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First-in-Human Study of the Safety and Efficacy of TOL101 Induction to Prevent Kidney Transplant Rejection

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TOL101 is a murine IgM mAb targeting the αβ TCR. Unlike other T cell targets, the αβ TCR has no known intracellular signaling domains and may provide a nonmitogenic target for T cell inactivation. We report the 6-month Phase 2 trial data testing TOL101 in kidney transplantation. The study was designed to identify a dose that resulted in significant CD3 T cell modulation (<25 T cell/mm^3), to examine the safety and tolerability of TOL101 and to obtain preliminary efficacy information. Thirty-six patients were enrolled and given 5–10 daily doses of TOL101; 33 patients completed dosing, while three discontinued after two doses due to a self-limiting urticarial rash. Infusion adjustments, antihistamines, steroids and dose escalation of TOL101 reduced the incidence of the rash. Doses of TOL101 above 28 mg resulted in prolonged CD3 modulation, with rapid recovery observed 7 days after therapy cessation. There were no cases of patient or graft loss. Few significant adverse events were reported, with one nosocomial pneumonia. There were five biopsy-confirmed acute cellular rejections (13.9%); however, no donor-specific antibodies were detected. Overall TOL101 was well-tolerated, supporting continued clinical development using the dose escalating 21–28–42–42–42 mg regimen.

Keywords: Acute rejection, induction, kidney transplantation

Abbreviations: AE, adverse events; ATG, anti-thymocyte globulin; BCAR, biopsy-confirmed acute rejection; BKV, BK virus; CMV, cytomegalovirus; DSA, donor-specific antibody; DSMB, data safety monitoring board; EBV, Epstein-Barr virus; ECD, expanded criteria deceased donor; eGFR, estimated GFR; ELISA, enzyme-linked immunosorbent assay; EOS, end of study; HAMA, human anti-mouse antibody; IFN-γ, interferon-γ; MABEL, minimum anticipated biologic effect level; MMF, mycophenolate mofetil; NO, nitric oxide; PD, pharmacodynamic; SAE, serious adverse events; TNF, tumor necrosis factor

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Introduction

Despite dramatic advances in clinical immunosuppression, a persistent goal in kidney transplantation remains the control of alloimmunity while minimizing the toxicities of immunosuppressive agents. Prophylaxis against T cell–mediated (cellular) acute rejection involves inhibition of alloreactive αβ T cells immediately following or within the first few weeks after engraftment (1–3). Contemporary maintenance immunosuppressive agents such as calcineurin inhibitors (cyclosporine, tacrolimus), mammalian target of rapamycin inhibitors (sirolimus, everolimus), anti-proliferative agents azathioprine, mycophenolate mofetil (MMF) or mycophenolic acid and agents capable of altering costimulation pathways (humanized IgG/CTLA4 fusion protein; belatacept) have all combined to provide effective tools to prevent rejection (4–6). In addition, the use of antibody induction therapy not only appears to decrease the severity and rate of acute rejection but also permits a delayed introduction and minimization of potentially nephrotoxic maintenance therapy and may improve outcomes (7–9). Prophylactic (induction) antibody agents include rabbit or equine anti-thymocyte globulin (ATG), anti-IL-2 receptor antibody

(basiliximab), anti-CD52 (alemtuzumab) and the recently discontinued murine anti-CD3 antibody OKT3 (10,11).

While the use of induction agents has significantly increased over the past decade, questions remain regarding the efficacy of anti-IL-2 receptor antibody and the risk/benefit balance of depletional agents. In patients with higher immunological risk, depletional therapies including ATG and alemtuzumab are typically utilized (11–13). These agents lead to prolonged mononuclear cell depletion, which is desirable under conditions of increased risk of rejection but may lead to untoward effects such as enhanced susceptibility to infection as well as the development of malignancy. They are also occasionally associated with a number of drug-related adverse events (AE) such as infusion reactions, and a first dose cytokine release syndrome characterized by IL-6 and tumor necrosis factor (TNF) release (14). Utilization is also limited in some cases in the acute setting by the development of thrombocytopenia, and in the chronic setting by the development of anti-drug antibodies (15). While the nondepleting anti-IL-2 receptor antibody basiliximab is associated with reduced side effects, its efficacy is not well defined under modern immunosuppression and is notably inferior to depletional agents when immune suppression minimization or steroid avoidance is attempted (13,16). Therefore, its use is usually restricted to patients at low risk for transplant rejection. Given the diverse spectrum of immunological risk profiles among kidney transplant recipients, there exists a therapeutic gap for an induction agent that rapidly and reliably modulates the T cell response but avoids long-term lymphodepletion.

Targeting the αβ TCR has been shown to be highly efficacious in animal models of transplantation and autoimmunity (17–20). Additionally, outcomes with predecessor anti-αβ agents in human studies not only suggest a reduced AE profile but also displayed similar efficacy to muromonab-CD3 (21–23). TOL101 is a murine IgM mAb to the αβ TCR under study as a T cell–modulating agent. Unlike predecessor anti-TCR agents including muromonab-CD3 and humanized monoclonal anti-CD3 IgG agents that targeted the CD3 subunit of the TCR, the novel targeting of TOL101 to the αβ subunits of TCR has the potential to modulate T cells without mitogenicity, since the αβ TCR has no known intracellular signaling domains.

In order to determine the safety and immunomodulatory effects of TOL101 as well as gather preliminary data regarding the efficacy of TOL101 in the prevention of acute T cell–mediated rejection, a first-in-human Phase 2 study of TOL101 as an induction agent for primary kidney transplant recipients was performed in 36 subjects, reported herein.

Methods

TOL101 development

TOL101 is an optimized version of the murine anti-human αβ TCR antibody originally named T10B9. This next-generation anti-αβ TCR antibody has two nucleotide mutations and a different glycosylation pattern to T10B9. In addition, the manufacturing process has been optimized, incorporating serum and animal-free components. TOL101 has been assigned a new IND #104,594.

Study design

This Phase 2 study sponsored by Tolera Therapeutics, Inc. (Kalamazoo, MI) was an open label, multicenter, first-in-human study designed to investigate the safety, preliminary efficacy and immunogenicity of TOL101 administered to primary kidney transplant recipients. The study was designed with a modified adaptive design, including an initial dose-escalation component. Subjects were enrolled from November 2010 until December 2012, ClinicalTrials.gov identifier NCT01154387. *De novo* renal transplant recipients (n = 36) were enrolled into successively higher dose levels with the goal of identifying two potentially therapeutic dose levels to be evaluated further in Phase 3. The initial dose levels started a one-tenth (0.28 mg) of the minimum anticipated biologic effect level (MABEL) of 2.8 mg. The follow-up period was 6 months for all 36 enrolled patients. Good Clinical Practice and Declaration of Helsinki ethical principles were followed throughout the study. Institutional Review Board approval was obtained at all participating centers, with patients receiving written informed consent upon enrollment. The sponsor, Tolera Therapeutics, monitored safety, with an independent data safety monitoring board (DSMB) used to provide safety oversight and to provide guidance on each cohort for dose escalation. Serious events and opportunistic infections were both investigator reported and confirmed by an independent monitor that reviewed the medical records of each enrolled patient.

Eligibility criteria

Eligible patients were aged 18–60 years and scheduled to receive a primary non-HLA identical kidney transplant from a living donor or standard criteria deceased donor, with cold ischemia time of <30 h. In the last cohort, two expanded criteria deceased donor (ECD) kidneys were enrolled. Only patients with a history of Epstein-Barr virus (EBV) exposure (positive IgG serology) and a panel reactive antibody of <20% were included in the study. Patients were excluded from participation if they had prior organ transplantation, positive flow cytometry T cell cross-match, an ABO-incompatible donor, white cell count <2000/mm^3, platelet count <100 000/mm^3, absolute neutrophil count <1000/mm^3, liver transaminases three times the upper normal value, HIV infection, hepatitis B or C virus infection. X-ray confirmed chest inflammation; co-morbid conditions thought to represent excessive risk or known hypersensitivity to rodent proteins or other protocol required medications.

Maintenance immune suppression

Maintenance immunosuppression consisted of MMF, tacrolimus and tapering steroids. Oral or intravenous (IV) MMF (minimum 750 mg twice daily) was initiated on the day of transplant. Tacrolimus was initiated orally between Study Day 1 and Day 6, at the discretion of the investigator. The starting dose of tacrolimus was 0.1–0.2 mg/kg/day in divided doses. Subsequent doses of tacrolimus were individualized to maintain whole blood C_0 levels in the range of 6–15 ng/mL for the first month posttransplant. Tacrolimus C_0 level measurements were done daily during TOL101 administration, weekly, Month 1 and on Days 90 and 180. The initial corticosteroid dose was 500 mg IV methylprednisolone at transplantation; 250 mg on Day 1; 125 mg on Day 2; 60 mg Day 3; then oral prednisone 0.5 mg/kg from Day 4; tapered to 5–10 mg/day by Month 1 and to ≥5 mg/day at Day 45 until Month 6.

Anti-infective prophylaxis

Oral valganciclovir (Valcyte®); Genentech, So. San Francisco, CA was recommended at starting dose of 450 mg within first 3 days of transplant in

cytomegalovirus (CMV)+ recipients or in recipients of kidneys from CMV+ donor, to be given daily for 6 months in those D+R−, and 3 months in the others. Oral trimethoprim/sulfamethoxazole (Septra SS®/Bactrim®) was required for 6 months for prophylaxis of *Pneumocystis carinii* pneumonia, and fungal prophylaxis per institution standard of care.

TOL101 administration and pharmacodynamics

TOL101 was provided in 14 mg lyophilized vials (Tolera Therapeutics, Inc.). Subjects received at least six daily doses of TOL101, beginning in the operating room on Day 0 through a central venous catheter. Initial infusions were administered over 1–2 h, and all subjects received 50 mg IV diphenhydramine and their daily methylprednisolone dose within 1 h of the first three TOL101 doses. Using CD3 T cell count as the primary marker of efficacy, this study was designed to test ascending doses of TOL101. Due to the potential immune stimulatory capacity of TCR targeting antibodies, the initial TOL101 dose used was one-tenth of the MABEL, which was determined to be 0.28 mg. The precise dosing regimens in successive cohorts were Group 1: 0.28 mg (n = 2); Group 2: 2.4 mg (n = 2); Group 3: 7 mg (n = 2); Group 4: 14 mg (n = 2); Group 5: 28 mg (n = 6); Group 6: 32 mg (n = 4); Group 7: 42 mg (n = 4) and two dose-escalation cohorts. The first dose-escalating cohort Group 8: (n = 6) tested 14 mg at Day 0, 21 mg at Day 1, 28 mg at Day 2, 42 mg at Day 3 and 42 mg at Day 4, with 42 mg administered daily until target tacrolimus levels were achieved. The second dose-escalating cohort Group 9: (n = 8) tested 21 mg at Day 0, 28 mg at Day 1, 42 mg at Day 2 and 42 mg at Day 3 and 42 mg until target tacrolimus levels were achieved. A 24-h hold between patients and regular DSMB review of patient data were performed as further safety precautions. A dose was considered to be efficacious if CD3 counts were <25 T cells/mm^3 throughout the dosing interval. Cessation of TOL101 was determined after a minimum of six doses, if the tacrolimus C_0 levels were therapeutic (8–15 ng/mL).

End points

Safety parameters: Multiple safety parameters were monitored. Events were classified by organ system as AE or serious adverse events (SAE) according to the Medra dictionary using Good Clinical Practice guidelines (http://www.who.int/medical_devices/innovation/MedDRAintroguide_version14_0_March2011.pdf). Subjects who suffered the same event more than once were recorded as suffering one event. Subjects who had more than one AE within a system organ class were counted only once in that system organ class. Immune safety parameters including symptoms that may suggest cytokine release syndrome were carefully monitored. Cytokine release syndrome was identified using a constellation of three observations after infusion, namely a fever greater than 101° Fahrenheit, combined with rigor (shaking chills) and shortness of breath. Serum levels of TNF, interferon-γ (IFN-γ), IL-2, IL-6 and IL-10 were determined at 0, 2, 8 and 24 h after the first dose, as well as upon recognition of an infusion reaction involving fever, chills, rigors, skin disorders or shortness of breath. Cytokines were measured by PRL laboratories (Overland Park, KS) using Luminex technology (Austin, TX). Nitric oxide (NO) levels were also determined using calorimetric assay by ABC Laboratories (Columbia, MI) at 0, 2, 8 and 24 h after the first dose as well as on Day 4. Human anti-mouse antibody (HAMA) was determined at baseline, Day 14 and Day 28, using sandwich enzyme-linked immunosorbent assay (ELISA) with TOL101 as the capture antibody (ABC Laboratories).

The incidence of malignancies including lymphoproliferative disorder was collected from clinical records. Screening for CMV (Days 28, 90 and 180), BK virus (BKV) (Days 90 and 180) and EBV (Days 28, 90 and 180) was performed using blood polymerase chain reaction detection. The incidence of other serious or opportunistic infections was also recorded.

Efficacy parameters: Clinical efficacy was determined by the pharmacodynamic (PD) effect of TOL101 on CD3+ T lymphocyte counts. Successful T cell modulation was considered present in patients with sustained daily CD3+ T cell numbers below 25 CD3+ counts/mm^3 for the continuous dosing interval. In addition, the triple end point of patient survival, graft survival and biopsy-confirmed acute rejection (BCAR) at any time during the 6 months was determined. Protocol biopsies were not required. Delayed graft function was defined as the need for dialysis within the first week posttransplant. Renal function was determined by estimated GFR (eGFR) at each study visit (MDRD method), with measured GFR determined by iothalamate clearance at Day 180/end of study (EOS). The spot urine protein to creatinine ratio as well as donor-specific antibody (DSA) was measured at Day 90 and Day 180/EOS.

CD3 counts

Flow cytometry was used to determine CD3 counts and the impact of TOL101 on memory and naïve T cell subsets. Blood samples were collected daily in the morning within 1–2 h of the next TOL101 dose in sodium heparin tubes and shipped overnight to a central flow cytometry facility (NeoGenomics Laboratories, Irvine, CA), with CD3 counts determined using the Beckman Coulter (Brea, CA) CD3 flow cytometry kit on whole blood samples.

Pharmacokinetics

Serum concentrations of TOL101 were measured at 0, 2, 4, 8 and 24 h after administration of Dose 1 (Day 0), Dose 4, the last dose, and on Day 14. An ELISA for murine mouse IgM was utilized (ABC Laboratories). A one-compartment IV infusion model was used and fit using Phoenix WinNonlin Version 6.2 (Certara, St. Louis, MO) and data were weighted by 1/Yhat, where Yhat is the predicted plasma concentration at each time.

Statistics

The number of subjects per cohort was not based on statistical considerations but intended to provide safety and PD data sufficient to escalate to the next dose level. Frequency tables have been presented for all infections, AEs, all AEs by maximum severity, drug-related AEs, SAEs and AEs resulting in study drug discontinuation. For quantitative laboratory tests, summary statistics are presented at each time point. Both measured and eGFR are summarized with descriptive statistics. Delayed graft function and episodes of BCAR are summarized in frequency tables. For the urine protein to creatinine ratio and the DSA assessments, summary statistics will be presented for the values obtained at Day 90 and Day 180/EOS.

Results

Patient characteristics

Patient enrollment began in February 2010 and ended in December 2012 with 13 US centers participating. A total of 36 patients were enrolled into this Phase 2 study, including a broad cross-section of patients (Table 1). The mean donor age was 40 years, with 28 living and 8 deceased donors. The mean recipient age was 44 years, with 75% male. The final dose-escalation cohorts included eight deceased donor transplants, two ECD donor kidneys and four African American recipients, with HLA mismatch being on average greater than 4. The most common causes of end-stage renal disease were glomerular diseases (38%) and polycystic kidney disease (25%).

Table 1: Demographics (N = 36)

	Recipients	Donors
Age at transplant, mean (years)	44.4	40.5
Height (cm)	177.2	N/A
Weight (kg)	91.58	N/A
BMI (kg/m^2)	29.47	N/A
Gender, n (%)		
Male	27 (75)	15 (41.7)
Female	9 (25)	21 (58.3)
Ethnic origin, n (%)		
White	27 (75)	21 (75)
Black	6 (16)	5 (18)
Asian	1 (2.8)	1 (3.5)
Other	1 (2.8)	1 (3.5)
Cause of renal failure, n (%)		
Hypertensive nephrosclerosis	5 (13.9)	N/A
Diabetes	5 (13.9)	N/A
Polycystic kidney disease	9 (25)	N/A
Glomerulonephritis	1 (2.8)	N/A
Focal segmental glomerulonephritis	3 (10.7)	N/A
IgA nephropathy	5 (17.9)	N/A
Other	8 (22.2)	N/A
Type of donor, n (%)		
Living, related	N/A	13 (46.4)
Living, unrelated	N/A	15 (41.6)
Deceased	N/A	8 (22.2)
Blood type, n (%)		
A	15 (41.7)	11 (30.5)
B	3 (8.3)	4 (11.1)
AB	3 (8.3)	1 (2.7)
O	15 (41.7)	20 (55.5)
HLA mismatch, n (%)		
0	0 (0)	N/A
1	2 (5.5)	N/A
2	2 (5.5)	N/A
≥3	32 (88.9)	N/A
Panel-reactive antibody at baseline		
Mean (%)	3.4	N/A
≥20, n (%)	1 (2.8)	N/A
Cold ischemia time		
Mean (min)	330	N/A
Pretransplant CMV antibody match, n (%)		
Donor+/recipient−	8 (22.2)	N/A
Donor+/recipient+	10 (27.8)	N/A
Donor−/recipient−	13 (36.1)	N/A
Donor−/recipient+	5 (13.9)	N/A

CMV, cytomegalovirus.

Serious adverse events

For the entire study, 27 SAEs were reported in 12 subjects (Table S1). No deaths were observed. All but one SAE was considered to be ''unrelated'' to study drug. The ''potentially related'' SAE was nosocomial pneumonia on POD 18 in a 42-year-old male. He was hospitalized with a cough and persistent chest X-ray infiltrate, but no organisms were cultured from blood or sputum. He was given antibiotics and the pneumonia resolved in 2 weeks. Otherwise, most other SAEs were commonly associated with transplant surgery and other non-TOL101-related issues.

Adverse events

For the entire study, a total of 653 AEs were reported in 36 subjects including 47 AEs in 22 subjects who were reported to be ''possibly,'' ''probably'' or ''definitely'' related to TOL101. All reported AEs regardless of causality observed in ≥10% of patients are shown in Table 2. The majority of AEs were reported in 28, 32, and 42 mg dose cohorts. Three subjects discontinued drug due to a drug-related AE (rash), all in the 42 mg dose group. The most commonly reported study drug-related AE observed in 11 (30%) of patients was a skin rash, which was variably described as urticarial, red, raised, hives and/or wheal like. The rash appeared on the trunk, abdomen and/or the extremities (Figure 1) and usually began at the end of the first or second infusion of TOL101 or a few hours thereafter (Tables 2 and 3). The rash resolved in all cases within a few hours spontaneously, or by the next day after treatment with additional diphenhydramine and acetaminophen. In no case was the rash necrotic or persistent, and it never progressed to more severe manifestations. The rash was most intense and only recurred in the initial 42 mg cohort. This led to the protocol of stepwise increases in the initial doses of TOL101 (the dose-escalating cohorts) and a prolongation of the infusion time to 6 h for the first two doses. This approach allowed for 42 mg dosing with reduced rash incidence. In the final cohort dose regimen of Day 0 (21 mg), Day 1 (28 mg), Day 2 (42 mg), Day 3 (42 mg) and Day 4 (42 mg), there was one transient rash, which occurred with inadvertent rapid infusion of the first dose (Table 3).

Infections and malignancies

Posttransplant infections observed during the study are presented in Table 4. Only one significant infection was considered potentially associated with TOL101: nosocomial pneumonia detected on chest X-ray. However, no culture was taken from this patient and as such a definitive diagnosis and causative agent cannot be established. Bacterial skin infections were described as incisional wound cellulitis or drainage, with one case of folliculitis reported. Five cases (13.9%) of BKV viremia were reported on protocol viral surveillance at 3 and 6 months. They were treated by immunosuppressive dose reduction according to local practice. There was one case (2.8%) of histologically confirmed BKV associated nephropathy (Patient 8-005). These six cases (16.7%) were spread across three dosing cohorts: Group 4, 14 mg = 1; Group 6, 32 mg = 2 and Group 8, dose-escalating chort 14–42 mg = 3, and as such a dose–response is not directly apparent. One case of CMV viremia with colitis occurred at Day 169. No EBV or pneumocystis pneumonia was observed. No malignancies have been reported to date in subjects who received TOL101.

Cytokine release and anti-drug antibody detection

As noted in the AE tables, symptoms associated with cytokine release syndrome were not observed. The lack of symptoms was supported by the low levels of IFN-γ, TNF, IL-2, IL-6 and IL-10 at baseline and 0, 2, 4, 8 and 24 h after

Table 2: Adverse events (all causality) >10% in any treatment group, N = 36

Adverse event	Patients, n (%)
All	36 (100)
Blood and lymphatic disorders	
Total	14 (38.9)
Anemia	5 (13.9)
Leukopenia	6 (16.7)
Cardiac disorders	
Total	9 (25)
Tachycardia	6 (16.7)
Eye disorders	
Total	4 (11.1)
Vision blurred	4 (11.1)
Gastrointestinal disorders	
Total	31 (86.1)
Abdominal distention	4 (11.1)
Abdominal pain	5 (13.9)
Constipation	16 (44.4)
Diarrhea	16 (44.4)
Nausea	20 (55.6)
Vomiting	10 (27.8)
General disorders and administration site disorders	
Total	24 (66.7)
Chills	4 (11.1)
Fatigue	10 (27.8)
Edema peripheral	9 (25)
Pain	4 (11.1)
Injury, poisoning and procedural complications	
Total	33 (91.7)
Incision site pain	26 (72.2)
Procedural pain	7 (19.4)
Investigations	
Total	19 (52.8)
Blood creatinine increased	5 (13.9)
Vitamin D decreased	4 (11.1)
Metabolic and nutritional disorders	
Total	28 (77.8)
Dehydration	4 (11.1)
Diabetes mellitus	4 (11.1)
Gout	4 (11.1)
Hyperglycemia	12 (33.3)
Hyperkalemia	5 (13.9)
Hyperlipidemia	5 (13.9)
Hypokalemia	7 (19.4)
Hypomagnesemia	20 (55.5)
Hypophosphatemia	11 (30.6)
Musculoskeletal and connective tissue disorders	
Total	12 (33.3)
Back pain	5 (13.9)
Nervous system disorders	
Total	23 (63.9)
Dizziness	4 (13.1)
Headache	6 (16.7)
Tremor	14 (38.9)
Psychiatric disorders	
Total	8 (22.2)
Insomnia	5 (13.9)
Renal and urinary disorders	
Total	12 (33.3)
Hematuria	6 (16.7)
Reproductive system	
Total	5 (13.9)
Scrotal edema/pain	4 (11.1)
Respiratory, thoracic and mediastinal disorders	
Total	15 (41.7)
Cough	5 (13.9)
Dyspnea	4 (11.1)
Oropharyngeal pain	6 (16.7)
Skin and subcutaneous tissue	
Total	23 (63.9)
Pruritus	11 (30.6)
Urticaria	6 (16.7)
Surgical and medical procedures	
Total	4 (11.1)
Incisional drainage	4 (11.1)
Vascular disorders	
Total	18 (50)
Hypertension	11 (30.6)
Hypotension	7 (19.4)

Table 2: Continued

Adverse event coding was done using the MedDRA Version 13.1 dictionary (http://www.who.int/medical_devices/innovation/MedDRAintroguide_version14_0_March2011.pdf). Subjects who have the same event more than once are counted only once for the preferred term. Subjects who have more than one adverse event within a system organ class are counted only once in that system organ class. Date produced: October 18, 2013.

infusion, respectively (Figure 2A–E). Furthermore, another potential inflammatory marker indicative of infusion reactions includes the production of NO, which was not significantly detected in TOL101-treated patients during or after infusions on Days 0 and 4. The mean percent change from baseline NO levels in micromolar during infusion, end of infusion and 2 h. Postinfusion were −24%,

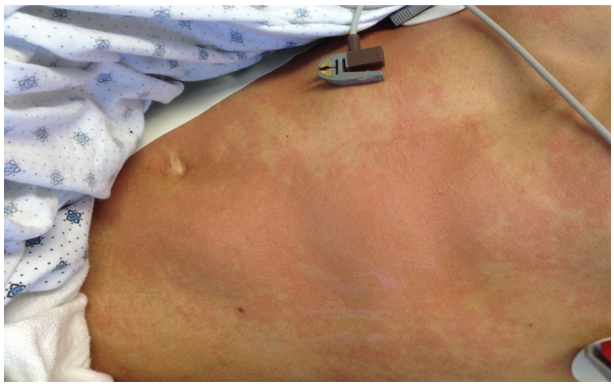

Figure 1: Post-TOL101 rash on trunk and abdomen after initial dose. In some cases, TOL101 infusion was associated with a urticarial rash. This is an example of the urticarial rash on the trunk and abdomen of a 22-year-old male treated with 50 mg diphenhydramine and 650 mg acetaminophen.

Table 3: Urticarial rash incidence; red cells denote TOL101 dose at time of rash

Treatment Group	Patient	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8
0.28 mg	06-001								
	08-001								
1.4 mg	02-001								
	04-001								
7.0 mg	02-002								
	04-002								
14.0 mg	05-001								
	08-002								
28.0 mg	01-001								
	04-003								
	07-001								
	03-001								
	07-003[1]								
	07-004								
32.0 mg	03-002[1]								
	08-004								
	12-001[1]								
	07-005								
42mg	06-002[1]								
	07-002								
	04-004								
	08-003								
Escalation Group 1 14-42mg	07-006	14mg	21mg	28mg	42mg	42mg	42mg		
	07-007	14mg	21mg	28mg	42mg	42mg	42mg		
	07-008	14mg	21mg	28mg	42mg	42mg	42mg		
	08-005[1]	14mg	21mg	28mg	42mg	42mg	42mg		
	07-009	14mg	21mg	28mg	42mg	42mg	42mg		
	02-003	14mg	21mg	28mg	42mg	42mg	42mg		
Escalation Group 2 21-42mg	07-010	21mg	28mg	42mg	42mg	42mg	42mg	42mg	42mg
	07-011	21mg	28mg	42mg	42mg	42mg	42mg	42mg	42mg
	07-012	21mg	28mg	42mg	42mg	42mg			
	07-013	21mg	28mg	42mg	42mg	42mg			
	02-004	21mg	28mg	42mg	42mg	42mg			
	03-003	21mg	28mg	42mg	42mg	42mg			
	03-004	21mg	28mg	42mg	42mg	42mg			

[1]Patients that had biopsy-confirmed acute rejection.

−2%, −19%, +12%, −31% and +4% for cohort Groups 4–9, respectively. Low titer HAMA (1/100) was detected in only 1/36 (2.8%) patient on Day 28. No high-titer HAMA (1/1000) was ever detected.

Efficacy as measured by CD3 count reduction

Peripheral blood CD3 T cell counts were measured daily in the morning within 1–2 h of the next TOL101 infusion. During TOL101 treatment, dosing of TOL101 was required for a minimum of 5 days (six doses), with continued dosing until therapeutic tacrolimus levels were reached (8–15 ng/mL). In seven patients, further doses were needed. In all patients, a primary reduction in leukocyte counts, including CD3 expressing cells was observed immediately after transplant, a potential result of IV steroid infusion (24). Within 48–72 h circulating CD3 counts increased above the 25/mm^3 target in patients receiving 0.28, 2.4, 7 and 14 mg TOL101 (Figure 3A). In the 28 mg cohort, CD3 counts remained mostly under 25/mm^3, with the exception of one patient who experienced a spike in CD3 numbers on Day 3 (Figure 3B). While 28 mg appeared to be a promising dose

Table 4: Infections and malignancies (N = 36)

Infections	Patients, n (%)
Bacterial	
Skin	5 (13.9)
Pneumonia	1 (2.8) nosocomial
Pharyngitis, rhinitis and sinusitis	5 (13.9)
Urinary tract	3 (8.3)
Urosepsis	1 (2.8) *Escherichia coli*
Viral (viremia)	
CMV	2 (5.6)
BK	5 (13.9)
BK associated nephropathy	1 (2.8)
EBV	0 (0)
Fungal	
Candida	0 (0)
Other	0 (0)
Opportunistic	
Pneumocystis	0 (0)
Cancer	
PTLD	0 (0)
Solid organ	0 (0)

CMV, cytomegalovirus; EBV, Epstein-Barr virus; PTLD, posttransplant lymphoproliferative disorder.

regimen, this one outlier triggered escalation of TOL101 to 32 and 42 mg. At both dosing regimens robust CD3 modulation was achieved; however, a rash was noted in patients in the 32 and 42 mg cohorts, respectively. To address possible preformed T cell soluble mediator release as a mechanism, two dose-escalation strategies were tested to exhaust these stores at sub-symptomatic levels. One strategy began at 14 mg on Day 0 with the other at 21 mg on Day 0. In each case, the dose was rapidly escalated to 42 mg by the third or fourth dose. The utilization of a dose-escalating regimen not only reduced the propensity for rash development but also resulted in robust CD3 modulation, meeting the PD target.

The recovery of CD3 expression after TOL101 dosing was observed to occur in all patients by Day 14 (Figure 3B). This recovery combined with stable white blood cell counts suggests the possibility of a nondepletional mechanism of action. The mean plasma elimination half-life of TOL101 in patients receiving greater than 28 mg or in the dose-escalating cohorts was 23.8 ± 9 h, supporting once daily infusions.

Efficacy composite triple end point

There were no patient deaths or graft losses reported in the study, and five (13.9%) subjects experienced BCAR episodes (Table 5). While no rejections occurred during TOL101 treatment, their occurrence was relatively soon after the drug was completed on Days 10–20, and three had a vascular component. The last tacrolimus trough blood level (ng/mL) prior to each rejection was Day 10 (13.1), Day 11 (7.8), Day 14 (6.3), Day 16 (7.6) and Day 20 (8.6). All rejection episodes were treated with thymoglobulin (4) or steroids (1), and resolved clinically without graft loss. No

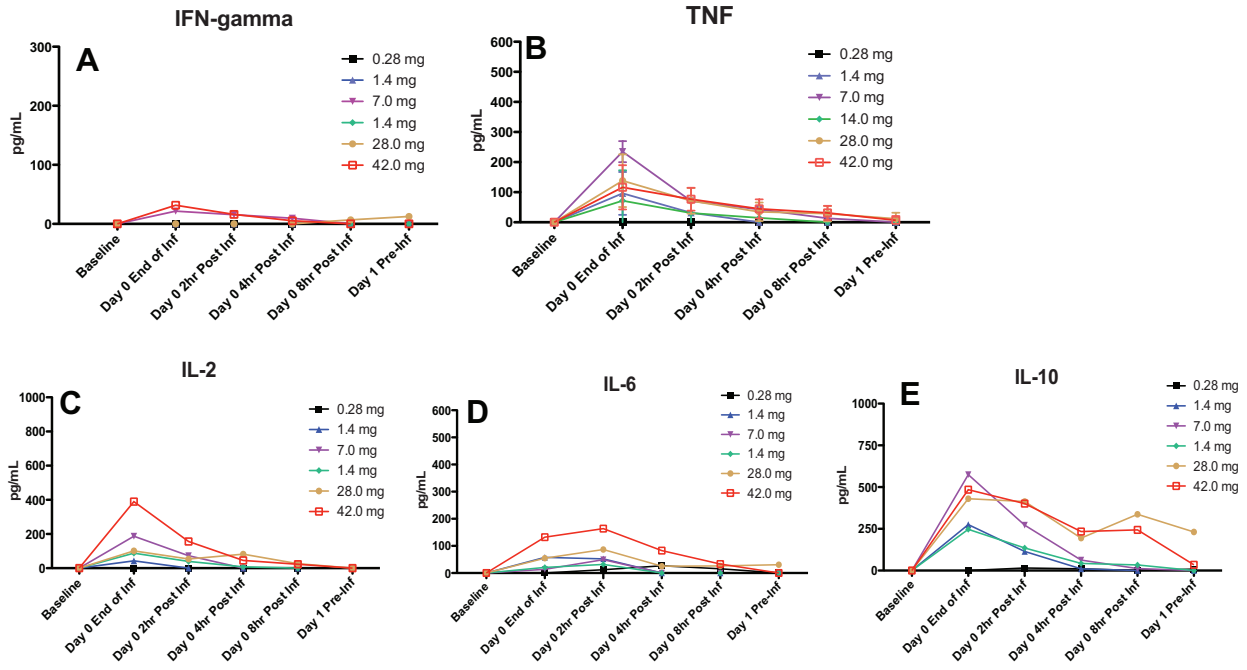

Figure 2: The production of cytokines after the first dose of TOL101. (A–E) The production of IFN-γ (A), TNF (B), IL-2 (C), IL-6 (D) and IL-10 (E) was measured 0, 2, 4, 8 and 24 h after the first infusion of TOL101. No difference in cytokine responses was observed across dose levels. The mean ± standard deviation are presented. IFN-γ, interferon-γ; TNF, tumor necrosis factor.

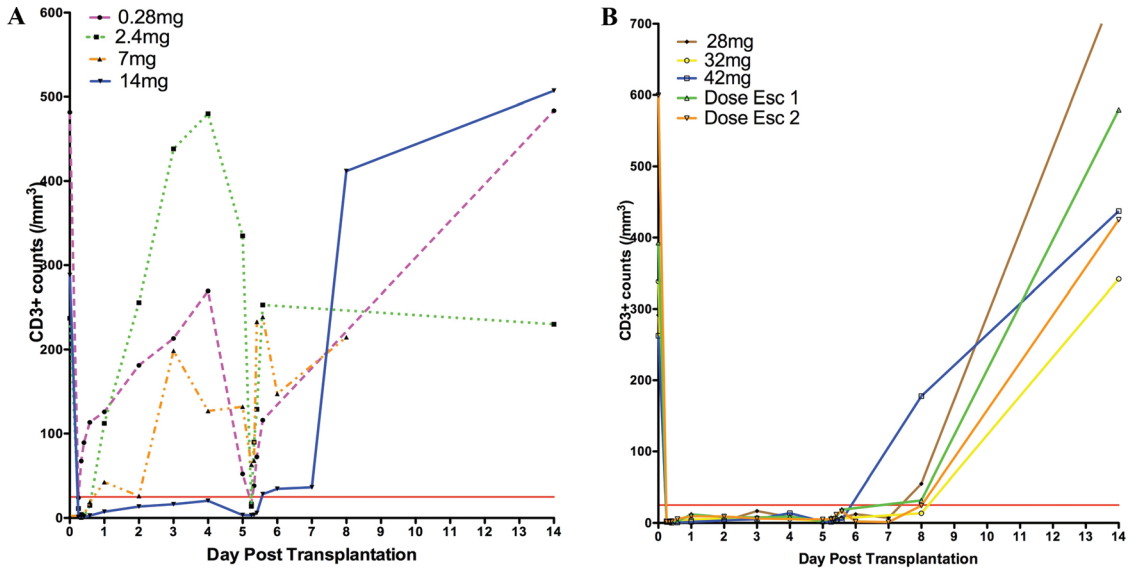

Figure 3: (A, B) Pharmacodynamic (CD3 absolute/mm^3) responses to increasing doses of TOL101 over 5–9 days. The mean of each cohort is presented.

donor-specific anti-HLA antibodies have been detected in the 22 patients tested at both 3 and 6 months posttransplant. Fourteen patients were not tested.

Kidney function

Two patients who received ECD kidneys in cohort 9 (07-010 and 07-011; Table 3) required first week dialysis. In general, kidney function was observed to improve throughout the study with increases in eGFR observed in all patients (Day 180 mean eGFR being 54 mg/dL) (Table 5). In addition, of the 23 subjects who completed a measured (iothalamate) GFR the mean was 65.7 mL/min/1.73 m^2. A voided urine protein to creatinine ratio was performed on Day 90 (n = 27) and Day 180/EOS (n = 27). Excluding three patients due to laboratory handling issues, ratios observed were 0.17 ± 0.12 and 0.13 ± 0.11, respectively (Table 5).

Table 5: Efficacy observations: N = 36 (end of study 6 months)

End point	N (%)
Patient survival	36 (100)
Graft survival	36 (100)
Treated BCAR	5 (13.9)
Banff scored	
1A (Day 10)	1
1B (Day 16)	1
2A (Days 11, 14 and 20)	3
2B or 3	0
Graft function	
Delayed graft function (all), n = 36	2 (5.6)
Delayed graft function (deceased donors), n = 8	2 (25)
Estimated GFR (cc/min) (Day 180), n = 36	55.6 ± 10
Measured GFR (cc/min/1.73 m^2) (Day 180), n = 23	65.7 ± 26
Urine protein/creatinine	
(Day 90)	0.17 ± 0.12
(Day 180) n = 27	0.13 ± 0.11
Donor-specific antibody	
n = 22 tested; 14 not tested	
Day 90	0
Day 180	0

BCAR, biopsy-confirmed acute rejection.

Discussion

Targeting the αβ TCR with TOL 101 is hypothesized to provide rapid and robust T cell modulation, low acute rejection rates and reduced AE compared with currently utilized induction agents. The results from this first-in-human Phase 2 clinical trial investigating the safety and efficacy of TOL101 in 36 primary renal transplant patients are reported. In particular, advancing from subtherapeutic to therapeutic dosing regimens did not result in diminished clinical outcomes or untoward events. These data show that TOL101 is a highly TCR-specific mAb capable of providing robust T cell modulation, without inducing significant cytokine release and/or other immunologic toxicity.

TOL101 was generally well tolerated across all groups, with urticarial rash the only significant AE resulting in study drug discontinuation in three subjects. Skin eruptions are commonly reported side effects in patients receiving biologics, and have been observed in some patients receiving rabbit ATG, alemtuzumab and anti-CD3 agents (25). In the case of TOL101, rash was not associated with any hemodynamic effects, skin breakdown or long-

lasting sequelae. Cytokine release and anaphylaxis are potential causes of rash; however, no patient in the current study appeared to have any anaphylactoid like reaction. The levels (pg/mL) of the examined pro-inflammatory cytokines IFN-γ, TNF, IL-2, IL-6 and IL-10 were relatively low in all TOL101-treated patients (Figure 2A–E). In comparison, infusion of rabbit ATG is accompanied by release at levels of 1000–3000 pg/mL of these cytokines (14). While the specific etiology of the rash is under further examination, one possible explanation for the rash was T cell release of preformed nonclassical soluble mediators with resultant cutaneous vasodilation. As such a dose-escalation strategy combined with a slower infusion rate was used to deplete these potential granule stores. This was viewed to be successful with patients receiving an escalating regimen of 21, 28, 42, 42 and 42 mg experiencing minimal rash symptoms, permitting the full infusion with the 42 mg dose.

Historically, other anti-αβ TCR antibodies have been tested in humans, namely T10B9 and BMA-031. Not only both these agents showed promising efficacy, but their safety profiles were also favorable, with less cytokine release syndrome and serious infections reported compared to muromonab-CD3 (21–23). However, the incidence and intensity of HAMA formation after treatment were similar to those recorded for muromonab-CD3. Unlike these predecessor antibodies, whereby immunogenicity was a major drawback, TOL101 utilization has been associated with very little anti-drug antibody formation, a result of different manufacturing processes and contemporary immune suppression (15,25,26). In this Phase 2 trial, only 1/36 (2.8%) of treated recipients demonstrated HAMA formation, a similar incidence to the chimerized IL-2 receptor blocker basiliximab, 1.5% (27). This potentially makes αβ TCR blockade an attractive target for the pharmacologic control of autoimmunity and transplant rejection (28).

This initial short-term (6-month) experience using TOL101 induction with tacrolimus, MMF and low-dose steroids in clinical kidney transplantation demonstrated excellent patient and graft survival with acceptable AE rates similar to conventional therapies. Together these initial data show TOL101 to be relatively safe and highly effective at modulating CD3+ T cells. This low to moderate risk population experienced a BCAR rate of 13.9%, with the majority of episodes occurring within the first 3 weeks of transplant. In addition, TOL101 induction permitted stable renal function and no reports of DSA development for up to 6 months. The plasma elimination half-life of 23.8 h permits once daily administration, and the apparent recovery of circulating CD3 T cells by 14 days suggests a narrower window of intense immunosuppression than other biologics. The rate of recovery of TOL101 treated T cells and their function should be an active area of future investigation. Finally, and when taken together, these data support the initiation of larger Phase 3 studies testing TOL101 against thymoglobulin and basiliximab, using the final dose-escalating regimen (cohort 9).

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. JPP, JJH, DRG, MTG, TJF, LO and FF are employees of and have stock holdings with Tolera. ACW is an employee of Tolera. SMF, LBM, SM, THW, AA, RS, SDM and FS are scientific advisors for Tolera.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: All reported serious adverse events.