Subject: ImmuKnow (Transplantation Immune Cell Function Assay)

Policy Number: NMP415

Effective Date*: March 2008

Updated: December 2015

This National Medical Policy is subject to the terms in the IMPORTANT NOTICE at the end of this document

For Medicaid Plans: Please refer to the appropriate State’s Medicaid manual(s), publication(s), citation(s), and documented guidance for coverage criteria and benefit guidelines prior to applying Health Net Medical Policies.

The Centers for Medicare & Medicaid Services (CMS)

For Medicare Advantage members please refer to the following for coverage guidelines first:

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<tr>
<th>Use</th>
<th>Source</th>
<th>Reference/Website Link</th>
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<tr>
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<td>National Coverage Determination (NCD)</td>
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Instructions
- Medicare NCDs and National Coverage Manuals apply to ALL Medicare members in ALL regions.
- Medicare LCDs and Articles apply to members in specific regions. To access your specific region, select the link provided under "Reference/Website" and follow the search instructions. Enter the topic and your specific state to find the coverage determinations for your region. *Note: Health Net must follow local coverage determinations (LCDs) of Medicare Administration Contractors (MACs) located outside their service area when those MACs have exclusive coverage of an item or service. (CMS Manual Chapter 4 Section 90.2)
- If more than one source is checked, you need to access all sources as, on occasion, an LCD or article contains additional coverage information than contained in the NCD or National Coverage Manual.
- If there is no NCD, National Coverage Manual or region specific LCD/Article, follow the Health Net Hierarchy of Medical Resources for guidance.
Current Policy Statement
Health Net, Inc. considers ImmuKnow, also known as the Transplantation Immune Cell Function Assay (ARUP Laboratories), investigational, because there is inadequate scientific evidence in the medical literature to support its validity in predicting the immunological events of graft failure and infection prior to their clinical manifestation in transplant patients.

Codes Related To This Policy
NOTE:
The codes listed in this policy are for reference purposes only. Listing of a code in this policy does not imply that the service described by this code is a covered or non-covered health service. Coverage is determined by the benefit documents and medical necessity criteria. This list of codes may not be all inclusive.

On October 1, 2015, the ICD-9 code sets used to report medical diagnoses and inpatient procedures have been replaced by ICD-10 code sets.

ICD-9 Codes

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<td>Complications of heart transplant</td>
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ICD-10 Codes

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<td>Z94.89 - Z94.9</td>
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CPT Codes

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<tr>
<td>86353</td>
<td>Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis (Lymphocyte Antigen Proliferation Assay)</td>
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HCPCS Codes
N/A

Scientific Rationale – Update December 2015
Ravaiol et al. (2015) completed a randomized controlled trial in which adult liver recipients were randomized to standard practice (control group; n = 102) or serial immune function testing (interventional group; n = 100) performed with a commercially available in vitro diagnostic assay (ImmuKnow; Viracor-IBT Laboratories, Lee's Summit, MO) before transplantation, immediately after surgery. ImmuKnow (Transplantation Immune Cell Function Assay) Dec 15
and at day 1, weeks 1 to 4, 6, and 8, and months 3 to 6, 9, and 12. The assay was repeated within 7 days of suspected/confirmed rejection/infection and within 1 week after event resolution. Based on immune function values, tacrolimus doses were reduced 25% when values were less than 130 ng/mL adenosine triphosphate (low immune cell response) and increased 25% when values were greater than 450 ng/mL adenosine triphosphate (strong immune cell response). The 1-year patient survival was significantly higher in the interventional arm (95% vs 82%; P < 0.01) and the incidence of infections longer than 14 days after transplantation was significantly lower among patients in the interventional arm (42.0% vs. 54.9%, P < 0.05). The difference in infection rates was because of lower bacterial (32% vs 46%; P < 0.05) and fungal infection (2% vs 11%; P < 0.05). Among recipients without adverse events, the study group had lower tacrolimus dosages and blood levels. Immune function testing may provide additional data which may help to optimize immunosuppression and improve patient outcomes. This study although promising was based on 12 month outcomes only. Long-term outcomes would be necessary to determine efficacy of this testing.

There is a Clinical Trial on ‘Randomized Controlled Trial of ImmuKnow in Liver Transplantation’ which has been completed but no study results are posted. ImmuKnow detects cell-mediated immunity in solid-organ transplant recipients undergoing immunosuppressive therapy. Increasing ImmuKnow values indicate a decrease of immunosuppression and decreasing ImmuKnow values suggest an increase of immunosuppression. The test measures the amount of ATP produced in CD4+ lymphocytes as a biomarker of lymphocyte activation. This study uses the ImmuKnow assay to proactively adjust immunosuppressive therapy in adult liver transplant recipients to reduce the risk of adverse events. ClinicalTrials.gov Identifier is NCT01764581 and this trial was related to the study by Ravaïoli et al. (2015) noted above.

An additional Clinical Trial on ‘Validation of a Novel Diagnostic Tool for the Evaluation of Post Renal Transplant Immunosuppression: The ImmuKnow Assay’ was also completed, with no study results posted. The ClinicalTrials.gov Identifier is NCT01859832 and it was last updated August 31, 2015. This study was designed to evaluate an in vitro assay (CylexImmuKnow Assay) for the measurement of cell-mediated immune response in renal transplant patients receiving immunosuppressive therapy. This assay measures ATP as an activation response of CD4+ cells to stimulation with phytohemagglutinin (PHA) in whole blood samples as a reflection of the immune system of the patient at any point in therapy. The natural history of the immune status of the renal transplant recipient as reflected by the ImmuKnow assay will be determined at specific time points of interest including: pre and post transplant, as an adjunct to therapeutic drug monitoring, and pre and post infectious or rejection episode. Analysis of the results of the assay at these time points will allow us to retrospectively study the effects of routine immunosuppressive agent modulation on immune function, and its subsequent effects in times of renal allograft insult.

There is another Clinical Trial on ‘Cytomegalovirus-Specific Response Measured by QuantiFeron and Overall Immunologic Response Measured by ImmuKnow in Lung Transplant Patients CMV-positive (REIVI)’ that is currently recruiting participants. The ClinicalTrials.gov Identifier is NCT02076971 and it was last updated March 6, 2015. The purpose of this study is to determine the sensitivity and specificity of QuantiFeron and ImmuKnow in combination for early detection of patients who will develop CMV infection in lung transplant patients with CMV-positive serology (R+) prior to transplant. The estimated primary completion date is March 2016.
Scientific Rationale – Update December 2014

Wozniak et al. (2014) The Cylex Immune Cell Function Assay measures cell-mediated immunity based on ATP production by stimulated CD4+ cells. We hypothesized that this test would discriminate acute cellular rejection (ACR) from infectious enteritis (IE) in pediatric intestinal transplant (ITx) recipients with allograft dysfunction. We retrospectively analyzed 224 Cylex assays drawn in 47 children who received 53 ITx. Samples were classified as stable, ACR, or IE based on clinical status. ATP values were analyzed using Kruskal-Wallis and t-tests. Overall, there was a statistically significant difference in ATP values based on clinical status (p = 0.03); however, overlap was observed between groups. The median ATP value during ACR was significantly greater than during stable periods (p = 0.02). No difference was seen in IE vs. stability (p = 0.8). The difference in median ATP value in ACR vs. IE approached significance (p = 0.1). Relative to previous levels, ACR episodes were associated with a median ATP increase of 101 ng/mL and IE episodes with a decrease of 3 ng/mL (p = 0.3). These data indicate that the Cylex assay has limited utility in differentiating ACR from IE, largely due to interpatient variability. Following longitudinal intrapatient trends may be an adjunctive tool in discriminating IE from ACR and guiding immunosuppression adjustments in select patients.

Quaglia et al. (2014) The Immuknow assay (IKA; Cylex) is a T-cell immune function assay that evaluates immunoreactivity in immunocompromised patients. The aim of this study was to analyze IKA values in a cohort of kidney transplantation (KT) recipients to investigate correlations between single-time point low IKA values and their trend over time with cytomegalovirus (CMV) or BK virus (BKV) reactivation. A total of 118 adult patients receiving deceased-donor KT were enrolled (55.6±11.9 years old; 79 [66.9%] male). IKA CMV and BKV viremia determinations and were performed at months 1, 3, and 6 after surgery. Overall, 272 IKA determinations were performed: IKA values significantly decreased from month 1 (422±184 ng/mL) to month 3 (330±159 ng/mL; P<.001) and from month 3 to month 6 (300±128 ng/mL; P=.030). IKA values did not correlate with renal function or viral reactivation at any time. However, patients with either CMV or BKV viremia had a trend to higher IKA values at month 1 and lower IKA values at month 6, even if the difference did not reach a statistical significance (P=.115). Our study suggests that presence of low immunologic reactivity (IKA<225 ng/mL) is not associated with an increased risk of CMV and BKV reactivation over the 1st 6 months after KT. However, a trend to a more pronounced drop in IKA values over time was observed in patients with viral reactivation. These preliminary results suggests that drop in IKA values within the 1st post-KT months, unlike single-time point immune function assay, may predict the risk of opportunistic viral infections.

Sageshima et al. (2014) The Cylex ImmuKnow assay measures the amount of stimulated ATP production by CD4+ T-cells, and has been used clinically, trying to predict rejection and infection episodes. However, predictive values of this assay after induction therapy with steroid-sparing maintenance protocols are unclear. In this single-center cohort study, the authors analyzed renal transplant recipients who received T-cell depleting +/-anti-IL2 receptor antibodies and tacrolimus/mycophenolate maintenance without steroids. A total of 4224 ImmuKnow levels in 306 patients were available for analysis. ImmuKnow levels (Mean ± SE) changed over time after induction therapy with a paradoxical initial increase: 419 ± 23, 461 ± 32, 519 ± 14, 411 ± 10, 344 ± 6, and 405 ± 3 for pre-transplant, 0-1 wk, 1 wk-1 mo, 1-3 mos, 3 mos-1 yr, and thereafter. This change was parallel to the evolution of peripheral WBC counts and ImmuKnow levels had weak but significant correlation with WBC counts (R(2)=0.264, P<0.0001). The levels for biopsy-proven rejection (389 ± 56) and borderlineclinical rejection (254 ± 41) were not significantly higher than the levels of quiescent patients. The levels for opportunistic infection (349 ±
48) and other infections (345 ± 27) were not significantly lower than the levels of quiescent patients. The longitudinal changes in ImmuKnow levels were not predictive of rejection or infection. In conclusion, ImmuKnow levels can vary after T-cell depleting induction therapies at various time points, even without significant clinical events. Since ImmuKnow levels seem to be affected by WBC counts, ImmuKnow results need to be interpreted with caution. The effects of leukocytosis or leukopenia caused by immunosuppressive medication on the ImmuKnow assay need further investigation.

**Scientific Rationale – Update December 2013**

Mizuno et al (2013) evaluated whether the combination of the peripheral blood CD4+ adenosine triphosphate activity (ATP) assay (ImmuKnow assay: IMK assay) and cytochrome P450 3A5 (CYP3A5) genotype assay is useful for monitoring of immunological aspects in the patient follow-up of more than one year after living donor liver transplantation (LDLT). Forty-nine patients, who underwent LDLT more than one year ago, were randomly screened by using IMK assay from January 2010 to December 2011, and the complete medical records of each patient were obtained. The CYP3A5 genotypes were examined in thirty-nine patients of them. The mean ATP level of the IMK assay was significantly lower in the patients with infection including recurrence of hepatitis C (HCV) (n = 10) than in those without infection (n = 39): 185 versus 350 ng/mL (P < 0.001), while it was significantly higher in the patients with rejection (n = 4) than in those without rejection (n = 45): 663 versus 306 ng/mL (P < 0.001). The IMK assay showed favorable sensitivity/specificity for infection (0.909/0.842) as well as acute rejection (1.0/0.911). CYP3A5 genotypes in both recipient and donor did not affect incidence of infectious complications. Investigators concluded in the late phase of LDLT patients, the IMK assay is very useful for monitoring immunological aspects including bacterial infection, recurrence of HCV, and rejection.

Mizuno et al (2013) evaluated whether the combination of the ImmuKnow assay (IMK) and cytochrome P450 3A5 (CYP3A5) genotype assay is useful for identifying the risk of morbidity and mortality in living donor liver transplantation (LDLT) patients, and also to investigate the optimal cutoff value of IMK level of immune status. Sixty six LDLT patients, who were randomly screened by using IMK between March 2002 and June 2011, were divided into 2 groups: patients in whom at least 1 IMK value was <175 ng/mL (Group A, n=16) and patients in whom all IMK values were >175 ng/mL (Group B, n=50). Both donors and recipients were evaluated for the CYP3A5 genotype. The frequencies of cytomegalovirus and bacterial infection in Group A were significantly higher than those in Group B. The short term mortality was 4 (25.0%) in Group A and none in Group B, and the IMK level in all four cases became <100 ng/mL at least one time before death. The rate of CYP3A5*1 allele (expressors) among recipients was significantly higher in Group A than in Group B (63.6% vs. 22.2%). The rates of CMV infection and bacterial infection, and the IMK levels was significantly higher in recipients with expressors. Investigators concluded the combination of the IMK and CYP3A5 genotype assay is useful for monitoring immune status after LDLT, and the IMK level <100 ng/ml might be the critical level to make a recovery from the severe immunesuppressive condition.

He et al (2013) reported that studies have shown correlations between ImmuKnow assay and adverse events, such as immunosuppression and low or high calcineurin inhibitor trough levels. The authors investigated the correlation between IK changes and rejection or infection in kidney transplant patients and studied the potential application of the IK assays in optimizing individual immunosuppressive therapy. ImmuKnow assay was used to determine dynamic intracellular ATP changes in CD4 cells in 193 samples from 42 kidney transplant patients and 25 healthy subjects.
Patients were categorized into rejection, infection, and event-free groups. The IK values were assayed and analyzed between kidney transplant patients and healthy controls. Most IK values fell between 200 and 599 ng/mL from pre-transplantation to 30 months post-transplantation. The mean IK values continuously increased throughout 30 months. Incidental allograft rejection patients had significantly higher IK values compared with the event-free patients and controls. However, infection patients had significantly lower IK values. Seven days after treatment, IK values in rejection/infection patients were different compared with the values in autograft patients, and there was a significant correlation between calcineurin inhibitor (FK506) trough levels and IK values in rejection/infection patients. Serum creatinine levels in the rejection patients were significantly higher than those in the event-free patients, and C-reactive protein levels were significantly higher in the infection patients compared with the event-free patients. Investigators concluded the IK assay combined with other biomarkers can be used to identify kidney transplant patients at high risk of rejection and infection.

The clinical utility of the Cylex ImmuKnow Assay continues to be investigated.

**Scientific Rationale – Update December 2012**

The clinical utility of the Cylex ImmuKnow Assay continues to be investigated in the peer review literature. In addition, clinical trials are ongoing.

Rodrigo et al (2012) conducted a systematic literature review to identify studies published up to March 2012 that documented the use of ImmuKnow for monitoring immune function in liver transplant recipients. The study quality was assessed with the Quality Assessment of Diagnostic Accuracy Studies 2 score. Reviewers identified 5 studies analyzing ImmuKnow performance for infection and 5 studies analyzing ImmuKnow performance for acute rejection. The pooled sensitivity, specificity, positive likelihood ratio, diagnostic odds ratio, and area under the summary receiver operating characteristic curve were 83.8%, 75.3%, 3.3,14.6 and 0.824 ± 0.034, respectively, for infection and 65.6 %, 80.4%, 3.4 8.8 and 0.835 ± 0.060, respectively, for acute rejection. Heterogeneity was low for infection studies and high for acute rejection studies. Reviewers concluded the ImmuKnow test is a valid tool for determining the risk of further infection in adult liver transplant recipients. Significant heterogeneity across studies precludes the conclusion that ImmuKnow identifies liver transplant patients at risk for rejection.

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

Moon et al (2012) assessed whether immuKnow ATP values predicted infectious syndromes. Investigators prospectively enrolled 71 kidney transplant patients between September 2008 and May 2011. ImmuKnow assay monitoring was performed at one day before as well as 4, 8, 12, 16, 20, 24, 36, and 52 weeks after the operation. ImmuKnow assay values were compared as well as BK viral infection pre-infection (PI), at first detection of infectious syndrome (DI), 4 weeks there after (4W), 8 weeks there after (8W) and 12 weeks there after (12W) and pre-recovery (PR), recovery (R) times. Serial ImmuKnow assays showed significant differences over time and BK viral infectious state. Interestingly, PI was significantly lower than DI and PR but PR significant greater than PI, 8W and 12W. However, the authors did not observe an adequate or absolute cutoff value of ImmuKnow by ROC curve: 377 ng/mL ImmuKnow showed 0.471 of AUC and 57.1% and 56.2%, of sensitivity and specificity. Investigators concluded longitudinal evaluation and adjustment of the value of ImmuKnow assay seemed to be a favorable modality to monitor infectious syndromes especially those involving BK virus.

Te et al (2012) evaluated the role of immune function test (IFT) in monitoring and adjustment of immunosuppression in orthotopic liver transplant (OLT) recipients. A total of 289 IFTs were obtained from 171 patients from March 2007 to June 2008. Graft/patient status was classified as stable, serious infection, or malignancy. IFT levels were analyzed with duration of follow-up after OLT, graft/patient status, and the presence of hepatitis C (HCV) infection. The mean age was 54 ± 14 yr, with 62% men. The median follow-up was 65 (2-249) months. Mean IFT levels were significantly lower in patients who were <24 months than in those ≥24 months post-OLT (220 ± 19.5 vs. 257 ± 11.3 ng/mL). Clinically stable patients had higher IFT levels than those with serious infection or malignancy (254 ± 11.1 vs. 162.5 ± 23.9). HCV-infected patients had lower IFT levels than uninfected patients (206.7 ± 15.7 vs. 273 ± 12.0 ng/mL). Immunosuppression was reduced in 58 patients with IFT levels <225 ng/mL, and 90% maintained stable graft function after a median follow-up of 22 (1-39) months. Investigators concluded IFT may be a useful tool in monitoring and lowering of immunosuppression in long-term OLT recipients.

Ling et al (2012) performed a meta-analysis to assess the efficacy of the Cylex ImmunKnow cell function assay (CICFA) in identifying risks of infection and rejection posttransplantation. After a careful review of eligible studies, sensitivity, specificity, and other measures of the accuracy of CICFA were pooled. Summary receiver operating characteristic curves were used to represent the overall test performance. Nine studies met the inclusion criteria. The pooled estimates for CICFA in identification of infection risk were poor, with a sensitivity of 0.58, a specificity of 0.69, a positive likelihood ratio of 2.37, a negative likelihood ratio of 0.39, and a diagnostic odds ratio of 7.41. The pooled estimates for CICFA in identifying risk of rejection were also fairly poor with a sensitivity of 0.43, a specificity of 0.75, a positive likelihood ratio of 1.30, a negative likelihood ratio of 0.96, and a diagnostic odds ratio of 1.19. Reviewers concluded the current evidence suggests that CICFA is not able to identify individuals at risk of infection or rejection. Additional studies are still needed to clarify the usefulness of this test for identifying risks of infection and rejection in transplant recipients.

Schulz-Juergensen et al (2012) investigated the correlations between intracellular adenosine-tri-phosphate (iATP) production(Cylex Immuknow) and adverse events, immunosuppression, calcineurin-inhibitor-trough levels, and age. In this prospective trial, 31 nontransplant pediatric subjects and 50 consecutive children were included ImmuKnow (Transplantation Immune Cell Function Assay) Dec 15
after they underwent liver transplantation (LTX). During the study period, 4 allograft rejections and 3 acute infections occurred. The patients were treated with cyclosporine, tacrolimus, mycophenolate mofetil, and everolimus either as monotherapy or in combinations. The reactivity of the immune system was measured as iATP concentration in CD4+ T-cells after in vitro stimulation by phytohemagglutinin. The iATP concentrations in patients with intercurrent, clinically significant infections were in the low immune response range (median iATP 181 versus 251 ng/mL), whereas the patients with incidental allograft rejection had significantly higher iATP concentrations as compared with the event-free group (median iATP 444 versus 251 ng/mL). However, there was a wide range of iATP concentrations in both nontransplant and LTX patient groups, and no clear iATP cut-off values for an increased risk of infection or rejection could be defined. Post LTX, stable-phase patients showed a significantly lower iATP compared with respective controls (median iATP 297 versus 384 ng/mL). No significant correlation between calcineurin-inhibitor-trough concentrations and iATP was found. iATP was not correlated with age, but was inversely correlated with time after transplantation. Investigators concluded the observed correlation between clinical events and iATP concentrations is similar to the findings previously reported in adult patients who underwent transplantation. The lack of correlation of iATP with trough drug concentrations suggests that the ImmuKnow assay provides independent information that may be useful to guide immunosuppressive therapy in pediatric (liver) transplant patients. However, the wide range of iATP levels in event-free patients suggests that serial iATP measurements will be necessary to assess and guide the individual immunosuppressive therapy. Further investigations are needed to evaluate and extend these findings.

Zhou et al (2011) sought to identify the correlation of a low ImmuKnow adenosine triphosphate (ATP) value with the development of invasive fungal infections (IFIs) and whether this is an independent risk factor for IFIs in liver recipients. Investigators followed up 248 liver recipients who developed 157 infectious episodes. Peripheral CD4(+) T cells were selected freshly for ATP detection. Percentages of T-helper (Th, CD3(+) CD4(+) ) and T-suppressor (Ts, CD3(+) CD8(+) ) lymphocyte subgroups were also examined. Overall 44 patients (17.7%) were diagnosed as IFIs, of whom 9 (20.5%) died. The average ImmuKnow ATP value in the IFI patients (109 ± 78 ng/mL) was significantly lower than that in common bacterial infections (174 ± 106 ng/mL) or stable liver recipients (314 ± 132 ng/mL), while there was no difference in the Th/Ts ratio among each group. Logistic regression analysis showed ImmuKnow ATP value less than 100 ng/mL was an independent risk factor of IFI (OR = 3.44). ImmuKnow ATP values had no correlation with lymphocytes or their subgroups, but tended to correlate with the number of neutrophils and total white blood cells. Investigators concluded ImmuKnow assay monitoring has the potential to identify the patients at risk of developing IFI after liver transplantation (LT), which may provide a feasible measure for optimizing liver recipients' immune cellular function after transplantation.

**Scientific Rationale – Update December 2011**

Zhou et al (2011) evaluated ATP levels reflecting the immune responses of Chinese kidney transplant recipients as a monitoring parameter to guide treatment after transplantation. 259 kidney transplant patients were divided into four groups: stable (n = 174), postoperative infection (n = 32), postoperative rejection (n = 16), and high-dose corticosteroid treatment (n = 33). The ImmuKnow assay was performed to measure CD4 T-cell ATP levels. Receiver operating characteristics measurements indicated an ATP predictive range of 238 to 497 ng/mL to monitor immune responses after transplantation and immunosuppressive therapy. To identify patients with infection, a cutoff ATP value of 238 ng/mL was used with 100% specificity and
positive predictive value and 92.9% sensitivity. To identify patients with rejection, a value of 497 ng/mL was used with 91.5% sensitivity. Compared with the 225 to 525 ng/mL ATP levels recommended by the FDA, the target values showed similar or better diagnostic accuracy.

Cheng et al (2011) sought to determine the utility of an immune function assay in assessing the risk of infection, rejection, and tumor recurrence in liver transplant recipients. Immune function was determined by ImmuKnow assay that measures the amount of adenosine triphosphate (ATP) produced by CD4 (+) T cells to monitor the global immune status in 342 whole blood samples from 105 liver transplant recipients. The association between ATP value and post-transplant tumor recurrence was evaluated in 60 HCC patients. The ATP value in predicting tumor recurrence in other independent cohort of 92 recipients with HCC was analyzed prospectively. The mean ATP values of liver transplant recipients with infection (145.2 ± 87.0 ng/ml) or acute rejection (418.9 ± 169.5 ng/ml) were different from those with stable state (286.6 ± 143.9 ng/ml, P < 0.05). In recipients with HCC who developed recurrent tumors, the values were significantly lower than those without recurrence (137.8 ± 66.4 vs. 289 ± 133.9 ng/ml, P < 0.01); the optimal threshold value to predict post-transplant tumor recurrence was 175 ng/ml. Comparing with the patients in lower immune group (ATP ≤ 175 ng/ml), patients in the higher immune group (ATP > 175 ng/ml) experienced significantly better disease-free survival (P < 0.01). Multivariate Cox regression analysis showed the ATP value was an independent predictor of HCC recurrence.

De Paolis et al (2011) evaluated the value of ImmuKnow (IK), a new tool to measure the net state of immunofunction among renal transplant recipients, in correlation with clinical and laboratory data among unselected renal transplant recipients, in a preliminary observational study. Forty-nine recipients of mean age of 51 years were enrolled and followed for 1 year after transplantation. All subjects received the same immunosuppressive strategy with basiliximab induction and tacrolimus, mycophenolate mofetil and steroid maintenance therapy. Samples for IK were collected before transplantation as well as at 7, 14, 21 and 42 days and after 3, 6, and 12 months. There were 54 samples with IK <225 ng/mL, 201 samples with normal IK values, and 135 samples with >525 ng/mL. Recipients were divided into 3 groups with respect to their basal IK values: Group 1 (Gr1; IK <225 ng/mL); Group 2 (Gr2; normal values of IK between 226 and 524 ng/mL); and Group 3 (Gr3; IK >525 ng/mL). At 1 year, a significant difference among IK values at the start and the end of the study was observed: Gr1 vs Gr2, P<.0001; Gr2 vs Gr3, P<.06 and Gr 1 vs Gr 3, P<.01). Reduced IK values to predict an increased risk of infection, was observed, particularly with cytomegalovirus (CMV) replication while higher IK value did not correlate with an increased risk of acute rejection episodes. Reduction of serum creatine levels occurred within 1 year in all groups (P<.005), but there was a significant difference between Gr 2 versus Grs 1 and 3 (P<.0001 and P<.0005, respectively). The authors noted the findings suggested that more stable IK values were associated with clinical quiescence and laboratory stability. They concluded, the preliminary analysis showed a beneficial capacity of this assay to represent the global depression of the immune system. They noted that reduced IK values, as a sign of excessive immunosuppressive therapy, were associated with an increased risk of infection. They did not confirm the predictive value of higher IK values for an increased risk of an acute rejection episode.

Scientific Rationale – Update March 2011
Huskey et al (2010) retrospectively analyzed 1330 ImmuKnow assay values in 583 renal transplant recipients at a single center and correlated these values with episodes of opportunistic infections (OI) or acute rejection (AR) in the subsequent 90
days. Assay values were compared with a control population matched for age, gender, and time post-transplantation. In patients with OI (n = 94), there were no differences in prior mean assay values compared with matched controls (386 versus 417 ng/ml, P = 0.24). In 47 patients with AR, again no differences were detected in prior assay results (390 versus 432 ng/ml, P = 0.25) when compared with controls. "Low" values (≤225 ng/ml) lacked sensitivity and specificity as a predictive test for subsequent OI, as did "strong" (≥525 ng/ml) values as a predictive test for subsequent AR. The investigators concluded the results fail to show an association between single time point ImmuKnow assay values and the subsequent development of an adverse event in the subsequent 90 days. The optimal use of the ImmuKnow assay in kidney transplantation has yet to be determined.

Hwang et al (2010) evaluated the clinical utility of peritransplant in vitro assays of immune cell function, measuring immune cell function, using the ImmuKnow assay, in 107 adult adult living donor liver transplant (LDLT) recipients and 200 potential living liver donors (control group). In the control group, the mean proportion of T-helper/inducer cells was 36.8% ± 8.2%. The degree of immune response was strong in 12%, moderate in 77%, and low in 11%. In the study group, the degree of immune response within the first month was strong in 4.6%, moderate in 38.2%, and low in 57.2%, thus significantly lower than in the control group (P < .001). ImmuKnow results and tacrolimus levels did not show a significant correlation. Although six patients showed biopsy-proven acute cellular rejection, none showed a strong immune response. Patients with overt infection showed a lower immune response. The authors concluded these results indicate that peritransplant assessment of immune response using the ImmuKnow assay does not reliably predict the occurrence of acute rejection. Additional studies are necessary to accurately assess the clinical utility of immune response monitoring.

Israeli et al (2010) evaluated the Immuknow assay for longitudinal immune monitoring of heart transplantation (HTx) patients throughout various clinical settings. The functional immune response as measured by the Immuknow assay was determined in 327 samples collected from 50 HTx patients at a single center and was analyzed together with common clinical parameters. The median Immuknow levels measured throughout the infection episodes and the episodes of biopsy-proven acute rejection were 129 and 619 ng ATP/mL, respectively. These values were significantly dissimilar to the median Immuknow level measured during clinical quiescence, which was 351 ng ATP/mL (P<0.05). Calcineurin inhibitors drug-level measurements did not provide a reliable depiction of the patients' immune function, because the median deviation from the recommended drug trough levels range was significantly higher than the median deviation of Immuknow levels from their expected immune response zones. Longitudinal monitoring of Immuknow levels through serial testing proved to be a reliable method for individual patient immune management. The investigators concluded the Immuknow assay reliably reflects the cellular immune function of HTx patients, thereby supporting the immune monitoring and management of these patients. Serial longitudinal Immuknow monitoring allows immune management of therapy according to the individual patient's immune status.

**Scientific Rationale – Update March 2010**

Kobashigawa et al (2010) sought to determine the utility of ImmunKnow (IM) in 296 heart transplant recipients. A total of 864 IM assays performed at 2 weeks to 10 years post-transplant and were correlated with infection and rejection events that occurred within 1 month after IM testing. All patients received standard triple-drug immunosuppressive therapy with tacrolimus, mycophenolate mofetil and corticosteroids, without induction therapy. The investigator reported that there were 38 infectious episodes and 8 rejection episodes. The average IM score was
significantly lower during infection than steady state (187 vs 280 ng ATP/ml, p < 0.001). The average IM score was not significantly different during rejection when compared with steady state (327 vs 280 ng ATP/ml, p = 0.35). Three of the eight rejection episodes were antibody-mediated rejections and had hemodynamic compromise and, for these, the mean IM score was significantly higher than for steady-state patients (491 vs 280 ng ATP/ml, p < 0.001). The investigators concluded that non-invasive IM test appears to predict infectious risk in heart transplant patients. The association between high IM scores and rejection risk is inconclusive due to the small number of rejection episodes. Further studies with larger sample sizes for rejection episodes are required.

Gesundheit et al (2009) evaluated 170 blood samples from 40 patients after allogeneic hematopoietic SCT (alloHSCT) and from 13 healthy controls using the ImmuKnow assay for CD4 ATP levels to compare known clinically immunocompromised vs immunocompetent patients after alloHSCT. The investigators compared the reconstitution of WBC count to the ImmuKnow results and clinical status. The patients' clinical course correlated with the stratification of immune response established by the ImmuKnow assay for solid organ transplantation (immunocompetent vs immunocompromised), and this often differed from their WBC count. The authors concluded that the ImmuKnow assay has the potential to predict clinical course and facilitate prompt management of post-HSCT complications, however, the assay should be evaluated prospectively in clinical trials.

Serban et al (2009) assessed the significance of immune cell function in 76 renal allograft recipients after Thymoglobulin induction and initiation of maintenance immunosuppression. Using the Immuknow (Cylex Inc) assay, the amount of adenosine triphosphate (ATP) produced by CD4+ cells in response to phytohemagglutinin (PHA) was measured in patients whole blood. In parallel, the frequency and phenotype of CD4+ T cells were determined by flow cytometry. The Immuknow assay yielded paradoxically high ATP values during the first 3 months post-transplantation, despite very low CD4+ T cell counts. High ATP values were caused by peripheral blood myeloid cells, did not predict rejection, and occurred primarily in transplant recipients who received darbepoietin. CD4+ T cells displayed predominantly an activated/memory phenotype and comprised a subpopulation of CD25+FOXP3+ cells. Over the first 5 months post-transplantation, mean ATP activity gradually decreased, whereas CD4+ T cell counts slowly increased. Low ATP values were predictive of infection. The authors concluded the Immuknow results need to be interpreted with caution in patients receiving Thymoglobulin induction therapy. Although low ATP levels identify patients at increased risk for infection, high ATP values fail to correlate with rejection and do not justify increased immunosuppression.

Macedo et al (2008) investigated the impact of EBV load on T-cell immunity from pediatric transplant (Tx) recipients, using using clinically applicable tests for improved assessment of T-cell immune competence. Thirty-five asymptomatic pediatric thoracic Tx patients were categorized into three groups according to their EBV load levels as follows: undetectable viral load (UVL), chronic low viral load (LVL) and chronic high viral load (HVL). Global and EBV-specific T-cell immunity were assessed by ATP release using Cylex Immuknow and T Cell Memory assays. UVL patients exhibited normal ATP release to Concanavalin A (ConA) and phytohemagglutinin (PHA; 190+/-86 ng/mL, 328+/-163 ng/mL) and detectable EBV-specific (37+/-34 ng/mL) ATP responses. LVL patients displayed significantly stronger responses to ConA (373+/-174 ng/mL), PHA (498+/-196 ng/mL) and EBV (152+/-179 ng/mL), when compared with UVL or to HVL patients (ConA 185+/-114 ng/mL, PHA 318+/-173 ng/mL, and EBV 33+/-42 ng/mL). Moreover, HVL patients displayed significant inverse correlation between CD4+ T-cell ATP levels and EBV ImmuKnow (Transplantation Immune Cell Function Assay) Dec 15
loads. The authors concluded that evaluation of global and EBV-specific T-cell immunity provides a rapid assessment of patients' immune competence. However, it is still unclear whether selective oversuppressed ATP release by CD4+ T cells reflects HVL patients at risk of posttransplant lymphoproliferative disease. They stated further longitudinal studies will determine the importance of Immuknow test in identifying asymptomatic HVL patients vulnerable to EBV complications.

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

At this time, the utility of immune monitoring assays such as the ImmuKnow assay remains unclear. Large prospective trials are needed to determine its role in the management of solid organ or stem cell transplant recipients.

**Scientific Rationale – Update March 2009**

Rossano et al. (2009) completed a case series on all children undergoing heart transplantation (1989-2006) for whom the Cylex ImmuKnow cell function assay (CICFA) levels were reviewed. The study noted that CICFA is not predictive of AR or significant infections in pediatric heart transplant patients. On the basis of the available evidence, this assay cannot be recommended as part of the routine management of pediatric heart transplant patients.

Bhorade et al. (2008) assessed the functional immune response by the ImmuKnow assay in 143 sequential blood samples from 57 lung transplant recipients. The average ImmuKnow assay in stable lung transplant recipients was 244 +/- 138 adenosine triphosphate (ATP) ng/ml and the median level was 236 ATP ng/ml (range 5 to 669 ATP ng/ml), approximately 703 +/- 695 days after lung transplantation. There was no correlation between ImmuKnow levels and tacrolimus trough levels. Stepwise multiple regression analysis identified African American race as an independent predictor of ImmuKnow assay levels when age, gender and underlying diagnosis were taken into account (p < 0.04). Fifteen infected lung transplant recipients had a lower ImmuKnow level at the time of their infections as compared with stable lung transplant recipients (111 +/- 83 vs 283 +/- 143 ATP ng/ml, respectively, p = 0.0001). Sixteen of the remaining 42 patients had low ImmuKnow assay values (<225 ATP ng/ml), but had no active infection. There were only 2 patients with acute rejection of Grade A1 in this cohort. There were no identifiable associations of the ImmuKnow level with either acute rejection episode. The Cylex ImmuKnow assay levels were lower in infected lung transplant recipients compared with non-infected recipients and increased with treatment of these infections. It remains unclear whether the Immuknow assay reflects over-immunosuppressed individuals at risk of infection or bone marrow suppression by infectious agents. Further investigation will determine the role of the Immuknow assay in tailoring immunosuppression in lung transplant recipients.
There continues to be insufficient evidence of the effectiveness of the ImmuKnow assay in the management of organ transplant rejection in individuals undergoing immunosuppressive therapy post solid organ transplant. This is also true for the identification of individual risk for rejection prior to kidney or any other solid organ transplant. Randomized controlled studies comparing ImmuKnow to established monitoring tests are needed to determine its role in the management of organ transplant recipients.

**Scientific Rationale – Initial**

The human immune system consists of two distinct yet interactive types of immunity: humoral immunity and cell-mediated immunity (CMI). Humoral immunity is mediated by B-lymphocytes and their production of antibodies. CMI is mediated by T lymphocytes and their effector responses and interactions with other immune cells. The measurement of CMI is valuable in a variety of applications including transplantation, management of infectious diseases (e.g. HIV, HCV), autoimmunity, cancer, as well as vaccine and drug development. Traditionally, in vivo methods such as the skin test have been used to measure CMI, but there is a need for in vitro methods to rapidly assess CMI. Current in vitro methods for assessing CMI include measuring cell activation signals, lymphoproliferation, cytotoxicity, and cytokine production. Most methods currently used for investigating immune function focus on lymphocytes. Exposure of lymphocytes in peripheral blood to stimulus results in activation and expansion of the population of lymphocytes reactive to the stimulus. Stimulated lymphocytes first undergo an influx of ions and increased ATP synthesis followed by surface receptor clustering, RNA synthesis, cytokine production and release, and DNA replication.

The survival of a transplanted organ is dependent on maintenance of continuous immunosuppression. However, even the strictest adherence to the recommended drug levels does not prevent the occurrence of numerous complications associated with immunosuppression. The efficacy of immunosuppression therapy protocols would be enhanced greatly by the availability of biotechnologies capable of identifying and predicting immunological events prior to the manifestation of clinical parameters indicating graft failure.

In ImmuKnow assay analysis ATP incremental changes indicative of rejection or infection were found in 75% and in 50% incidences, respectively. In stable patients, the ATP deviation from the preoperative baseline, indicative of stable engraftment, was much less pronounced than in other habitual clinical tests. Because of the necessary immuno-suppression, transplant recipients have a high risk of infection. Conversely, under-immunosuppression carries with it the risk of rejection. It would be quite useful to have a test that could differentiate between infection and rejection in renal transplant patients and better still, to predict which patients are at risk of complications.

ImmuKnow, also known as the Cylex Immune Cell Function Assay, detects CMI by measuring the concentration of ATP from CD4 cells following stimulation. This measurement is made on heparin anticoagulated whole blood using a luminometer and luciferin/luciferase. The assay is used for the detection of cell-mediated immunity in an immunosuppressed population. The patented Cylex technology combines cell stimulation, cell selection, and quantification of metabolic markers (e.g. ATP) to measure cell-mediated immunity. The assay utilizing these principles was first described to assess T cell response to bacterial antigens in mice following immunization. ImmuKnow measures the early response to stimulation by detecting intracellular ATP synthesis in CD4 cells selected from blood by monoclonal antibody coated magnetic beads. The amount of ATP present in stimulated blood specimens is
a measure of lymphocyte activity. Because the CD4 lymphocytes orchestrate CMI responses through immunoregulatory signaling, the measurement of CD4 activation reflects the degree of immune cell function. As response to immunosuppressive therapy varies among individuals, assessment of a patient's immune cell function may provide useful information to the clinician in the course of individual patient management.

Kowalski et al (2007) performed a meta-analysis of 504 solid organ transplant recipients (heart, kidney, kidney-pancreas, liver and small bowel) from 10 U.S. centers on the Cylex ImmuKnow assay. In this analysis, 39 biopsy-proven cellular rejections and 66 diagnosed infections occurred. They found that a recipient with an immune response value of 25 ng/ml adenosine triphosphate (ATP) was 12 times more likely to develop an infection than a recipient with a stronger immune response. Similarly, a recipient with an immune response of 700 ng/ml ATP was 30 times more likely to develop a cellular rejection than a recipient with a lower immune response value. Of note is the intersection of odds ratio curves for infection and rejection in the moderate immune response zone (280 ng/ml ATP). This intersection of risk curves provides an immunological target of immune function for solid organ recipients. These data show that the Cylex ImmuKnow assay has a high negative predictive value and provides a target immunological response zone for minimizing risk and managing patients to stability.

Cadillo-Chávez et al (2006) examined the records of all patients who received a kidney transplant in their program between August 2004 and January 2005. Of 64 patients, 58 had pretransplant and posttransplant ATP level determinations. They searched for associations between ATP levels and immunosuppression type, doses, and levels; creatinine levels; white blood cell count; tissue typing; preformed antibodies; as well as ATP levels on infection and rejection, and changes in ATP levels with time. They found that there was no relation between ATP levels and immunosuppression type, doses, or levels; creatinine levels; white blood cell counts; HLA; and panel-reactive antibody. However, patients with moderate or high pretransplant ATP levels had more rejection episodes (8/10) while patients with ATP levels in the low immune response had more infections (6/11). The mean ATP levels for rejection was 423.3 ng/mL versus 268.45 ng/mL for infection and 277.15 ng/mL for no events. Although acute rejections occurred mostly above 300, this was not significant. Infections were more frequent with ATP under 300 and severe infection (endocarditis, meningitis, peritoneal abscesses, pneumonia, etc) were more frequent under 200. Comparing pretransplant with posttransplant values at the second week an increase correlated with rejection, while a decrease did not correlate with the infection. Patients who received antirejection treatment had a decrease in their ATP levels at 5 days. They thought that this ATP release assays is helpful in determining the risk of developing infection or rejection, as well as follow-up in the response to therapy.

Visner and Goldfarb (2007) in their review of current trends in pediatric lung posttransplant management, reveals pitfalls that exist, and introduces additional parameters that may have an impact on long-term survival. A number of parameters are monitored after transplantation to prevent or identify early complications related to lung transplantation in hope of reducing morbidity and mortality. These include routine laboratory studies, imaging, and monitoring of drug levels and lung function. Drug monitoring allows individualization of a patient's immunosuppressive therapy; however, drug levels alone may not reflect the patient's immune status. Two major complications are rejection and infection, and bronchoscopy is used to differentiate these two entities. Silent rejection may occur and increase the chance of developing bronchiolitis obliterans; therefore, many centers perform surveillance bronchoscopies. Recently, de-novo anti-histocompatibility locus antigen antibodies and ImmuKnow (Transplantation Immune Cell Function Assay) Dec 15
gastroesophageal reflux have been associated with poor outcomes, and many centers are monitoring these entities as part of care following lung transplantation. Despite these monitoring methods, there has been little improvement in long-term outcomes of lung transplantation. Current monitoring methods are utilized to maintain or improve outcomes and recently additional monitoring parameters have been identified which hopefully will improve long-term outcomes. ImmuKnow is mentioned as a general immune-monitoring assay that may help guide therapy.

Thai et al (2006) used ImmuKnow to compare pancreas recipient clinical states (stable, rejection, infection) with T cell responses. Blood samples were taken from pancreas recipients pretransplant and at approximately three-month intervals posttransplant for analysis of T cell responses. When possible, T cell responses were also quantified during changes in clinical status (infection or rejection). A range between 100-300 ng/ml adenosine triphosphate (ATP) was found in stable patients with good graft function and no infection or rejection. A low T cell response was highly correlated with infectious states. The fourteen patients with infections/posttransplant lymphoproliferative disease had a mean ATP of 48 ng/ml. Risk hazard analysis showed that patients with ATP levels <100 ng/ml were four to seven times more susceptible to infection compared to stable patients. Four patients with rejection showed a T cell response of 550 ng/ml ATP, which was statistically significant compared to stable patients, although the sampling numbers (9) were too small to be conclusive. The authors came to the conclusion that the Cylex ImmuKnow assay is a valuable tool to more precisely modulate immunosuppression in pancreas transplant patients. In particular, the assay is extremely useful in detecting overly immunosuppressed patients vulnerable to infections.

Gautam et al (2006) assessed cell mediated immunity (CMI) by the ImmuKnow assay in 12 patients after kidney transplantation, who presented with viral infection. Treatment included lowering of immunosuppression in all cases and antiviral treatment if indicated. The assay was repeated during the follow up. The ImmuKnow assay at time of presentation of viral infections had a median value OF 22 ATP ng/ml. With the clearance of viral infection and lowering of immunosuppression, the assay showed an increase in the level of CMI to a median of 150 ATP ng/ml. There was viral clearance or stabilization in all cases and there was no incidence of allograft rejection. They surmised that the ImmuKnow assay of CMI can be used to titrate initial immunosuppression reduction and its subsequent increase in patients with viral infection after transplantation.

Hooper et al (2005), acknowledging that the in vitro assay, the Cylex ImmuKnow ATP assay provides a global assessment of cellular immune function to help monitor the immune status of immunosuppressed patients by measuring the degree of activation of CD4 T cells, also reveals that the normal values for this assay were developed with healthy adult patients. In their study, they determined the normal ranges for the ImmuKnow assay in healthy children and compared those values to levels obtained in healthy adults and in stable pediatric renal transplant patients. They found that healthy children 12 yr of age and older showed immune function levels indistinguishable from adults, while healthy children under 12 had significantly lower immune function levels than adults. For adults, the ImmuKnow assay zones in ng/mL ATP of strong, moderate and low immune function correspond to >525, 225 to 525, and <225, respectively. In children under 12, they found the corresponding zones to be >395, 175-395 and <175 ng/mL, respectively. The median value for normal adults was 415, whereas it was only 295 for children <12 yr of age and this value decreases to 165 in stable renal transplant patients <12 yr of age (compared with 258 for stable adult renal transplant patients). Thus, this study provided critical information necessary to utilize the ImmuKnow assay with pediatric patients. In adults, the degree of immune function as assessed by the ImmuKnow assay helps to ImmuKnow (Transplantation Immune Cell Function Assay) Dec 15
predict patients at risk for infection or rejection. If further studies in pediatric patients document the same and is true for children, then the ImmuKnow assay will provide a useful adjunct tool to prevent over- or under-immunosuppression as newly developed drugs are utilized or drug treatment is altered because of drug side effects, toxicity, concurrent illnesses or rejection.

Although recent studies using ImmuKnow have suggested that this test may provide useful information to the clinician in the course of individual management of transplant patients on immunosuppression therapy as regards early rejection and risk for infection, additional clinical use data are required to conclusively establish the predictive capacity of ImmuKnow as an instrument of monitoring for rejection, risk of infection and regulation of the dosage of immunosuppression drugs. To date, there have been no randomized studies directly comparing ImmuKnow versus established monitoring tests as regards patient outcomes. It must be proven that ImmuKnow provides equivalent or better health outcomes and quality of life, in addition to providing the potential to fine-tune immunosuppressive medication.

Review History
March 2008 Medical Advisory Council initial approval
March 2009 Update. No revisions. Codes reviewed
March 2010 Update – no revisions to policy statement. Added new CPT code for ImmuKnow, 86352
March 2011 Update – no revisions
December 2011 Update – no revisions
December 2012 Update – no revisions
December 2013 Update – no revisions
December 2015 Update - no revisions. Codes Updated.

References – Update December 2015

References – Update December 2014


References – Update December 2013


References – Update December 2012


5. Schulz-Juergensen S, Burdelski MM, Oellerich M, Brandhorst G. Intracellular ATP production in CD4+ T cells as a predictor for infection and allograft rejection in
trough-level guided pediatric liver transplant recipients under calcineurin-inhibitor therapy. Ther Drug Monit. 2012 Feb;34(1):4-10.


References – Update December 2011


References – Update March 2011


References – Update March 2010


References – Update March 2009

References – Initial

**Important Notice**

**General Purpose.**

Health Net's National Medical Policies (the "Policies") are developed to assist Health Net in administering plan benefits and determining whether a particular procedure, drug, service or supply is medically necessary. The Policies are based upon a review of the available clinical information including clinical outcome studies in the peer-reviewed published medical literature, regulatory status of the drug or device, evidence-based guidelines of governmental bodies, and evidence-based guidelines and positions of select national health professional organizations. Coverage determinations are made on a case-by-case basis and are subject to all of the terms, conditions, limitations, and exclusions of the member's contract, including medical necessity requirements. Health Net may use the Policies to determine whether under the facts and circumstances of a particular case, the proposed procedure, drug, service or supply is medically necessary. The conclusion that a procedure, drug, service or supply is medically necessary does not constitute coverage. The member's contract defines which procedure, drug, service or supply is covered, excluded, limited, or subject to dollar caps. The policy provides for clearly written, reasonable and current criteria that have been approved by Health Net's National Medical Advisory Council (MAC). The clinical criteria and medical policies provide guidelines for determining the medical necessity criteria for specific procedures, equipment, and services. In order to be eligible, all services must be medically necessary and otherwise defined in the member's benefits contract as described this "Important Notice" disclaimer. In all cases, final benefit determinations are based on the applicable contract language. To the extent there are any conflicts between medical policy guidelines and applicable contract language, the contract language prevails. Medical policy is not intended to override the policy that defines the member's benefits, nor is it intended to dictate to providers how to practice medicine.

**Policy Effective Date and Defined Terms.**

The date of posting is not the effective date of the Policy. The Policy is effective as of the date determined by Health Net. All policies are subject to applicable legal and regulatory mandates and requirements for prior notification. If there is a discrepancy between the policy effective date and legal mandates and regulatory requirements, the requirements of law and regulation shall govern. * In some states, prior notice or posting on the website is required before a policy is deemed effective. For information regarding the effective dates of Policies, contact your provider representative. The Policies do not include definitions. All terms are defined by Health Net. For information regarding the definitions of terms used in the Policies, contact your provider representative.

**Policy Amendment without Notice.**

Health Net reserves the right to amend the Policies without notice to providers or Members. In some states, prior notice or website posting is required before an amendment is deemed effective.
**No Medical Advice.**
The Policies do not constitute medical advice. Health Net does not provide or recommend treatment to members. Members should consult with their treating physician in connection with diagnosis and treatment decisions.

**No Authorization or Guarantee of Coverage.**
The Policies do not constitute authorization or guarantee of coverage of particular procedure, drug, service or supply. Members and providers should refer to the Member contract to determine if exclusions, limitations, and dollar caps apply to a particular procedure, drug, service or supply.

**Policy Limitation: Member’s Contract Controls Coverage Determinations.**
Statutory Notice to Members: The materials provided to you are guidelines used by this plan to authorize, modify, or deny care for persons with similar illnesses or conditions. Specific care and treatment may vary depending on individual need and the benefits covered under your contract. The determination of coverage for a particular procedure, drug, service or supply is not based upon the Policies, but rather is subject to the facts of the individual clinical case, terms and conditions of the member’s contract, and requirements of applicable laws and regulations. The contract language contains specific terms and conditions, including pre-existing conditions, limitations, exclusions, benefit maximums, eligibility, and other relevant terms and conditions of coverage. In the event the Member’s contract (also known as the benefit contract, coverage document, or evidence of coverage) conflicts with the Policies, the Member’s contract shall govern. The Policies do not replace or amend the Member’s contract.

**Policy Limitation: Legal and Regulatory Mandates and Requirements**
The determinations of coverage for a particular procedure, drug, service or supply is subject to applicable legal and regulatory mandates and requirements. If there is a discrepancy between the Policies and legal mandates and regulatory requirements, the requirements of law and regulation shall govern.

**Reconstructive Surgery**
CA Health and Safety Code 1367.63 requires health care service plans to cover reconstructive surgery. "Reconstructive surgery" means surgery performed to correct or repair abnormal structures of the body caused by congenital defects, developmental abnormalities, trauma, infection, tumors, or disease to do either of the following:

1. To improve function or
2. To create a normal appearance, to the extent possible.

Reconstructive surgery does not mean "cosmetic surgery," which is surgery performed to alter or reshape normal structures of the body in order to improve appearance.

Requests for reconstructive surgery may be denied, if the proposed procedure offers only a minimal improvement in the appearance of the enrollee, in accordance with the standard of care as practiced by physicians specializing in reconstructive surgery.

**Reconstructive Surgery after Mastectomy**
California Health and Safety Code 1367.6 requires treatment for breast cancer to cover prosthetic devices or reconstructive surgery to restore and achieve symmetry for the patient incident to a mastectomy. Coverage for prosthetic devices and reconstructive surgery shall be subject to the co-payment, or deductible and coinsurance conditions, that are applicable to the mastectomy and all other terms and conditions applicable to other benefits. "Mastectomy" means the removal of all or part of the breast for medically necessary reasons, as determined by a licensed physician and surgeon.

**Policy Limitations: Medicare and Medicaid**
Policies specifically developed to assist Health Net in administering Medicare or Medicaid plan benefits and determining coverage for a particular procedure, drug, service or supply for Medicare or Medicaid members shall not be construed to apply to any other Health Net plans and members. The Policies shall not be interpreted to limit the benefits afforded Medicare and Medicaid members by law and regulation.