. This idea was supported by a 1994 study of CD4-positive T-cell responsiveness to 3 stimulants (including phytohemagglutinin in HIV-positive patients).[26] The authors suggested that assays performed in clinical laboratories should profile immunoregulatory cytokines (e.g., interleukin 2), which modulate the complex interplay between cellular and humoral immune mechanisms.

Liver Transplants

Cheng et al (2011) evaluated the capability of ImmuKnow to predict recurrence of hepatocellular carcinoma (HCC) in Chinese patients undergoing liver transplantation for HCC.[27] A threshold ATP production of 175 ng/mL was initially determined from 176 assays of 60 patients with HCC (mean age, 49.8 years), 60 (34%) from patients with recurrent HCC posttransplant and 116 (66%) from stable patients without HCC recurrence, infection, or biopsy-

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proven rejection. Mean (SD) ATP production levels in patients with recurrent HCC (137.8 [6.4] ng/mL) were lower than those without recurrence (289.2 [133.9] ng/mL; p<.01). The sensitivity and specificity for the 175-ng/mL threshold value were 83% and 84%, respectively (AUC, 0.869). ImmuKnow was then administered to the second cohort of 92 patients with HCC undergoing liver transplantation (mean age, 50.1 years). 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<.001). Mean (SD) ATP production levels were 295.3 (85.4) ng/mL and 126.6 (37.9) ng/mL in the high- and low-immune response groups, respectively (p<.001). High immune response was associated with recurrence-free survival (OR, 7.28; 95% CI, 3.23 to 16.13) but not OS (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 level during infection (145.2 [87.0] ng/mL) and rejection (418.9 [169.5] ng/mL) differed from mean (SD) production level during stability (286.6 [143.9] ng/mL, p<.01 for both comparisons). ROC analysis showed that optimum cutoff for infection was 200 ng/mL, with a sensitivity of 79% and specificity of 75% (AUC, 0.842). The optimum cutoff for rejection was 304 ng/mL, with a sensitivity of 80% and specificity of 76% (AUC, 0.806). Another retrospective study (2011) of 87 liver transplant recipients used a cutoff for rejection of 407 ng/mL based on ROC curve analysis, with a sensitivity and specificity of 86% and 81%, respectively (AUC, 0.869).[28,]

To assess ImmuKnow's ability to differentiate ACR from recurrent hepatitis C virus (HCV) infection in patients with liver transplanted due to HCV-related liver disease, Hashimoto et al (2010) retrospectively reviewed 54 allograft liver transplant recipients who had concomitant ImmuKnow results available (mean age, 52 years; range, 40 to 63 years).[29,] 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 blinded to ImmuKnow results. Polymerase chain reaction detection of HCV RNA was not used. Immunosuppressive regimens included basiliximab, calcineurin inhibitors, and mycophenolate mofetil. ImmuKnow assays were collected before the biopsy. Results were divided into 4 groups based on biopsy findings: ACR (n=11), recurrent HCV (n=26), normal biopsy (n=12), and overlapping features of both ACR and recurrent HCV. Mean (SD) ATP production levels in ACR (365 [130] ng/mL; range, 210 to 666) was higher than in normal biopsy (240 [71] ng/mL; range, 142 to 387; p=.006). Mean (SD) ATP production levels in recurrent HCV (152 [100] ng/mL; range, 20 to 487) were lower than in both ACR (p<.001) and normal biopsy (p=.019). Mean (SD) ATP production of patients with overlapping features of both ACR and recurrent HCV (157 [130] ng/mL; range, 25 to 355) did not differ statistically from the other groups. Further, 73% of patients with ACR had ATP production within the manufacturer-defined moderate range; 88% of patients with recurrent HCV had ATP production in the low range (p<.001). ROC curve analysis yielded a cutoff level of 220 ng/mL with a sensitivity of 89% and specificity of 91% (AUC, 0.93; 95% CI, 0.85 to 1.00).

Cabrera et al (2009) assessed the ability of ImmuKnow to differentiate between ACR and recurrent HCV infection in 42 adults with liver transplant due to HCV-related end-stage liver disease.[30,] All patients had liver enzyme abnormalities posttransplant and underwent a liver biopsy to diagnose both ACR and recurrent HCV. The most sensitive indicator of HCV infection (HCV RNA detection by polymerase chain reaction) was not used to diagnose HCV. ImmuKnow was performed with blood collected before the biopsy, and biopsy samples were interpreted by histopathologists blinded to ImmuKnow results. Median ATP production in 12 patients diagnosed with ACR was 283.3 ng/mL (range, 241.1 to 423.0 ng/mL), and median ATP production in 15 patients diagnosed with recurrent HCV was 148.0 ng/mL (range, 33.7 to 186.0 ng/mL), a statistically significant difference (p<.001). Median ATP production levels in 15 patients with

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mixed biopsy features of both ACR and recurrent HCV, but the predominance of neither, was 234.0 ng/mL (range, 155.3 to 325.0 ng/mL), a statistically significant difference for both the ACR group (p=.02) and the recurrent HCV group (p<.001). Of note, although 100% of patients with recurrent HCV had ATP production within the manufacturer's range for increased risk of infection (<225 ng/mL), all patients with ACR had ATP production outside of the manufacturer's cutoff for increased risk of rejection (>525 ng/mL).

Lung Transplants

Narasimhan et al (2021) conducted a retrospective cohort study evaluating effects of the 2-dose SARS-CoV-2 messenger RNA vaccination series (Moderna vs. Pfizer) on humoral response in immunocompromised lung transplant patients through various antibody response measurements using SARS-CoV-2 anti-nucleocapsid protein Immunoglobulin G (IgG) assay (IgG_{NC}), SARS-CoV-2 anti-spike protein Immunoglobulin M (IgM) assay (IgM_{SP}), and SARS-CoV-2 anti-spike protein IgG II assay (IgG_{SP}).[31] As a marker of immunocompetence, CD4-positive T-cell activity was assessed with ImmuKnow testing, measured in 56 of the 73 lung transplant recipients included in the study. Results were interpreted based on manufacturer ATP ranges of low (≤225 ng/mL), moderate (226 to 524 ng/mL), or strong (≥525 ng/mL). In patients who received the Moderna vaccine series, a positive IgG_{SP} response was demonstrated in 44% (4 out of 9) of patients found to have moderate ImmuKnow values and 50% (1 out of 2) of patients with strong ImmuKnow values. In patients who received the Pfizer vaccine series, a positive IgG_{SP} response was demonstrated in only 18% (3 out of 17) of patients with a moderate ImmuKnow response and no patients (0 out of 6) with strong ImmuKnow levels. The ImmuKnow assay did not give any insight into predicting which patients may have a better antibody response for IgG_{SP}, IgM_{SP}, or IgG_{NC} for either vaccine.

Piloni et al (2016) reported on a retrospective cohort study evaluating the immunosuppressive association between oversuppression (ImmuKnow score, corresponding to intracellular ATP, ≤226 ng/mL) and adequate or under suppression (ImmuKnow score, >226 ng/mL) in a sample of 61 patients in follow-up for lung transplantation.[32] ImmuKnow testing had been performed at a 6-month follow-up for patients who entered the study at the time of transplant (n=28); for other patients, testing was obtained on an as-needed basis because of acute graft dysfunction or suspected immune oversuppression. Being in the immune oversuppression group was associated with higher odds of infection (51 cases of infection/71 ImmuKnow tests vs. 25/56; OR, 2.754; 95% CI, 1.40 to 5.39; p=.003). However, given that many patients tested in the as-needed group might have been tested because of suspected immune oversuppression, the risk of bias is very high.

Husain et al (2009) assessed the correlation between ImmuKnow results and different types of infections (bacterial, fungal, viral) in 175 adult lung transplant recipients receiving immunosuppression induction with alemtuzumab.[33] 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 levels drawn within 1 month before the episode were analyzed. Median ATP production during stability was 175 ng/mL (25th-75th percentile, 97 to 306 ng/mL). Significantly lower median ATP production levels were seen in 13 cytomegalovirus infections (49 ng/mL; p<.001) and 14 bacterial pneumonias (92 ng/mL; p=.002). Median ATP production for fungal disease (85 ng/mL) did not differ significantly from that in stability (p-value not reported). Four patients who developed invasive pulmonary aspergillosis all had ATP levels of less than 50 ng/mL. Generalized estimating logistic regression analysis demonstrated odds of 2.81 (95% CI, 1.48 to 4.98) for increased risk of infection with ATP levels less than 100 ng/mL; moreover, the analysis demonstrated an OR of 9 (95% CI not reported) with values less than 50 ng/mL. In comparison, a diagnosis of cystic fibrosis yielded odds of 2.66 (95% CI, 1.26 to 5.63); cytomegalovirus mismatch (donor positive, recipient negative) yielded an OR of 2.97 (95% CI, 1.52 to 5.80). Note that all ImmuKnow levels, both during periods of stability and within the month before infectious episodes, fell below the manufacturer's cutoff for increased risk of infection (225 ng/mL).

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Bhorade et al (2008) assessed the relation between low posttransplant ATP production (≤225 ng/mL) and recent infection in 57 immunosuppressed adult lung transplant recipients.[34] 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, cytomegalovirus infection); 14 (93%) of the 15 had ATP production levels 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 with preinfection ATP production, it is impossible to determine whether low ATP production levels contributed to or resulted from the development of infection. In a 2012 U.S. single-center study on 175 adult lung transplant recipients, Shino et al (2012) reported the ImmuKnow test had some predictive ability but was unlikely to be sufficiently accurate for use in clinical care.[35] The AUC was relatively low (0.61). At a cutoff of 525 ng/mL, there was a significant increase in the risk for ACR (OR, 2.1; 95% CI, 1.1 to 3.8). However, at this cutoff, sensitivity was 35% and specificity was 82%. When a cutoff of 425 ng/mL was used, sensitivity was 53% and specificity was 65%.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs).

The only study identified comparing patients managed with and without immune response assays is a study by Ravaioli et al (2015).[36] 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 were reduced by 25% when ImmuKnow values were less than 130 ng/mL, and increased by 25% when ImmuKnow values were greater than 450 ng/mL. In the control group, ImmuKnow testing was performed but not revealed to treating physicians, and tacrolimus was managed according to standard practice. Declared study outcomes were survival, infection rate, rejection rate, and graft loss. One-year survival was 95% in the ImmuKnow group and 82% in the control group ($p<.01$). 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 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, we have concerns about the study's validity. The standard of care monitoring practice is not described. The study was performed at a single-center. The control mortality rate might not be representative of modern liver transplant outcomes. The difference in mortality rates seems implausibly large given the known characteristics of ImmuKnow in discriminating risk of infection. Although the study suggested a benefit of monitoring immunosuppression with ImmuKnow in liver transplant patients, many trial limitations indicated that it needs to be replicated.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of ImmuKnow testing has not been established for solid organ transplants, a chain of evidence supporting the test's clinical utility cannot be constructed.

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Section Summary: ImmuKnow Test for Solid Organ Transplants
For solid organ transplants, the ImmuKnow test has shown variable associations with infection and rejection, depending on the type of transplant and context of the study. 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 the heterogeneity of the studies. The predictive characteristics of the test are still uncertain and do not allow a strong chain of evidence for clinical utility. The trial of the ImmuKnow test in liver transplant patients showed improvement in OS; however, the trial had several limitations.

ImmuKnow Test for Hematopoietic Cell Transplants
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Two studies examined the association between ImmuKnow and prognosis in HCT, 1 in autologous transplants and 1 in allogeneic transplants. Manga et al (2010) assessed ATP production in 16 adults (mean age, 52 years) with hematologic malignancies (multiple myeloma, B- or T-cell lymphoma, acute myeloid leukemia) undergoing mobilization with a granulocyte-colony stimulating factor with or without granulocyte-macrophage-colony stimulating factor for autologous HCT.[37.] Mean (SD) ATP production on day 5 of granulocyte-colony stimulating factor 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=.014). The ROC curve analysis identified a cutoff of 522 ng/mL for predicting patient survival, with a 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 to 64 years) after engraftment of allogeneic HCT 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.[38.] ImmuKnow results were categorized “low” or “normal” according to the manufacturer’s ATP cutoff values and correlated with post-engraftment clinical course. Overall survival for the immunocompetent ("normal") group was 83% (10/12 patients) at 13 months of follow-up and OS for the immunocompromised (“low”) group was 12% (3/25 patients) at 12 months of follow-up. Although test results were associated with the outcome, it is unclear how such information could be used to improve patient outcomes.

Section Summary: Clinically Valid
Two studies evaluated the association between ImmuKnow and prognosis in HCT. In autologous and allogeneic transplant populations, higher ImmuKnow levels were associated with patients with longer OS at 2 years and 12 months, respectively. However, it cannot be determined from these studies whether the discrimination of risk is clinically important and whether there is a compelling chain of evidence that treatment modifications based on predicted risk would improve patient outcomes

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

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No studies assessing the clinical utility of the ImmuKnow test were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of ImmuKnow testing has not been established for HCTs, a chain of evidence supporting the test's clinical utility cannot be constructed.

Section Summary: ImmuKnow Test for Hematopoietic Cell Transplants
For HCTs, the ImmuKnow test has shown associations with longer OS for both autologous and allogeneic transplant populations. However, no clinical utility studies were identified. Therefore, it cannot be determined whether the discrimination of risk is clinically important and could potentially alter treatment that would improve patient outcomes

Pleximmune Test for Solid Organ Transplants
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
The U.S. Food and Drug Administration (FDA) documents have described 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 (2009) evaluated the association between CD154 expression and rejection among pediatric liver transplant patients.[39,] 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 Immunoreactivity Index (IR) than the current test, IR was associated with the risk of rejection.

A study by Ashokkumar et al (2017) reported on the preclinical development and validation of an allogeneic-specific CD154-positive T-cytotoxic memory cell test to predict ACR after liver or intestine transplantation in patients with pediatric liver or lung transplantation.[40,] Plexision (manufacturer of Pleximmune) was involved in the study design and assay standardization. A total of 127 patients (120 analyzable samples) were included in the training set (enrolled from 2006 to 2010), and 87 patients (72 analyzable samples) were included in the validation set (enrolled from 2009 to 2012). The training and test sets differed significantly in terms of organ type composition, with a higher proportion of those in the training set represented by liver or liver/small bowel transplant (e.g., 83% liver in training set vs. 71% in validation set; p=.007 for the difference between groups). The IR was defined as the ratio of the reaction of donor-induced CD154-positive T-cytotoxic memory cell to the reaction exceed those induced by reference peripheral blood leukocytes; a ratio above 1 was considered to indicate an increased risk of rejection. An IR of 1.1 or greater as a cutoff in posttransplant samples was associated with an area under the summary ROC curve of 0.878 in the test set (0.791 in the validation set), while a pretransplant IR of 1.23 or greater was associated with a ROC curve of 0.82 in the training set (0.842 in the validation set). The association test performance characteristics are shown in Table 1.

Table 1. Test Performance Characteristics

Cutpoint	Performance Measures	Measure, %	95% Confidence Interval, %
Posttransplant IR ≥1.1	Sensitivity	84	60 to 96
	Specificity	80	65 to 90
	Positive predictive value	64	43 to 81
	Negative predictive value	92	78 to 98
Pretransplant IR ≥1.23	Sensitivity	57	30 to 81

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Cutpoint	Performance Measures	Measure, %	95% Confidence Interval, %
	Specificity	89	65 to 98
	Positive predictive value	80	44 to 96
	Negative predictive value	74	51 to 89

Adapted from Ashokkumar et al (2017).[40.]
IR: Immunoreactivity Index.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No studies directly demonstrating improved patient outcomes were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

An argument for clinical utility using a chain of evidence would rely on both a demonstration of clinical validity and a rationale that specific clinical interventions based the results of the test decrease the risk of a poor health outcome. At present, the clinical interventions that would occur as a result of the test result are uncertain, and the clinical validity is uncertain. Therefore, the clinical utility of Pleximmune is unknown for solid organ transplants.

Section Summary: Pleximmune Test for Solid Organ Transplants
For the use of the Pleximmune test in the solid organ transplant population, extremely limited evidence is available and includes a study with a small number of patients described briefly in the FDA approval documents and a second study in which the CI bounds for sensitivity and specificity estimates were wide. No direct studies of clinical utility were identified.

Pleximmune Test for Hematopoietic Cell Transplants
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
No evidence for the clinical validity of the Pleximmune test for HCT populations was identified.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No evidence for the clinical utility of the Pleximmune test for HCT populations was identified.

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Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

An argument for clinical utility using a chain of evidence would rely on both a demonstration of clinical validity and a rationale that specific clinical interventions based the results of the test decrease the risk of a poor health outcome. At present, the clinical interventions that would occur as a result of the test result are uncertain, and the clinical validity is uncertain. Therefore, the clinical utility of Pleximmune is unknown for HCTs.

Section Summary: Pleximmune Test for Hematopoietic Cell Transplants

No evidence for the clinical validity or clinical utility of the Pleximmune test for HCT populations were identified.

Summary of Evidence

For individuals with a solid organ transplant or HCT who receive immune cell function assay testing with ImmuKnow, the evidence includes numerous studies on the association between assay test values and subsequent rejection or infection, and an RCT in liver transplant patients. Relevant outcomes are OS, other test performance measures, and morbid events. The ImmuKnow test has shown variable associations with infection and rejection, depending on the type of transplant and context of the study. 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 the heterogeneity of the studies. The predictive characteristics of the test are still uncertain and do not allow a strong chain of evidence for clinical utility. The trial of the ImmuKnow test in liver transplant patients showed improvement in OS; however, the trial had several limitations. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with a solid organ transplant or HCT who receive immune cell function assay testing with Pleximmune, the evidence includes the U.S. FDA documentation and a report on the test's development and validation. Relevant outcomes are OS, other measures of test performance, and morbid events. Small studies have shown that Pleximmune values correlate with long-term survival. 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 the FDA approval documents and a second study, in which the CI bounds for sensitivity and specificity estimates were wide. No direct studies of clinical utility were identified. An argument for clinical utility using a chain of evidence would rely on both a demonstration of clinical validity and a rationale that specific clinical interventions based the results of the test decrease the risk of a poor health outcome. At present, the clinical interventions that would occur as a result of the test result are uncertain, and so the clinical validity is uncertain. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

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American Society of Transplantation Infectious Diseases Community of Practice

In 2019, the American Society of Transplantation Infectious Diseases Community of Practice updated guidelines on post-transplant lymphoproliferative disorders in solid organ transplant.[41] A statement indicated: "Simpler rapid assays to measure global and [Epstein-Barr virus] EBV-specific T-cell immunity using commercial ATP release assays (Cyclex ImmuKnow and T-cell Memory) have undergone preliminary evaluation as adjunct markers of [post-transplant lymphoproliferative disorders] PTLD risk when combined with viral load testing in pediatric thoracic transplant recipients but require further validation." Routine immunologic monitoring was not recommended.

Transplantation Society

In 2018, the International Cytomegalovirus Consensus Group of the Transplantation Society updated its consensus statement on the management of cytomegalovirus in solid organ transplant.[42] The statement indicated that “there are no clinical studies demonstrating that management decisions based on immunologic monitoring affect patient outcomes.” Routine immunologic monitoring was not recommended.

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

A search of ClinicalTrials.gov in November 2021 did not identify any ongoing or unpublished trials that would likely influence this review.

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Documentation for Clinical Review

- No records required

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

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The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	86352	Cellular function assay involving stimulation (e.g., mitogen or antigen) and detection of biomarker (e.g., ATP)
	81560	Transplantation medicine (allograft rejection, pediatric liver and small bowel), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score ***(Code effective 1/1/2022)***
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
07/02/2010	New Policy Adoption
06/28/2013	Title changed from Immune Cell Function Assay in Solid Organ Transplantation with position change
06/30/2015	Coding update
02/01/2016	Policy revision without position change
02/01/2017	Policy revision without position change
02/01/2018	Policy revision without position change
03/01/2019	Policy revision without position change
04/01/2020	Annual review. No change to policy statement. Literature review updated.
02/01/2021	Annual review. No change to policy statement. Literature review updated.
02/01/2022	Annual review. Policy statement and literature updated.
03/01/2022	Coding update

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment,

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procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

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Appendix A

POLICY STATEMENT	
BEFORE **Red font: Verbiage removed**	**AFTER** **Blue font: Verbiage Changes/Additions**
Immune Cell Function Assay 2.04.56 **Policy Statement:** Use of the immune cell function assay to monitor and predict immune function is considered **investigational** in **either** of the following: I. After solid organ transplantation II. After hematopoietic cell transplantation Use of the immune cell function assay for all other indications is considered **investigational**.	**Immune Cell Function Assay 2.04.56** **Policy Statement:** Use of immune cell function assay testing to monitor and predict immune function is considered **investigational** in **either** of the following: I. After solid organ transplantation II. After hematopoietic cell transplantation Use of immune cell function assay testing for all other indications is considered **investigational**.

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