THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lum EL, Nassiri N, Kogut N, et al. Delayed immune tolerance through donor haematopoietic stem cell infusion 14 months after kidney transplantation. *Lancet* 2024; **404:** 1346.

Delayed immunological tolerance in the setting of pre-existing kidney transplantation
Appendix #1: Clinical Trial Protocol details and Monitoring Schedule

The following logistic activities have already been achieved for the Delayed Tolerance trial: IND clinical protocol approval, IRB approval, study start-up, study initiation visit, CRF development.

The overall study flow is as follows: patient identification and pre-screening, consent, recipient and donor screening, recipient and donor eligibility confirmation, donor cell mobilization and collection, cell manufacturing and processing, recipient rATG and TLI conditioning regimen, stem cell infusion, recipient safety follow-ups, recipient chimerism and immune assessment follow-ups. Each step is detailed, along with the study timeline below.

Patient Identification/Pre-screening: Patients will be identified via UCLA chart review, transplant coordinator meetings, or through advertising. Utilizing an approved HIPAA waiver, patient charts will be screened by study coordinators and or transplant coordinators to determine possible candidates. If patients are candidates, a sub-investigator reaches out to the potential donor to determine interest. If the donor is interested, the recipient is then separately contacted to determine interest. Once this interest is confirmed, a consent visit is scheduled for both donor and recipient.

Consent: Consent takes place between 4-12 weeks pre-infusion depending on the pace at which screening, and eligibility occurs. Consent is scheduled with a nephrology investigator and a hematology investigator. Both donor and recipient are present for the initial consent discussion. After discussion with both the nephrologist and hematologist, the donor and recipient are separated to complete consent. This ensures no coercion from either party. Consent takes place either in person at the UCLA Connie Frank Kidney Transplant Center or remotely via teleconference and utilizing 21 CFR part 11 DocuSign to obtain written consent. The donor and recipient also receive the California Experimental Bill of Rights, sign a HIPAA waiver, and receive copies of all documents signed.

Screening: Once consent is obtained, the screening process begins. A hematologist co-investigator will present the proposed case to the BMT review committee which reviews all proposed standards of care and research BMT transplants. This committee will reject the case or allow screening and review to move forward.

The recipient is screened first. The recipient comes to the UCLA Bowyer oncology center for a visit with a study hematologist in which a complete physical examination and medical history is obtained. The recipient then goes through the standard UCLA allogeneic transplant screening process which includes the following: hematology tests, infectious disease blood tests, echocardiogram, pulmonary function test, chest x-ray, high resolution HLA typing, allogeneic transplant consent, and a physical exam with a hematologist co-investigator. This includes multiple appointments which is structured over the course of 1-2 days. Once results are received, an eligibility checklist is completed. If a patient is preliminarily eligible, the donor is then brought in for screening. This includes the following: hematology tests, infectious disease blood tests, high resolution HLA typing, a physical exam with a different hematologist co-investigator, and a consult with hemapheresis. The consult with hemapheresis includes a vein

assessment to determine whether a central line placement is necessary for the upcoming apheresis procedure. Once all assessments are completed, eligibility is determined.

Eligibility: The research coordinator will compile all results and document on two eligibility checklists which mirrors the protocol inclusion and exclusion criteria: one for the donor and one for the recipient. Each criterion will have a matching source to verify the potential patients meet all protocol inclusion/exclusion criteria. The PI or delegated sub-investigator reviews the eligibility documents and signs off to confirm eligibility. This is then verified by the UCLA data and safety monitoring board (DSMB). Once DSMB confirms final eligibility, the recipient and donor are enrolled onto the study.

Donor Activities:

Apheresis: The donor proceeds with starting GCSF injections which are prepared by the UCLA investigational pharmacy. The first injection is scheduled a nurse visit in the Bowyer oncology center, where a nurse instructs the patient how to self-inject. Then the patient proceeds with at-home injections for a total of 6 days of GCSF injections. Should a patient desire, he or she can elect to come in and complete all injections via nurse visit at the Bowyer oncology center. On day 5, after the morning GCSF injection, donor comes to the hemapheresis center to start apheresis. If a central line was indicated, donor first goes to the surgical center to have a central venous catheter inserted in the groin – the surgical suite is in the same building as the hemapheresis clinic. After labs are collected, donor begins apheresis. The donor completes apheresis for approximately 4 hours. They are monitored by the hemapheresis staff for side effects and any adverse events. The product is then sent to the UCLA HSCT Stem Cell Laboratory to begin the cell processing and selection. If the stem cell lab confirms that enough CD34 cells were collected, the donor does not come back for day 6 of apheresis and GCSF. If not, the donor comes back on day 5 for a dose of plerixafor to ensure enough product is collected. Then the same procedure is followed on day 6 – if the patient had a central line inserted, it is then removed.

Stem Cell Processing: When participants are found to be eligible, the study team immediately orders the required amount of processing materials to be shipped to the stem cell lab. If additional products are required, the stem cell lab team consults with other labs across the Southern California region to obtain product. The products from apheresis are brought to the UCLA stem cell lab – the cells are separated and counted. If enough cells are harvested, day 6 apheresis is cancelled. If not, more cells are collected on day 6. After both products are collected, the products are processed, selected for, counted, and tested for contamination. A sample is sent out for endotoxin testing. Following processing, the CD34 cells and CD3 cells are prepared and frozen according to protocol.

Follow-up: After collection of the stem cells, the donor is then followed for 1-4 weeks after hemapheresis for adverse event monitoring and collection. The donor may return for follow up with the transplant group and labs may be completed if clinically indicated.

Recipient Activities

Radiation Planning Visit: The recipient is seen by a radiation oncologist for consultation and planning – this usually occurs in conjunction with the screening process to minimize visits

for the patient. Approximately 1-4 weeks prior to the start of conditioning, the recipient comes in and completes a CT-scan for radiation therapy simulation and treatment mapping.

Pre-HSPC Infusion Visit: Approximately 1-4 weeks prior to treatment, the recipient is seen by the transplant team in the Connie Frank Kidney Transplant Center for a final review of HSPC transplant evaluation and to answer any questions the recipient or his/her family may have. Labs are drawn for safety and any additional clinical labs are added as clinically indicated.

Conditioning: Starting at Day 0, over two weeks, the recipient comes in every day for the conditioning regimen. On a Monday (or other day if necessary), the recipient begins the conditioning regimen. To maximize ease for the patient, the conditioning regimen is started in the Connie Frank Kidney Transplant Center. A peripheral venous catheter is placed. Safety labs are taken in the morning consisting of CBC w/differential and CMP to review possible significant thrombocytopenia or the development of infection of neutropenic fever – this will occur every day over the next two weeks to ensure safety of the patient for radiation therapy. Methylprednisolone is infused as pre-medication at a dose of 5 mg/kg IV with a maximum dose of 250mg. Then rATG infusion is administered at a dose of 1.5 mg/kg. This process takes approximately 4-5 hours. Once infusion is complete and the patient is monitored for adverse events, the patient proceeds downstairs in the same building to the radiation oncology suite. There, the first fraction of TLI at 120cGY is administered – this visit takes approximately one hour. For the rest of the first week,(I.e. Monday through Friday), this same pattern takes place each day until all 5 doses of rATG are completed. The steroid dose as pre-medication tapers each day. After completion of rATG, on days 5 and 6 (or the weekend), the patient does not come into clinic nor receives TLI. Patient continues the taper of steroids, and takes oral prednisone – 60 mg on day 5 and 40 mg on day 6. The recipient is asked to record this on a diary.

On week 2, patient begins TLI once per day again. The recipient comes early in the morning to complete safety labs – prior to TLI start, an investigator confirms safety parameters are met. Each day the recipient receives a 120cGy fraction – a total of 10 fractions are given over the course of conditioning. The patient continues oral steroids on a taper until their last dose on day 10. During the course of the conditioning, the recipient may be seen by either the nephrologist or the hematologist investigator as needed, but will have at least two visits over the course of the weeks. Additionally, on treatment visits with the radiation oncologist will also occur once per each week during the conditioning regimen.

Over these two weeks, the recipient begins taking tacrolimus 0.075 mg/kg orally twice per day – the target trough level is 10/12 ng/ml. The study nephrologist manages the dose to adhere to the protocol desired trough levels. The recipient will also begin prophylactic medications – valganciclovir, acyclovir, trimethoprim/sulfamethoxazole, nystatin, and fluconazole if required. These doses will be adhered to as per protocol unless the recipient has an adverse toxicity to any of these medications – doses may be changed or removed entirely as per clinical discretion.

Infusion: On day 11 (i.e. Friday if treatment begins Monday the week before) patient comes in for their infusion visit. The day begins with safety labs in the morning at Connie Frank, then a visit with the study nephrologist. Immediately following, the recipient receives the final

fraction of TLI. Once completed, the patient then goes to the Bowyer infusion center. (Of note, all of these clinics are located in the same building – should a patient need transport, transportation services are scheduled). Standard premeds are administered. Following pre-meds, the patient receives the CD34 cell infusion. Once completed, the patient waits an hour. After at least one hour, the next infusion of CD3 cells occurs. The nursing team then monitors the patient for at least two hours for any adverse events. The patient is seen by the BMT hematologist either bedside or after infusion in the nearby clinic. After confirming stability of patient, the patient heads home.

Chimerism and Immune Assessment Testing: The donor will provide DNA and blood samples for chimerism analysis and associated immune assessment assays as discussed in secondary endpoint 7. The recipient will also provide blood samples for chimerism testing and immune assessment assays during screening to provide a baseline sample. The recipient will be monitored for donor chimerism in whole blood and cell subsets including CD3+, CD33, CD19, and CD56 at the following timepoints: Weeks 6 and 10, and months 4, 5, 6, 8, 10, and 12. Whole blood chimerism is monitored only at Months 15, 18, 24, 36, and 48 post-HSPC infusion.

The chimerism results provided guide recipient treatment. If the patient maintains chimerism for at least 6 months (defined as at least 1% of donor cells in at least 1 cell line), the patient continues tapering and eventual removal of immunosuppression, even if they lose chimerism later. If the patient gains and then loses chimerism in the first 6 months, they are withdrawn from study treatment and placed back on standard immunosuppression. If the patient never obtains chimerism, a bone marrow biopsy is completed within a week of the second negative result for safety purposes. The stored samples during this period of time will then be analyzed to evaluate the mechanism predicting loss or maintenance of donor mixed chimerism and clinical immune tolerance.

Appendix #2: Schedule for immunosuppression withdrawal

Immune Suppression Tapering and Subject Follow-up: The recipient begins the follow-up and tapering process starting on day 14, or week 3 of the study treatment period. The recipient completes safety labs and sees the nephrologist at the Connie Frank clinic and also sees the study hematologist for stem cell transplant follow-up. At this point, the recipient has appointments with the study team twice a week, then tapers to once a week starting on week 5, then every two weeks starting on week 12 through month 6. During this time, they are receiving safety laboratory tests at the same schedule. Tacrolimus is dosed to 10-12 ng/ml through week 12, 8-10 ng/mL in months 4 and 5, and 8 ng/ml in month 6.

After month 6, patients can leave the local Los Angeles area if they should choose – if so, the study team works with the patient's local physician/lab to obtain required study safety labs. Starting month 7, recipient sees the study team monthly. Safety labs are taken every other week. Tacrolimus continues to be tapered to 6 ng/mL in Month 7, 4 ng/mL in Month 8, 3 ng/mL in Month 9, 2 ng/mL in month 10, and <1.5 ng/mL in Month 11. If all is set and patient is doing well, they are then removed from tacrolimus during the second or third week of month 12. All other prophylactic medications are also stopped. Patient continues slowly tapered follow up visits through month 48.

Appendix #2: Chimerism level over time

IMMUNOGENETICS CENTER REPORT

Chimerism Testing Report

Donor: P101D, Date of Bone Marrow Transplant: 02/24/2023

Donor: P101D Date of Kidney Transplant: 01/03/2022

Sample Date	Received Date	Sample ID	Specimen Source	Cell Type	Days Post Transplant	Donor Cells	Recipient Cells
07/17/2024	07/17/2024	806593	PB	Unfractionated PBMC	509	2%	98%
07/17/2024	07/17/2024	806593	PB CD3+	CD3	509	2%	98%
07/17/2024	07/17/2024	806593	CD19+	CD19	509	2%	98%
07/17/2024	07/17/2024	806593	CD56+	CD56	509	2%	98%
07/17/2024	07/17/2024	806593	CD33+CD66+	CD33	509	2%	98%
05/15/2024	05/15/2024	804127	PB	Unfractionated PBMC	446	4%	96%
05/15/2024	05/15/2024	804127	PB CD3+	CD3	446	4%	96%
05/15/2024	05/15/2024	804127	CD19+	CD19	446	4%	96%
05/15/2024	05/15/2024	804127	CD56+	CD56	446	4%	96%
05/15/2024	05/15/2024	804127	CD33+CD66+	CD33	446	4%	96%
01/10/2024	01/10/2024	799039	PB	Unfractionated PBMC	320	3%	97%
01/10/2024	01/10/2024	799039	PB CD3+	CD3	320	2%	98%
01/10/2024	01/10/2024	799039	CD19+	CD19	320	4%	96%
01/10/2024	01/10/2024	799039	CD56+	CD56	320	3%	97%
01/10/2024	01/10/2024	799039	CD33+CD66+	CD33	320	2%	98%
11/15/2023	11/15/2023	797214	PB	Unfractionated PBMC	264	4%	96%
11/15/2023	11/15/2023	797214	PB CD3+	CD3	264	2%	98%
11/15/2023	11/15/2023	797214	CD19+	CD19	264	10%	90%
11/15/2023	11/15/2023	797214	CD56+	CD56	264	5%	95%
11/15/2023	11/15/2023	797214	CD33+CD66+	CD33	264	3%	97%
09/13/2023	09/13/2023	794741	PB	Unfractionated PBMC	201	16%	84%
09/13/2023	09/13/2023	794741	CD3+	CD3	201	4%	96%
09/13/2023	09/13/2023	794741	CD19+	CD19	201	41%	59%
09/13/2023	09/13/2023	794741	CD56+	CD56	201	21%	79%
09/13/2023	09/13/2023	794741	CD33+CD66+	CD33	201	15%	85%
07/12/2023	07/13/2023	792359	PB	Unfractionated PBMC	138	26%	74%
07/12/2023	07/13/2023	792359	PB CD3+	CD3	138	4%	96%

IMMUNOGENETICS CENTER REPORT

Chimerism Testing Report

07/12/2023	07/13/2023	792359	CD19+	CD19	138	51%	49%
07/12/2023	07/13/2023	792359	CD56+	CD56	138	28%	72%
07/12/2023	07/13/2023	792359	CD33+CD66+	CD33	138	26%	74%
06/14/2023	06/14/2023	791390	PB	Unfractionated PBMC	110	32%	68%
06/14/2023	06/14/2023	791390	CD3+	CD3	110	3%	97%
06/14/2023	06/14/2023	791390	CD19+	CD19	110	54%	46%
06/14/2023	06/14/2023	791390	CD56+	CD56	110	29%	71%
06/14/2023	06/14/2023	791390	CD33+CD66+	CD33	110	31%	69%
05/17/2023	05/17/2023	790364	PB	Unfractionated PBMC	82	36%	64%
05/17/2023	05/17/2023	790364	PB CD3+	CD3	82	4%	96%
05/17/2023	05/17/2023	790364	CD19+	CD19	82	60%	40%
05/17/2023	05/17/2023	790364	CD56+	CD56	82	23%	77%
05/17/2023	05/17/2023	790364	CD33+CD66+	CD33	82	35%	65%
04/19/2023	04/19/2023	789252	PB	Unfractionated PBMC	54	43%	57%
04/19/2023	04/19/2023	789252	PB CD3+	CD3	54	4%	96%
04/19/2023	04/19/2023	789252	CD19+	CD19	54	63%	37%
04/19/2023	04/19/2023	789252	CD56+	CD56	54	17%	83%
04/19/2023	04/19/2023	789252	CD33+CD66+	CD33	54	46%	54%
03/20/2023	03/20/2023	788049	PB	Unfractionated PBMC	24	26%	74%
03/20/2023	03/20/2023	788049	PB CD3+	CD3	24	2%	98%
03/20/2023	03/20/2023	788049	CD19+	CD19	24	21%	79%
03/20/2023	03/20/2023	788049	CD56+	CD56	24	3%	97%
03/20/2023	03/20/2023	788049	CD33+CD66+	CD33	24	28%	72%

This short tandem repeat based engraftment test has a high degree of accuracy in distinguishing the cells of origin from two individuals or in detecting mixtures provided the minority population is present in a proportion of at least 1%. Purity of the cell subsets is >90% as determined through protocol validation studies.

BM = Bone Marrow.
PB = Peripheral Blood.