Published in final edited form as:

Clin Transplant. 2011 ; 25(1): E32–E37. doi:10.1111/j.1399-0012.2010.01303.x.

Immune functional assay for immunosuppressive management in posttransplant malignancy

Tadahiro Uemura^a, Thomas R. Riley^b, Akhtar Khan^a, Christopher Hollenbeak^{c,d}, Ian Schreiber^b, Nasrollah Ghahramani^e, Brian Reeves^e, Ronald E. Domen^f, Dani S. Zander^f, and Zakiyah Kadry^a

^aDivision of Transplantation, Department of Surgery, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

^bDivision of Gastroenterology, Department of Internal Medicine, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

^cDepartment of Public Health Sciences, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

^dDivision of Outcomes Research and Quality, Department of Surgery, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

^eDivision of Nephrology, Department of Internal Medicine, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

^fDepartment of Pathology, Penn State Milton S. Hershey Medical Center, College of Medicine, Penn State University, Hershey, PA, USA

Abstract

Immunosuppression management in post-transplant malignancy is challenging because of a lack of objective immunologic assessment tools. The ImmuKnow assay measures the ATP level from CD4 T cells, quantifying cell-mediated immunity and providing an insight into the immune status of transplant recipients. Its potential use in patients with post-transplant *de novo* malignancy was evaluated. Thirteen adult transplant patients with *de novo* malignancy were divided into survivors ($n = 9$) and non-survivors ($n = 4$) after malignancy treatment. Tacrolimus and the ImmuKnow levels were monitored before, during, and after malignancy treatment. The ImmuKnow level in non-survivors group was significantly lower before and after malignancy treatment compared to survivors group ($p = 0.013$ and 0.0014 respectively). In survivor group, the ImmuKnow level was significantly decreased during malignancy treatment ($p = 0.019$) but recovered to the initial level after the treatment. However, in non-survivor group, the ImmuKnow level remained suppressed throughout the observed period despite a reduction in immunosuppressive drug levels. The ImmuKnow assay can be an objective means evaluating immune status of patients with *de novo* malignancy. The ImmuKnow assay can express the degree of immune suppression induced by chemotherapeutic or radiation therapy and may be a useful tool in optimizing the timing of re-introduction of immunosuppression after malignancy treatment.

© 2010 John Wiley & Sons A/S.

Corresponding author: Zakiyah Kadry, MD, Division of Transplantation MCH062, Department of Surgery, Penn State Milton S. Hershey Medical Center, PO Box 850, 500 University Drive, Hershey, PA 17033, USA. Tel.: +717 531 5921; fax: +717 531 5851; zkadry@psu.edu.

Conflict of interest: None.

Keywords

Cylex ImmuKnow assay; *de novo* malignancy; immunosuppression

Post-transplant malignancy is a critical issue after organ transplantation, and the incidence of malignancy in transplant patients is significantly higher than that of age-matched controls (1, 2). Reduction in immunosuppression is considered to be an important mainstay of therapy in patients who have developed *de novo* malignancy (3, 4). However, the timing of resumption and dosage of immunosuppression after treatment of post-transplant malignancy has been a difficult challenge, with the risk of development of late rejection and ultimate poor long-term graft function if re-initiation of immunosuppression is delayed too long (5, 6).

Immunosuppressant drug monitoring has traditionally been the mainstay of immunologic monitoring, in conjunction with the patient's clinical status and graft function, but it does not take into account the high degree of patient variation in the metabolism of the immunosuppressant medication nor does it accurately reflect the synergistic effect of combination immunosuppressant therapy. Pharmacokinetic monitoring has been recognized as being more effective in preventing drug toxicity and as being less effective in predicting functional efficacy. Thus, single trough levels of immunosuppressive agents do not reflect the overall degree of immunosuppression in a patient. In fact, until recently, no clinical tool has been available for the direct assessment of the net status of a patient's immune function. However, in 2002, the FDA approved the Immune Cell Function Assay (Cylex ImmuKnow assay) to quantify cell-mediated immunity by measuring the concentration of ATP from CD4 T cells. The Cylex ImmuKnow assay has been reported to be clinically useful in assessing the relative risk of infection and rejection (7). This assay has the potential to serve as an index of the immunologic status of patients in a variety of clinical scenarios, which include transplant recipients or any patient suffering from an immune deficiency. We recently introduced the Cylex ImmuKnow assay at our institution and utilized it on decisions involving immunosuppressive management. This is the first report of immunosuppressive management with the Cylex ImmuKnow assay in the setting of post-transplant *de novo* malignancy.

Materials and methods

Subjects

Demographic, diagnostic, and treatment-related data were obtained from a prospectively maintained database of liver, kidney, and pancreas transplant recipients at the Penn State Milton S. Hershey Medical center. Adult patients (>18 yr old) were included in this study. Thirteen patients with pathologically established *de novo* malignancies arising after transplantation were identified as the Cylex ImmuKnow assay was introduced in our institution in December 2005. The patient demographics are shown in Table 1. The patients were divided into two groups. Group A consisted of nine patients who are currently alive after treatment of malignancy (n = 9), and Group B consisted of four patients who did not survive after treatment of malignancy (n = 4).

Laboratory evaluation

Blood for the Cylex ImmuKnow testing was collected during outpatient or inpatient visits at the same time as the calcineurin trough level was drawn prior to the administration of immunosuppressive medication. Briefly, the Cylex ImmuKnow assay (ImmuKnow™, Cylex Inc.®, Columbia, MD, USA) was undertaken in our core laboratory according to the manufacturer's instructions (8). Patient samples were tested within 30 h of collection.

Cylex ImmuKnow and tacrolimus level

The Cylex ImmuKnow level was monitored weekly or biweekly. The Cylex ImmuKnow level and tacrolimus level were compared before, during, and after treatment of malignancy in Group A (survivors) and B (non-survivors). The Cylex ImmuKnow level before treatment of malignancy represents the most recent level before the diagnosis of malignancy. The Cylex ImmuKnow level after treatment of malignancy represents the level at three months of completion of malignancy treatment.

Management of immunosuppression

Maintenance immunosuppression was composed of tacrolimus and mycophenolate mofetil. Prednisone was given postoperatively and tapered off in the first three to six months. Immunosuppression was significantly reduced or discontinued after diagnosis of malignancy.

Treatment of malignancy

In addition to reduction or discontinuation of immunosuppression, surgical resection was performed if clinically indicated. Rituximab was used for CD20-positive post-transplant lympho-proliferative disorder (PTLD), and chemotherapy was used for high histological grade of PTLD.

Statistical analysis

For descriptive statistics, the Cylex ImmuKnow assay (ATP) levels and tacrolimus levels were expressed as mean $\pm$ standard deviation. Undetectable level of tacrolimus is considered as 0 ng/mL. Comparisons between continuous variables were made using Student's *t*-test, and comparisons between categorical variables were made using a chi-square test.

Results

Fig. 1 shows the Cylex ImmuKnow level before and after malignancy treatment in two groups. Group B (non-survivors) showed a significant lower level of the Cylex ImmuKnow assay before and after the treatment compared to Group A (survivors) ($p = 0.013$ and 0.0014 , respectively). Fig. 2 shows the Cylex ImmuKnow and tacrolimus levels in Group A (survivors). The Cylex ImmuKnow level prior to malignancy therapy was 280 ± 51 ng/mL ATP and it significantly decreased at 124 ± 27 ng/mL ATP upon malignancy treatment ($p = 0.019$) (Fig. 2A), reflecting a significant immune depression in spite of the concomitant significant reduction in the mean tacrolimus level ($p = 0.028$) (Fig. 2B). This decrease in Cylex ImmuKnow level was a paradoxical change in the setting of reduced tacrolimus levels. The Cylex ImmuKnow level significantly increased after oncologic therapy ($p = 0.007$) (Fig. 2A) in spite of increased tacrolimus levels ($p = 0.037$) (Fig. 2B). In Group A, no acute or chronic rejection occurred during and after malignancy treatment. All grafts are currently functional. No cytomegalovirus infection was experienced, but two cases of bacterial infections (bacteremia and urinary tract infection) occurred (Table 1). All patients except one are currently in remission from their malignancies.

In the non-survivor group B, the Cylex ImmuKnow level before malignancy treatment was 113 ± 19 ng/mL ATP and it remained low during and after malignancy treatment (Fig. 3A) in spite of the low tacrolimus level after treatment (Fig. 3B), indicating continued significant immune depression in this group of patients. In Group B, no acute or chronic rejection occurred during and after malignancy treatment. Three patients died from malignancy progression, while one patient died from pneumonia and sepsis (Table 1).

Fig. 4 shows a case study to illustrate the dynamics involved. The patient was a 49-yr-old liver transplant recipient who received chemotherapy for a squamous cell carcinoma of the tongue. As shown in Fig. 4, the Cylex ImmuKnow level was markedly decreased after chemotherapy despite the decrease in tacrolimus dosage and level. No rejection occurred with reduction in immunosuppression. After completion of chemotherapy, the Cylex ImmuKnow level gradually increased to its previous level over a period of two months although the same dose of tacrolimus was maintained. The tacrolimus dose was then increased to 1.5 mg twice daily resulting in a slight decrease in the ImmunoKnow level. The patient has shown no signs of cancer recurrence on outpatient follow-up.

Discussion

This is the first study showing immune monitoring with the Cylex ImmuKnow assay in immunosuppressed solid organ transplant recipients with *de novo* malignancies. The Cylex ImmuKnow assay was recently designed to allow measurement of the net state of immune function in an immunosuppressed population (7). Clinical validity of this assay as an objective tool for assessing immune function has been validated in previous reports looking at its effectiveness in tailoring immunosuppression to optimize the treatment of rejection and infections as well as in immunosuppression-weaning protocols (7, 9–12). It has been reported that other immunological assays were useful for monitoring several malignancies. CD3 response *in vitro* assay has been published as the single greatest predictor of reduced disease-free survival in head and neck cancer (13). Interleukin 2 production in lymphocyte culture has been reported to correlate with tumor recurrence or severity in cervical disease (14, 15). However, it is unclear how useful these assays are monitoring malignancy in the setting of immunosuppressed transplant patients. On the other hand, the Cylex ImmunoKnow assay has been established as a useful assay in transplant patients and has been published as a tool for the management of immunosuppressive therapy in transplant recipients (7, 16, 17). Our data show that it may also be a useful and important assay in the setting of immunosuppression and cancer therapy. The Cylex ImmuKnow assay is a routine blood test, whereas CD3 *in vitro* assay nor Interleukin 2 production in lymphocyte culture are not routinely available in a laboratory.

Results from this study add to what has been previously published by documenting the substantial additive immune suppression introduced by chemotherapeutic agents and radiation therapy, the variability of the patient immune status in such situations, and the relatively small impact of reduction in immunosuppressant drug levels on the degree of immunosuppression. The data also suggest that the Cylex ImmuKnow assay can be used to measure the immunologic outcome of therapeutic interventions, a key step to the creation of an optimized drug regimen. Until recently, transplant recipients suffering from *de novo* malignancy were treated by either a drastic reduction or complete temporary discontinuation of immunosuppressant therapy. Decisions on the timing of reinstitution of immunosuppressant regimens once tumor control was achieved have been difficult and have resulted on occasion either in the loss of the transplanted allograft or in the development of rejection (5). The decision to re-institute immunosuppressants post-malignancy treatment is frequently left to the individual physician's experience. However, in this study, we have shown that the Cylex ImmuKnow assay can be used as an objective tool to monitor a patient's overall immune level thus improving the decision-making behind the timing of re-institution of immunosuppression.

The pharmacokinetic monitoring of immunosuppressive agents does not automatically correlate with immunosuppressive effect. The net patient immune status level was dramatically impacted during the treatment of malignancy, resulting in greater variability in the immune function of these patients (Fig. 2A) despite the overall reduction in

immunosuppressant drug levels (Fig. 2B). This was further corroborated in the case study shown in Fig. 4 where pharmacokinetic monitoring of immunosuppressant levels did not correlate with the net degree of patient immune activity as reflected by the Cylex ImmuKnow level.

In most centers, current clinical practice of immunosuppressant dosing is protocol driven with pharmacokinetic monitoring of the serum level of immunosuppressant drugs. The failure to measure the net immune level may be associated with excessive immunosuppression in the presence of stable graft function. This has been recognized as a risk factor for the development of *de novo* malignancy after transplantation (6, 18). The Cylex ImmuKnow assay may allow a more preventive role in the future by further reducing immunosuppression through the monitoring of the overall net immune status with concomitant clinical correlation of graft function. This in turn may minimize the risk of developing *de novo* malignancy after transplantation by allowing us to administer the minimum effective immunosuppressive dosage at an earlier stage post-transplantation. Follow-up with future prospective trials using the Cylex ImmuKnow assay in larger patient cohorts may provide further data to that effect.

In this study, the mean Cylex ImmuKnow level before malignancy treatment was maintained at ~300 ng/mL ATP in patients who survived after the treatment (Fig. 1). However, the Cylex ImmuKnow level was significantly lower at ~100 ng/mL in patients who did not survive after the treatment (Fig. 1). The Cylex ImmuKnow assay may provide insight into the prognosis with malignancy treatment. The Cylex ImmuKnow level in non-survivor patients after malignancy treatment was at a significantly lower value (Fig. 1), and one patient died because of sepsis after malignancy treatment in group B. These results suggest that immunosuppression could have been further reduced or even safely discontinued during treatment for malignancy until the immune-level monitoring showed restoration to the baseline immune levels, at which point immunosuppressive therapy could have been either increased or resumed.

In summary, the utilization of the Cylex ImmuKnow Assay in patients with *de novo* malignancy facilitates the management of immunosuppression by providing an accurate evaluation of the patient's immune status in addition to immunosuppressive drug monitoring. It allows for the safe manipulation of immunosuppression in the setting of *de novo* malignancy after transplantation by reducing the risk of under- or over-immunosuppression.

References

1. Feng S, Buell JF, Chari RS, DiMaio JM, Hanto DW. Tumors and transplantation: the 2003 Third Annual ASTS State-of-the-Art Winter Symposium. *Am J Transplant.* 2003; 3:1481. [PubMed: 14629278]
2. Miao Y, Everly JJ, Gross TG, et al. De novo cancers arising in organ transplant recipients are associated with adverse outcomes compared with the general population. *Transplantation.* 2009; 87:1347. [PubMed: 19424035]
3. Danovitch, G. *Handbook of Kidney Transplantation.* 4. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 240
4. Busuttil, RW.; Klintmalm, GB. *Transplantation of the Liver.* 2. Philadelphia: Elsevier Saunders; 2005. p. 1151
5. Uemura T, Ikegami T, Sanchez EQ, et al. Late acute rejection after liver transplantation impacts patient survival. *Clin Transplant.* 2008; 22:316. [PubMed: 18190550]
6. Grimm P. Use of an immune function assay to monitor immunosuppression. *Pediatr Transplant.* 2006; 10:533. [PubMed: 16856988]

7. Kowalski RJ, Post DR, Mannon RB, et al. Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. *Transplantation*. 2006; 82:663. [PubMed: 16969290]
8. Kowalski R, Post D, Schneider MC, et al. Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. *Clin Transplant*. 2003; 17:77. [PubMed: 12709071]
9. Zeevi A, Britz JA, Bentlejewski CA, et al. Monitoring immune function during tacrolimus tapering in small bowel transplant recipients. *Transpl Immunol*. 2005; 15:17. [PubMed: 16223669]
10. Cadillo-Chavez R, de Echegaray S, Santiago-Delpin EA, et al. Assessing the risk of infection and rejection in Hispanic renal transplant recipients by means of an adenosine triphosphate release assay. *Transplant Proc*. 2006; 38:918. [PubMed: 16647510]
11. Gautam A, Fischer SA, Yango AF, Gohh RY, Morrissey PE, Monaco AP. Cell mediated immunity (CMI) and post transplant viral infections—role of a functional immune assay to titrate immunosuppression. *Int Immunopharmacol*. 2006; 6:2023. [PubMed: 17161357]
12. Gautam A, Morrissey PE, Brem AS, et al. Use of an immune function assay to monitor immunosuppression for treatment of post-transplant lymphoproliferative disorder. *Pediatr Transplant*. 2006; 10:613. [PubMed: 16856999]
13. Shibuya TY, Nugyen N, McLaren CE, et al. Clinical significance of poor CD3 response in head and neck cancer. *Clin Cancer Res*. 2002; 8:745. [PubMed: 11895904]
14. Radhakrishna Pillai M, Balaram P, Bindu S, Hareendran NK, Padmanabhan TK, Nair MK. Interleukin 2 production in lymphocyte cultures: a rapid test for cancer-associated immunodeficiency in malignant cervical neoplasia. *Cancer Lett*. 1989; 47:205. [PubMed: 2699725]
15. Tsukui T, Hildesheim A, Schiffman MH, et al. Interleukin 2 production in vitro by peripheral lymphocytes in response to human papillomavirus-derived peptides: correlation with cervical pathology. *Cancer Res*. 1996; 56:3967. [PubMed: 8752165]
16. Hashimoto K, Miller C, Hirose K, et al. Measurement of CD4+ T-cell function in predicting allograft rejection and recurrent hepatitis C after liver transplantation. *Clin Transplant*. 2009 Dec 28. [Epub ahead of print].
17. Husain S, Raza K, Pilewski JM, et al. Experience with immune monitoring in lung transplant recipients: correlation of low immune function with infection. *Transplantation*. 2009; 87:1852. [PubMed: 19543064]
18. Dantal J, Hourmant M, Cantarovich D, et al. Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. *Lancet*. 1998; 351:623. [PubMed: 9500317]

Fig. 1.
The Cylex ImmuKnow level before and after malignancy treatment between the survivors (Group A) and non-survivors (Group B). The non survivor group showed a significantly decreased the Cylex ImmuKnow level before and after malignancy treatment.

Fig. 2.
 (A) The Cylex ImmuKnow level in survivor patients (Group A). The Cylex ImmuKnow levels are shown before, during and after completion of malignancy treatment. (B) Tacrolimus level in patients who survived after malignancy treatment (Group A).

Fig. 3. (A) The Cylex ImmuKnow level in patients who did not survive after malignancy treatment (Group B) before, during and after completion of malignancy treatment. (B) Tacrolimus level in patients who did not survive after malignancy treatment (Group B).

Fig. 4.

The change in the Cylex ImmuKnow and tacrolimus levels during treatment for *de novo* malignancy in one patient.

Table 1The demographic of patients with *de novo* malignancies after transplantation

	Survivors (Group A, n = 9)	Non-survivors (Group B, n = 4)
Median age (yr)	57 (22–69)	55 (42–74)
Gender (M/F)	4/5	3/1
Type of TXP	KTx/LTx (4/5)	KTx/LKTx (3/1)
Type of malignancy	4 PTLT, 1 colorectal ca., 1 RCC, 1 oral/larynx ca., 1 skin ca., 1 lung ca.	2 PTLT, 1 colorectal ca., 1 RCC
Median F/U malignancy	2.6 yr (0.5–3.0 yr)	0.9 yr (0.4–1.9 yr)
Median time between TXP and malignancy	7.1 yr (1.0–16.8 yr)	4.8 yr (0.6–13.4 yr)
ACR during treatment	0/9	0/4
Chr. rejection	0/9	0/4
CMV infection	0/9	0/4
Bacterial infection	2/9	1/4

PTLT, post-transplant lymphoproliferative disorder; RCC, renal cell carcinoma; TXP, transplantation; LTx, liver transplant; KTx, kidney transplant; LKTx, liver and kidney transplant.