# Preserved Kidney Allograft Function and Unique Urinary Biomarker Profiles in Living Donor Kidney Transplant (LDKT) Patients Tolerized with an Investigational Allo-HSCT Cell Therapy

Joseph R. Leventhal[1], John P. Galvin[1], Michael G. Ison[1], John R. Lee[2], James M. Mathew[1], Lorenzo G. Gallon[1], Meg Gibson[1], Dianne S. Belshe[1], David Tollerud[3], Manikkam Suthanthiran[2], Suzanne Ildstad[3]

[1]Northwestern University, Chicago, IL, USA, [2]Weill Cornell Medicine, New York, NY, USA, [3]Talaris Therapeutics, Louisville, KY, USA

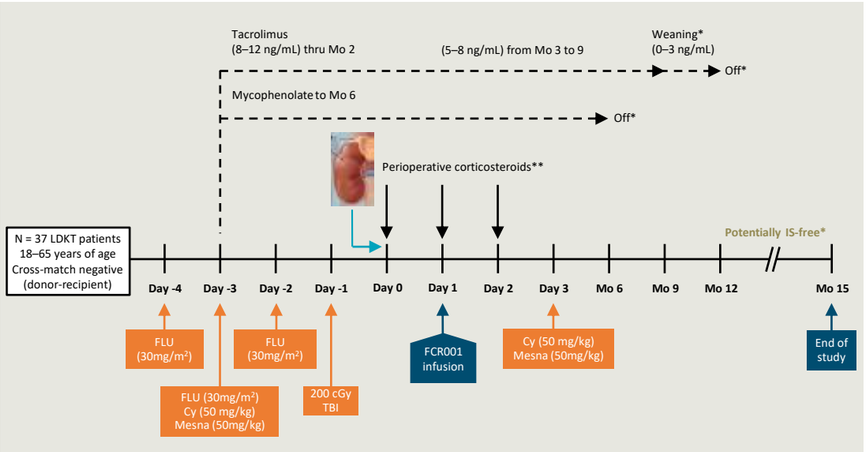
## INTRODUCTION

- Long-term outcomes in living donor kidney transplant (LDKT) patients are suboptimal due to the complications associated with lifelong chronic immunosuppressive medications[1,2]
- Talaris Therapeutics is developing FCR001, a single-dose, investigational cell therapy intended to induce durable immune tolerance to transplanted solid organs without the need for chronic immunosuppression (IS)
- An open-label, single-arm, phase 2 trial of FCR001 in 37 adult LDKT patients treated with FCR001 found that durable chimerism was induced in 26 of 37 patients (70%) and allowed complete discontinuation of IS without any organ rejection for the duration of their follow-up (median 6 years)[3]
- Urinary cell mRNA profiling has emerged as a powerful tool for the noninvasive monitoring of renal allografts. We hypothesized that tolerance in kidney allograft recipients conditioned with FCR001 would be associated with a unique urinary cell mRNA signature, one that could distinguish FCR001 recipients from kidney allograft recipients treated with conventional immunosuppressive regimens
- Here, urinary cell mRNA profiling was examined for signatures of tolerance

## METHODS

- The protocol was based upon tolerogenic CD8+/TCR- facilitating cell-enriched hematopoietic stem cell allografts (FCR001) administered to human leukocyte antigen-mismatched recipients of LDKT following nonmyeloablative conditioning (**Figure 1**)
  - Tacrolimus/mycophenolate mofetil-based IS was weaned and discontinued at 1 year if durable chimerism and normal kidney function and transplant biopsy were confirmed
- Absolute levels of mRNA in urine samples collected from the FCR001 cohort and the CTOT-04 cohort were measured via preamplification enhanced real-time quantitative PCR assays[4]

### Figure 1. Phase 2 trial design




*Assuming no biopsy-proven acute rejection; stable kidney function; >50% donor chimerism; no Graft vs Host Disease.
**Methylprednisolone 500 mg IV on Day 0 in operating room; 250 mg Day 1 and 125 mg Day 2.
cGy, centigray; Cy, cyclophosphamide; FLU, fludarabine; IS, immunosuppression; IV, intravenous; LDKT, living donor kidney transplant; Mesna, mercapto-ethyl sulfonate; Mo, month; TBI, total body irradiation.

## RESULTS

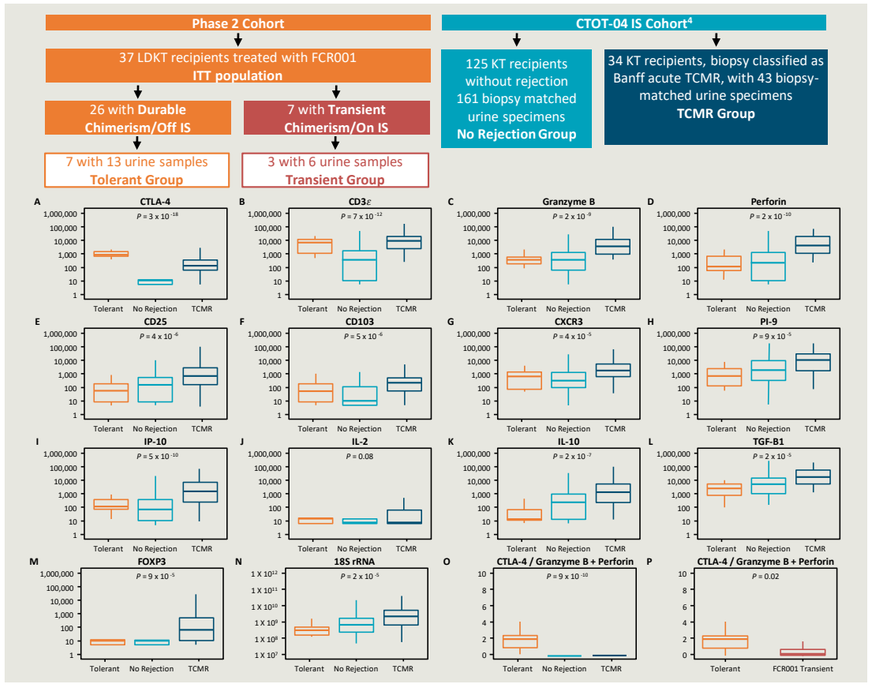
- Through October 1, 2021, for FCR001 patients, on an ITT basis, five-year graft and patient survival were 92% and 89%, respectively.
- Through October 1, 2021, graft function, as assessed by estimated glomerular filtration rate, was more stable for the FCR001 cohort compared to SOC, with the difference due to patients with durable chimerism and off IS (**Table 1**)

### Table 1. eGFR in FCR001 recipients and a SOC cohort

| | eGFR values (mL/min) | | | | | |
|---|---|---|---|---|---|---|
| **Cohort** | **Month 1** | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
| Chimeric/Off IS (n = 26) | 58.8 | 60.6 | 65.4 | 65.0 | 64.3 | 66.1 |
| ITT (n = 37) | 55.0 | 59.9 | 63.7 | 63.7 | 62.9 | 62.6 |
| Northwestern SOC (n = 132) | | 58.9 | 58.1 | 55.4 | 51.5 | |

eGFR, estimated glomerular filtration rate; IS, immunosuppression; ITT, intent-to-treat; SOC, standard of care

### Figure 2. Urinary cell mRNA profiling in the Phase 2 and CTOT-04 cohorts




**A-N)** Box and whisker plots show the median, 25th, and 75th percentile values, and the largest value no more than 1.5 times the interquartile range from the hinge of mRNA copy number per microgram of total RNA and 18S rRNA copy number per microgram of total RNA. *P*-values within each box are based on KW test comparing the three groups, with mRNA copy numbers used as the dependent variable. **O-P)** The ratio of CTLA-4 mRNA copy number to the sum of granzyme B mRNA copy number and perforin mRNA copy number was also evaluated. **O)** *P*-value shown was calculated using the KW test, using the ratio as the dependent variable. **P)** *P*-value was calculated using the Wilcoxon rank sum test.
IS, immunosuppression; ITT, Intent-to-treat; KT, Kidney Transplant; KW, Kruskal-Wallis; LDKT, Living donor kidney transplant; TCMR, T-cell mediated rejection.

- Urinary cell mRNA profiling of the Tolerant Group of FCR001 patients identified a potential signature of tolerance, characterized by increased levels of CTLA-4 mRNA, and a higher ratio of CTLA-4 mRNA to mRNA for granzyme B and perforin mRNA (**Figure 2**)
  - Urinary cell levels of mRNA for CTLA-4 (adjusted $P = 3 \times 10^{-9}$) and CD3$\varepsilon$ (adjusted $P = 0.002$) were significantly higher in the Tolerant Group than in the No Rejection Group (**Figure 2 Panels A, B**)
  - Urinary cell levels of mRNA for granzyme B, perforin, CD25, PI-9, IP-10, IL-10, TGF-B1, and 18S rRNA were significantly lower in the Tolerant Group compared to the T-cell Mediated Rejection (TCMR) Group (adjusted $P < 0.05$) (**Figure 2, Panels C-E,H,I,K,L,N**)
  - The ratio of CTLA-4 mRNA to the sum of granzyme B mRNA and perforin mRNA was significantly different among the Tolerant Group, No Rejection Group, and the TCMR Group ($P = 9 \times 10^{-10}$, Kruskal Wallis test) (**Figure 2, Panel O**)
    - Tolerant Group vs No Rejection Group: median ratio 2.00 vs 0.01, $P = 3 \times 10^{-9}$
    - Tolerant Group vs TCMR Group: median ratio 2.00 vs 0.02, $P = 8 \times 10^{-8}$
- The ratio of CTLA-4 mRNA to the sum of granzyme B mRNA and perforin mRNA was significantly higher in the FCR001 Tolerant Group with durable chimerism compared to the FCR001 Transient Group (median ratio 2.00 vs 0.35, $P = 0.02$) (**Figure 2, Panel P**)

## CONCLUSIONS

- In this Phase 2 study, kidney allograft function in the FCR001-treated patients was preserved over the 5-year follow up period, indicating a potential clinical benefit to inducing tolerance.
- Urinary profiling of a subgroup of FCR001 subjects identified a potential signature of tolerance, characterized by increased levels of CTLA-4 mRNA, and a significantly higher ratio of CTLA-4 to granzyme B and perforin mRNA species. Urinary profiling of several other mRNAs did not differ between FCR001 treatment and the No Rejection biopsy group. This signature may help to identify patients who receive FCR001 that could be safely taken off chronic IS
- As of October 1, 2021, we have accumulated a total of approximately 252 patient-years of exposure to FCR001 in LDKT, and the safety profile observed in the Phase 2 patients remains generally consistent with that expected of a patient who receives both a living donor kidney transplant and an allo-HSCT with nonmyeloablative conditioning
- We continue to monitor patients in the Phase 2 trial for long-term safety and durability of immune tolerance and graft function. We are currently enrolling patients in FREEDOM-1, a randomized, controlled, open-label Phase 3 trial in the US in adult LDKT recipients

### References

**1)** Calogero et al., 2020. *Open Medicine*. 15:623–34; **2)** Piotrowski et al., 2017. *Folia Neuropatholigca*. 55(2):86–109; **3)** Leventhal et al., 2021. *American Transplant Congress*. 21(suppl 3): Abstract 685; **4)** Suthanthiran et al., 2013. *N Engl J Med.* 369(1):20–31.

### Acknowledgments

Editorial support provided by Kelly Hamilton, PhD, of AlphaScientia, and supported by Talaris Therapeutics.