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Profile of the Pleximmune blood test for transplant rejection risk prediction

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Summary

The Pleximmune™ test (Plexision Inc., Pittsburgh, PA, USA) is the first cell-based test approved by the US FDA, which predicts acute cellular rejection in children with liver- or intestine transplantation. The test addresses an unmet need to improve management of immunosuppression, which incurs greater risks of opportunistic infections and Epstein–Barr virus-induced malignancy during childhood. High-dose immunosuppression and recurrent rejection after intestine transplantation also result in a 5-year graft loss rate of up to 50%. Such outcomes seem increasingly unacceptable because children can experience rejection-free survival with reduced immunosuppression. Pleximmune test sensitivity and specificity for predicting acute cellular rejection is 84% and 81% respectively in training set–validation set testing of 214 children. Among existing gold standards, the biopsy detects but cannot predict rejection. Anti-donor antibodies, which presage antibody-mediated injury, reflect late-stage allosensitization as a downstream effect of engagement between recipient and donor cells. Therefore, durable graft and patient outcomes also require an accurate management of cellular immune responses in clinical practice.

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Keywords

Acute cellular rejection; cell-based assay; prognostic; Liver transplantation; intestine transplantation; children; risk of rejection; T-cytotoxic memory cells; CD154; flow cytometry; index-based

Introduction

Roughly 500 children receive liver transplantation (LTx) and <50 children receive intestine transplantation (ITx) in the United States each year [1]. Up to half of these children will experience acute cellular rejection over their lifetimes (Figure 1). Acute cellular rejection (ACR) is caused by cell-mediated cytotoxicity to the allograft independent of graft type [2]. Mediated predominantly by recipient T-cells, this inflammatory immune response can progress to graft loss if unchecked. Lifelong prevention is the mainstay of management. Potent drugs such as tacrolimus and mycophenolate mofetil have reduced the occurrence of refractory rejection episodes and all but eliminated systemic signs of inflammation such as fever, during acute rejection [3,4]. For this and several other reasons, invasive sampling of the allograft or a biopsy is the gold standard for diagnosis [5,6]. Elevated liver function tests or serum creatinine seen with rejection of liver and renal allografts can also be caused by non-specific viral infections or mechanical obstruction. Diarrhea with ITx rejection can also result from several other causes including infections. In this regard, rejection of heart, lung, and pancreas allografts is relatively silent on several fronts. The added immunosuppression required to control rejection can be associated with life-threatening infections and post-transplant lymphoma-like disorders (PTLD), and is also best undertaken after biopsy confirmation [3,7].

The resource-intensive biopsy procedure has drawbacks including cost and the possibility of bleeding and organ perforation. These drawbacks are not trivial because ‘for cause’ biopsies precipitated by evidence of organ dysfunction may reveal ACR in only half of the biopsied recipients [8]. This diagnostic yield and the risk: benefit ratio is lower with surveillance biopsies aimed at detecting ongoing ‘silent’ rejection, whose incidence is lower than rejection episodes which cause graft dysfunction. *Non-invasive prediction of rejection* can potentially reduce the incidence of ACR and attendant invasive procedures through better management of immunosuppression.

The donor-specificity of the cellular rejection response poses challenges and opportunities. Preventive immunosuppression is based on small molecule immunosuppressants, which exert non-specific antiproliferative effect (mycophenolate mofetil, rapamycin, azathioprine) or suppress cytokine production in donor-specific and non-specific T-cells (tacrolimus, cyclosporine) [9,10]. Among children, whose immune systems are still maturing, the side effects of non-specific immunosuppression include opportunistic viral infections with cytomegalovirus or Epstein-Barr virus in up to a fifth of all children who have received LTx or ITx [3,4]. The incidence of PTLD varies from roughly 2% in LTx recipients to over 10% after ITx, because of greater cumulative exposure to immunosuppression in the rejection-prone ITx allograft [3,4]. Non-immunological side effects have also included gradual loss of kidney function, neurotoxicity, hypertension and diabetes with tacrolimus and cyclosporine.

Biologics offer more options having evolved from polyclonal lymphocyte or T-lymphocyte depleting effects to targeted inhibition of receptor-mediated signaling via the IL-2 receptor, CD25, or the costimulatory T-cell receptor, CD28 [10–11]. These agents also induce significant perturbations, such as T-cell anergy to mitogen-stimulation after exposure to polyclonal antilymphocyte antibodies [12]. This effect has implications for the performance of non-specific cell-based predictors of acute cellular rejection. However, an enhanced donor-specific T-cell response can be demonstrated before transplantation in rejection-prone individuals [13–15]. Measuring rejection-risk before transplantation may overcome the limitations of measuring rejection-risk during the cellular reconstitution phase immediately after induction with lymphocyte ablative antibodies.

Market Overview and current approaches

Prior to the advent of potent immunosuppressants, monitoring of the host immune system consisted of establishing compatibility between the donor and recipient. The cross-match blood test and matching of HLA antigens between the donor and recipient have been used as lab developed tests for this purpose [16]. A positive cross-match contraindicated kidney transplantation, a practice which eliminated hyperacute rejection due to preformed anti-graft antibodies. In contrast, a zero HLA-mismatch, which augured rejection-free outcomes led to allocation of a renal allograft to its suited recipient as a matter of national policy. Since then, the cross-match blood test has evolved from detection of preformed anti-donor antibodies to higher resolution detection of anti-HLA antibodies to any number of polymorphic single antigens in the class I and class II major histocompatibility loci [17,18]. Coupled with intragraft deposition of complement fragments, these lab developed test systems have advanced the recognition of antibody-mediated rejection in the clinic.

The non-invasive detection and prediction of ACR, the most common type of rejection after transplantation, is evolving in concert with biotechnological innovations. Mitogen-stimulated production of adenosine triphosphate by T-helper cells, a measure of general immune responsiveness has been used clinically in recent years [19]. Enhanced general immune responsiveness has not been seen consistently with ACR. One can speculate that mitogen-stimulation may not be a perfect surrogate for alloantigen-stimulated events, or that mitogen-stimulated T-helper cells are rendered less informative when subjected to certain immunosuppressants [12]. Efforts are also underway to apply specific multi-gene algorithms, one of which detects heart transplant rejection, to predict rejection of several other organs [20]. No such approach has been implemented to predict ACR in children who receive LTx or ITx.

Pleximmune™

The Pleximmune™ blood test measures the inflammatory immune response of recipient T-cells to the donor in co-culture of lymphocytes from both sources [14,15,21]. Flow cytometry is used to measure the frequency of recipient T-cytotoxic memory cells (TcM), which express the inflammatory marker CD154, or CD40 ligand (CD154+TcM) in response to donor stimulation (Figure 2). These results are expressed as a fraction of CD154+TcM induced by a reference alloantigen in a parallel reaction. The reference alloantigen consists of human cells, which are non-identical to the recipient and donor at the HLA-A, -B, and -

DR loci. The resulting immunoreactivity index (IR) if >1 implies enhanced donor-specific alloreactivity relative to reference alloreactivity, and increased risk of rejection (Figure 2, upper panel). An IR less than 1 is seen among those at decreased risk of rejection (Figure 2, lower panel). In practice, an IR ≥ 1.1 in post-transplant samples predicts ACR and has been identified with logistic regression analysis.

These *general principles* are derived from the classical mixed lymphocyte co-culture. In these co-cultures, proliferative alloresponses were measured with incorporation of tritiated thymidine in 5–7-day culture, with rejection-prone recipients demonstrating enhanced donor-specific alloreactivity independent of immunosuppression [22,23]. Compared with proliferation, the stimulated expression of CD154 peaks in 16 hours in overnight culture, an incubation period suited to clinical reporting and decision-making [14]. This expression is measured with flow cytometry, which uses fluorochrome-labeled antibodies to specific markers in lieu of radioisotopes. The specific markers are CD8 for the T-cytotoxic cell (Tc), CD45RO or the memory marker, CD3 of the T-cell marker, and CD154 or CD40 ligand. A viability dye, 7-actinomycin-D, stains non-viable cells, which are excluded from the final assessment. Logistic regression analysis has been used to select CD154+TcM as the predictor of ACR with the best sensitivity and specificity. The other cell types considered in these analyses were CD154-expressing memory and naïve T-helper and naïve T-cytotoxic cells. This selection was performed independently in each of three types of recipients, pediatric recipients of the liver and intestine, and adult renal transplant recipients [15,21,24]. The emergence of alloantigen-specific CD154+TcM as predictors of ACR in these different organ systems has mechanistic support in large animal studies where the TcM emerged as the sole barrier to durable tolerance [25].

The test system also incorporates a practical solution to the limitations in accessing an indefinite supply of stimulator cells from cadaveric donors. ‘Surrogate’ donor stimulators can be obtained from the same bank of cryopreserved HLA-typed peripheral blood lymphocytes, which are used as reference allostimulators. ‘Surrogate’ donor stimulators must match the donor at a minimum of one HLA-DR antigen, and a total of two antigens distributed in any combination on the HLA-A, and HLA-B loci. Each of the three loci has two antigens, one from each parent. Sample requirements are a minimum of 3 ml, ideally 5 ml, collected in sodium heparin (green top) tubes. Samples should be shipped overnight at ambient temperature, to ensure arrival within 30 hours of phlebotomy at Plexision’s reference laboratory. Test results have not been compromised with this approach [24].

Disease-specificity and personalized output are inherent attributes of the Pleximmune™ test design. The co-culture of donor and recipient cells simulates the in vivo interaction between donor and transplant recipient. Compared with other T-cell subsets, the CD154+TcM subset predicts ACR with a high sensitivity and specificity and adds to disease-specificity. The output is personalized, because donor-induced CD154+TcM are expressed as a fraction of those induced by reference allostimulus in a parallel reaction. The result is an absolute number called the immunoreactivity index, which characterizes individuals on a common dynamic scale. This normalization approach also minimizes the effect of several confounders, for example simultaneous infections, or non-immunosuppressive drugs, which are expected to affect the donor and reference alloresponse in the same way.

Sensitivity and specificity clinical profile

Pre-clinical development—The test system was conceived during a prospective single-center protocol (NCT#01163578) aimed at identifying predictors of transplant outcomes. Blood samples were obtained from children <21 years with LTx or ITx, before transplantation, and between days 1–60, 61–199 and days 200 onwards. These test periods were named IR0, IR1, IR2, and IRx respectively.

In 58 children with LTx, predictive IR values were developed in a training subset, and predictions validated in the remaining children [14]. These studies demonstrated that ACR could be predicted with high sensitivity and specificity in the 60-day period after sampling if a threshold IR was reached or exceeded. Further, T-cytotoxic memory cells (TcM), which expressed CD154 after stimulation achieved the best sensitivity and specificity for prediction of ACR from among naïve and memory T-helper and T-cytotoxic cells in logistic regression analysis. Finally, in most children who went on to develop ACR during the first 60 post-transplant days, pre-transplant samples demonstrated IR values at or above the rejection threshold, attesting further to the predictive nature of this test system. The results of evaluating this test system in 32 ITx recipients were similar [21]. A validation cohort could not be assembled in this rare transplant population. On the strength of these data, the lack of a predicate device, and an unmet need, allospecific CD154+TcM were designated a Humanitarian Use Device (HUD#08-0206) by the FDA's Office for Orphan Products in 2009 [26].

The *pre-marketing evaluation* of this test system under the Humanitarian Device Exemption route requires that Pleximmune™ 1) addresses an unmet need and has no predicate for the intended use, 2) does not pose an unreasonable or significant risk of injury, and 3) demonstrates *probable benefit* which outweighs the risk of injury or illness related to its intended use [27].

Study Design

The three-phase pre-marketing evaluation consisted of a) Identifying a threshold IR value predictive of ACR, and its performance in training set samples. These samples were tested with research grade fluorochrome-labeled antibodies and the LSRII flow cytometer (BDBiosciences, San Jose, CA), b) Assay standardization and reproducibility testing per guidelines of the National Committee of Clinical Laboratory Standards [28]. This phase was performed with cGMP-synthesized versions of antibodies and the FDA-approved FACS-CANTO flow cytometer (BD Biosciences, San Jose, CA), and c) Evaluating performance of the standardized test in independent validation set samples using predictive IR thresholds established in the training set. Testing of training set and validation set samples was separated by a period during which the assay system was standardized. This work has been described elsewhere [29].

Statistical analyses

Single blood samples obtained from independent subjects were used to determine predictive thresholds. Separate thresholds for predicting rejection were defined for pre-transplant samples (IR0) when no immunosuppression is used, and post-transplant samples, which are

obtained when immunosuppression is being given. To enhance cohort size for this rare subject population, single samples obtained from independent subjects during the early (IR1) and late (IRx) post-transplant period were combined for this analysis. A small and inconsistent collection of samples during the 61–199 day period, when most patients return to referring institutions led to the exclusion of these samples from analysis. Subjects were termed rejectors or non-rejectors based on the presence of biopsy-proven ACR within the 60-day post-sampling period for every pre- and post-transplant sample, using established biopsy criteria [5,6]. When a biopsy could not be performed in rare instances of LTx recipients, elevated liver function tests and the absence of bile duct obstruction on ultrasound served as clinical confirmation of ACR.

Pre-transplant IR thresholds which predicted ACR within the first 60 days after transplantation were identified with logistic regression [15]. Post-transplant IR thresholds were identified 60-day post-sampling period. The logistic model included the covariates age, gender, race (Caucasian vs non-Caucasian), type of stimulator cell (actual donor or surrogate donor), organ transplant type (liver, intestine, combined liver-intestine or combined liver-kidney), tacrolimus whole blood concentrations, type of induction therapy rabbit antihuman thymocyte globulin (rATG, Genzyme), campath (alemtuzumab, Genzyme), or none, and time between transplantation and outcome. Test performance was summarized as sensitivity, specificity, positive and negative predictive values (PPV, NPV) with 95% confidence intervals, as well as area under the receiver-operating-characteristic curve (AUC).

Results

Two hundred and eighty samples from 214 children <21 years with LTx or ITx were collected at a single institution, the Children's Hospital of Pittsburgh after informed consent under protocol NCT#01163578. After excluding 36 samples for failed stimulation or inadequate cell counts, 147 samples were analyzed in the training set, and 97 samples were analyzed in the validation set.

Standardization and reproducibility testing confirmed that test outputs were below the preset mean % coefficient of variation (CV) limit of 20% under a variety of conditions [29]. The mean $\pm$ SD CV% was $6 \pm 3.1\%$ for 20 samples tested in duplicate in each of two runs on the same day, $8.2 \pm 4.8\%$ in three-instrument-three-operator same-day testing of 21 samples, $8.9 \pm 7\%$ in 20 samples tested before and 30 days after cryopreservation in liquid nitrogen, $4.8 \pm 3\%$ for 5 samples tested simultaneously by two operators, and $3.2 \pm 3\%$ for 5 samples tested before and after overnight storage in the reference lab at ambient temperature and after overnight shipment at ambient temperature.

Test performance

For each type of training set sample, pre- or post-transplant, two predictive models were identified. The first incorporated significant covariates and the IR of CD154+TcM, and the second consisted of the single variable, IR of CD154+TcM. For the single variable post-transplant or IR1+IRx model, the threshold IR value at or above which rejection was predicted was 1.10. The threshold IR value for the single variable pre-transplant or IR0 model was 1.23.

When applied to the validation set, the predictive training set models incorporating multiple variables demonstrated inferior performance. The single variable IR threshold for pre- and post-transplant samples established in the training set demonstrated consistent performance for predicting rejection in corresponding validation set samples (Table 2). Test sensitivity is 92% and 82% respectively in post-transplant training set samples and 84% and 81% respectively in corresponding validation set samples (Table 2a). Test sensitivity is 80% and 71% respectively in pre-transplant training set samples and 57% and 89% respectively in corresponding validation set samples (Table 2b). The lower sensitivity in pre-transplant samples may result from smaller numbers of rejectors in this cohort (n=14). Pre-transplant test sensitivity demonstrated overlapping 95% confidence intervals of 59–92% in training set and 30–81% in the validation set.

Clinical test performance during viral infections or non-viral inflammation

During the preclinical phase. The effect of common opportunistic viruses like cytomegalovirus and Epstein–Barr virus (EBV) on test performance could not be established during the preclinical phase. The single sample obtained during tissue-invasive EBV infection (enteritis) failed stimulation. Further, viral load detection methods changed repeatedly during this phase of testing, so that the effect of viremia on test performance also could not be assessed reliably. An evaluation of the first 63 children who have been tested with the Pleximmune™ assay clinically shows that test performance is not confounded by infection which was present at sampling [29]. Infections consisted of liver allograft cholangitis in one, adenoviral allograft enteritis in another, and EBV load of 10926 copies per ml (range 120–31000) by PCR in nine children. Test predictions concurred with absence of rejection in children with cholangitis and enteritis. When children with EBV viral load were compared with those without EBV load with polymerase chain reaction testing, no differences were seen in test sensitivity or specificity. CMV viremia was not reported or detected in this clinical cohort on the day of sampling.

Precautions

The Pleximmune™ test predicts acute cellular rejection and has not been tested for the prediction of antibody-mediated rejection. Also, the performance of Pleximmune™ during ischemia-perfusion injury and graft-versus-host disease is not known. Opportunistic infections seen in children with liver or small bowel transplantation do not appear to confound test performance in early clinical evaluation.

Regulatory Status

The test system was designated a Humanitarian Use Device in 2009 by the US-FDA's Office for Orphan Products. Following submission of training-set-validation-set and assay standardization data, Pleximmune was approved for the prediction of ACR after liver or intestine transplantation in children <21 years under the Humanitarian Device Exemption regulatory track of the FDA in August 2014 [30]. The test should be used as an adjunct to all available clinical information. Pleximmune™ is marketed as a service from Plexision's reference laboratory.

Conclusions

Children with liver or intestine transplantation are an orphan population whose immature immune systems are relatively naïve to common opportunistic viruses but can also accommodate the transplanted organ with greater ease. As a result, children can accept a transplanted organ with reduced immunosuppression over time, but are less able to resist EBV-induced malignant transformation of B-lymphocytes, which results in PTLTD. Therefore, managing lifelong immunosuppression to prevent acute cellular rejection and life-threatening side effects is essential in order to ensure durable long-term survival in an era of declining organ donation and shrinking healthcare resources.

The Pleximmune™ blood test is the only index-based cell-based blood test approved by the FDA, which predicts ACR in this unique high-risk transplant population. Test results should be used as an adjunct to available clinical information. Early clinical experience shows that test predictions are particularly useful in planning immunosuppression in the setting of indeterminate biopsy findings, or in modifying protocol-mandated treatment when combined with all other available clinical information about an individual patient. The personalized output of this test system, and the disease-specificity of the functional T-cell subset measured in this test yields performance metrics, which are comparable to other high-complexity multivariate algorithms in several therapeutic areas.

Expert commentary

Alloantigen-specific CD154+TcM measured in the Pleximmune™ blood test provide a personalized measure of donor-specific cellular alloreactivity, a universal mechanism of acute cellular rejection. Therefore, the test system can potentially serve as a surrogate for this event, and provide non-invasive detection or prediction of this event in other organ systems.

Surrogate endpoints can facilitate drug development in children, in whom clinical endpoints dictate unachievable sample sizes. The ability of CD154+TcM to fulfill surrogacy criteria described by Prentice and Fleming were evaluated in a simulation using outcomes data from 30 ITx recipients, 14 induced with the immunosuppressant alemtuzumab, and 16 induced with rabbit anti-human thymocyte globulin (rATG, Genzyme, Cambridge, MA) [11]. In this simulation, CD154+TcM correlated significantly with the incidence and severity of ACR with high sensitivity and specificity independent of either immunosuppressant. Further, low rejection-risk measured with CD154+TcM predicted a shorter time to minimization of the maintenance immunosuppressants, tacrolimus and steroids. A simulated trial was modeled using longitudinal IR data from these two treatment groups. In this simulated trial, lower incidence of ACR with alemtuzumab compared with rATG, 50% vs 69%, did not achieve statistical significance because of the small sample size. However, an IR value above the rejection-risk threshold and consistent with increased rejection-risk declined more rapidly with alemtuzumab (46 ± 20 vs. 158 ± 59 days, $p=0.009$, Kaplan-Meier analysis) compared with rATG, supporting the potentially greater efficacy of alemtuzumab for management of rejection. As a surrogate end-point, time-to-rejection-risk resolution measured with CD154+TcM also predicted a 50% reduction in sample sizes in a simulated comparison of alemtuzumab and rATG.

The potential of allospecific CD154+TcM to detect acute cellular rejection in other organ systems is illustrated by evaluation of 43 adult renal transplant recipients [24]. All subjects were sampled at the time of 'for cause' biopsies for allograft dysfunction. The IR of CD154+TcM, which was associated with ongoing ACR in 32 of 43 training set subjects demonstrated a sensitivity and specificity of 88% each. In the remaining subjects test sensitivity and specificity of 100% and 88% replicated test performance confirming additional uses of allospecific CD154+TcM. This experience suggests that it will be possible to assess rejection-risk in adult recipients using the principles of the test system described here.

Five-year view

The Pleximmune™ test is an affordable tool for rejection-risk assessment in a high-risk population, because its cost is based on existing reimbursement codes for flow cytometry, a standard technology to evaluate immune cell distribution and function. Like any new product, which fulfills unmet needs and for which there is no predicate, the adoption of a novel prognostic test like Pleximmune™ will highlight the spectrum of scenarios where the additional information is desirable to exclude the likelihood of ACR with confidence. These situations include allograft dysfunction of uncertain origin, continuing dysfunction during management of opportunistic infection, and biopsy findings with overlapping features of rejection, infection or mechanical obstruction. Assessing long-term allograft health after LTx with surveillance biopsies at 5-year intervals has raised several questions, which can be better addressed by the Pleximmune™ test. Inflammatory infiltrates, which do not approach the threshold of ACR but are accompanied by mild fibrosis have suggested ongoing low-grade cellular and humoral alloreactivity, respectively [31]. If confirmed, these features may preclude additional minimization of immunosuppressants. Simultaneous evaluation of such patients with Pleximmune™ and donor-specific anti-HLA antibodies can provide the additional information necessary to manage immunosuppression with confidence [32]. The Pleximmune™ test is currently offered in the United States. The test system is expected to be marketed outside the United States in the near future.

T-cell help to B-cells via receptor-ligand systems such as CD40-CD154 is essential for antibody production [31]. Precise control of the T-cell response is necessary in order to limit the synthesis of donor-specific antibody, an important source of chronic injury and graft loss. Therefore, an assessment of cellular rejection-risk provided by Pleximmune™ is inseparable from an assessment of the risk of antibody-mediated rejection with anti-HLA antibodies [32].

Abbreviations

ACR	Acute cellular rejection
AUC	Area under the receiver-operating-characteristic curve
ITx	Intestine transplantation
LTx	Liver transplantation

NPV	Negative predictive values
PPV	Positive predictive values
PTLD	Post-transplant lymphoma-like disorders
rATG (Genzyme)	Rabbit antihuman thymocyte globulin
TcM	T-cytotoxic memory cells

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. 2014 Annual Report of the US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1998–2014. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation; Rockville, MD: United Network for Organ Sharing; Richmond, VA: University Renal Research and Education Association; Ann Arbor, MI:
2. Ingulli E. Mechanism of cellular rejection in transplantation. *Pediatr Nephrol.* 2010; 25(1):61–74. [PubMed: 21476231]
3. Shepherd RW, Turmelle Y, Nadler M, et al. Risk factors for rejection and infection in pediatric liver transplantation. *Am J Transplant.* 2008; 8(2):396–403. [PubMed: 18162090]
4. Nayyar N, Mazariegos G, Ranganathan S, et al. Pediatric small bowel transplantation. *Semin Pediatr Surg.* 2010; 19(1):68–77. [PubMed: 20123276]
5. International Panel: Demetris AJ, Batts KP, Dhillon AP, et al. Banff schema for grading liver allograft rejection: An international consensus document. *Hepatology.* 1997; 25(3):658–663. [PubMed: 9049215]
6. Wu T, Abu-Elmagd K, Bond G, Nalesnik MA, Randhawa P, Demetris AJ. A schema for histologic grading of small intestine allograft acute rejection. *Transplantation.* 2003; 75(8):1241–1248. [PubMed: 12717210]
7. Abu-Elmagd KM, Costa G, Bond GJ, et al. Five hundred intestinal and multivisceral transplantations at a single center: major advances with new challenges. *Ann Surg.* 2009; 250(4):567–581. [PubMed: 19730240]
8. Al-Awwa IA, Hariharan S, First MR. Importance of allograft biopsy in renal transplant recipients: correlation between clinical and histological diagnosis. *Am J Kidney Dis.* 1998; 31(6 Suppl 1):15S–18S.
9. Kaltenborn A, Schrem H. Mycophenolate mofetil in liver transplantation: a review. *Ann Transplant.* 2013; 8:685–696. [PubMed: 24346057]
10. Safa K, Riella LV, Chandraker A. Beyond calcineurin inhibitors: emerging agents in kidney transplantation. *Curr Opin Nephrol Hypertens.* 2013; 22(6):689–697. [PubMed: 24100221]
11. Spada M, Petz W, Bertani A, et al. Randomized trial of basiliximab induction versus steroid therapy in pediatric liver allograft recipients under tacrolimus immunosuppression. *Am J Transplant.* 2006; 6(8):1913–1921. [PubMed: 16771811]
12. Merion RM, Howell T, Bromberg JS. Partial T-cell activation and anergy induction by polyclonal antithymocyte globulin. *Transplantation.* 1998; 65(11):1481–1489. [PubMed: 9645807]
13. Augustine JJ, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. *Am J Transplant.* 2005; 5(8):1971–1975. [PubMed: 15996247]
- 14**. Sindhi R, Ashokkumar C, Higgs BW, et al. Allospecific CD154 + T-cytotoxic memory cells as potential surrogate for rejection risk in pediatric intestine transplantation. *Pediatr Transplant.*

- 2012; 16(1):83–91. Erratum in: *Pediatr. Transplant.* 16(8), 913 (2012). First article to evaluate a biomarker of transplant rejection as a surrogate endpoint in a simulation. [PubMed: 22122074]
- 15**. Ashokkumar C, Talukdar A, Sun Q, et al. Allospecific CD154+ T cells associate with rejection risk after pediatric liver transplantation. *Am J Transplant.* 2009; 9(1):179–191. First report describing alloantigen-specific CD154+T-cytotoxic memory cells as predictors of acute cellular rejection in children with liver transplantation. [PubMed: 18976293]
16. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med.* 1969; 280(14):735–739. [PubMed: 4886455]
17. Farmer DG, Venick RS, Colangelo J, et al. Pretransplant predictors of survival after intestinal transplantation: analysis of a single-center experience of more than 100 transplants. *Transplantation.* 2010; 90(12):1574–1580. [PubMed: 21107306]
18. Wozniak LJ, Hickey MJ, Venick RS, et al. Donor-specific HLA antibodies are associated with late allograft dysfunction after pediatric liver transplantation. *Transplantation.* 2015; 99(7):1416–1422. [PubMed: 26038872]
19. Wozniak LJ, Venick RS, Gordon Burroughs S, Ngo KD, Duffy JP, Farmer DG. Utility of an immune cell function assay to differentiate rejection from infectious enteritis in pediatric intestinal transplant recipients. *Clin Transplant.* 2014; 28(2):229–235. [PubMed: 24433466]
20. Fang KC. Clinical utilities of peripheral blood gene expression profiling in the management of cardiac transplant patients. *J Immunotoxicol.* 2014; 4(3):209–217. [PubMed: 18958730]
- 21**. Ashokkumar C, Gupta A, Sun Q, et al. Allospecific CD154+ T cells identify rejection-prone recipients after pediatric small-bowel transplantation. *Surgery.* 2009; 146(2):166–173. First report describing alloantigen-specific CD154+T-cytotoxic memory cells as predictors of acute cellular rejection in children with intestine transplantation. [PubMed: 19628070]
22. Sindhi R, Magill A, Bentlejewski C, et al. Enhanced donor-specific alloreactivity occurs independently of immunosuppression in children with early liver rejection. *Am J Transplant.* 2005; 5(1):96–102. [PubMed: 15636616]
23. Khera N, Janosky J, Zeevi A, Mazariegos G, Marcos A, Sindhi R. Persistent donor-specific alloreactivity may portend delayed liver rejection during drug minimization in children. *Front Biosci.* 2007; 12:660–663. [PubMed: 17127326]
- 24**. Ashokkumar C, Shapiro R, Tan H, et al. Allospecific CD154+ T-cytotoxic memory cells identify recipients experiencing acute cellular rejection after renal transplantation. *Transplantation.* 2011; 92(4):433–438. First report describing the ability of alloantigen-specific CD154+T-cytotoxic memory cells to detect acute cellular rejection in adult renal transplant recipients. [PubMed: 21747326]
25. Koyama I, Nadazdin O, Boskovic S, et al. Depletion of CD8 memory T cells for induction of tolerance of a previously transplanted kidney allograft. *Am J Transplant.* 2007; 7(5):1055–1061. [PubMed: 17286617]
26. [Accessed January, 23, 2015] Designating Humanitarian Use Devices. <http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/DesignatingHumanitarianUseDevicesHUDS/default.htm>
27. Eydelman MB, Chen EA. The FDA's humanitarian device exemption program. *Health Aff (Millwood).* 2011; 6(30):1210–1212. author reply (1212). [PubMed: 21653975]
28. National Committee for Clinical Laboratory Standards (NCCLS). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition. NCCLS document EP5-A2. 2004
- 29**. Ashokkumar C, Soltys K, Mazariegos G, et al. Predicting cellular rejection with a cell-based assay: Pre-clinical and clinical evaluation in children. *Transplantation.* (In Press). Detailed summary of pre-marketing evaluation of Pleximmune™ and its clinical implementation.
30. FDA U.S. Food and Drug Administration. Recently-Approved Devices. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfTopic/pma/pma.cfm?num=h130004>
31. Miyagawa-Hayashino A, Yoshizawa A, Uchida Y, et al. Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts. *Liver Transpl.* 2012; 18(11):1333–1342. [PubMed: 22888064]

32. Ranganathan S, Celik N, Mazariegos G, Sindhi R. Liver allograft fibrosis and minimization of immunosuppression. *Pediatr Transplant*. 2015; 19(7):667–668. [PubMed: 26333572]

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Key Issues

- Lifelong immunosuppression is necessary to prevent and treat acute cellular rejection, which can affect half of all recipients during a lifetime.
- Cellular rejection also signals the immune system to produce antibodies, which produce graft loss through chronic injury.
- Children who have received liver or intestine transplantation can experience life-threatening infections and EBV-infection-induced malignant transformation of lymphocytes due to immunosuppression.
- There are currently no FDA-approved tests to predict cellular rejection in the target population. Biopsies detect but do not predict cellular rejection.
- The Pleximmune™ blood test is the first FDA-approved test to predict acute cellular rejection in children with liver or intestine transplantation.
- Test results show high sensitivity and specificity exceeding or approaching 80%, are delivered within a day, and should be used with all available clinical information.

Figure 1.

Histologic sections of

Fig 1A: Liver allograft biopsy showing small inconspicuous portal areas with no inflammation. (H&E $\times$ 100)

Fig 1B: Liver allograft biopsy showing portal and pericentral lymphocytic infiltrate in acute cellular rejection (H&E $\times$ 40)

Fig 1C: Normal intestine allograft biopsy (H&E $\times$ 100)

Fig 1D: Intestine allograft biopsy showing moderate to severe acute cellular rejection with multiple crypt apoptosis and surface ulceration. (H&E $\times$ 100).

Figure 2.

Flow cytometry scatterplots from a child with increased risk of rejection (upper panel) show increased frequency of CD154+TcM (orange dots) induced by stimulation with donor allostimulus (left upper) compared with those induced by reference allostimulus (right upper). In the lower panel, donor-induced CD154+TcM (lower left) are exceeded by those induced by reference allostimulus (lower right). SSC=side scatter.

Table 1

FDA cleared tests for immune monitoring after transplantation

Manufacturer	Name	Technology	Readout	Uses	Regulatory Status	Organ Type
Viracor	Immuknow	Mitogen-stimulated T-helper cell function	Adenosine triphosphate	General immune function	510k cleared	Not specified
CareDx	Allomap	qRT-PCR	Multiple gene algorithm	Predicts rejection	510k cleared	Heart transplant rejection

Table 2

Performance of Pleximmune™ IR thresholds for predicting rejection in post-transplant (upper table) and pre-transplant training and validation set samples.

Post-transplant Samples	AUC	IR threshold	Sensitivity	Specificity	PPV	NPV
Training set (n=98)	0.878	1.10	92% (n=24) CI ₉₅ 72–99%	84% (n=74) CI ₉₅ 73–91%	65% (n=34) CI ₉₅ 46–80%	97% (n=64) CI ₉₅ 88–99%
Validation set (n=68)	0.791	1.10	84% (n=19) CI ₉₅ 60–99%	80% (n=45) CI ₉₅ 65–90%	64% (n=25) CI ₉₅ 43–81%	92% (n=39) CI ₉₅ 78–98%

Pre-transplant Samples	AUC	IR threshold	Sensitivity	Specificity	PPV	NPV
Training set (n=49)	0.82	1.23	80% (n=25) CI ₉₅ 59–92%	71% (n=24) CI ₉₅ 49–87%	74% (n=27) CI ₉₅ 53–88%	77% (n=22) CI ₉₅ 54–91%
Validation set (n=33)	0.842	1.23	57% (n=14) CI ₉₅ 30–81%	89% (n=19) CI ₉₅ 65–98%	80% (n=10) CI ₉₅ 44–96%	74% (n=23) CI ₉₅ 51–89%