Study protocol

Immune monitoring to facilitate belatacept monotherapy

1. Synopsis

Title	Immune monitoring to facilitate belatacept monotherapy
Study design	Prospective single-center, single-arm pilot study
Study center	Massachusetts General Hospital
Principal investigator	Dr Hannah Gilligan
Co-investigators	Dr Thomas Vanhove
Objectives	1. To determine the percent of belatacept-treated renal transplant patients that can be safely converted to belatacept monotherapy 2. To determine the utility of novel immune monitoring tools to facilitate belatacept monotherapy
Primary endpoint	Occurrence of biopsy-proven acute rejection
Secondary endpoints	- Change in eGFR (CKD-EPI) - Appearance of proteinuria - Appearance of de novo donor-specific antibodies - Overall and death-censored graft survival
Sample size	20
Summary of inclusion criteria	- Age 18 years or older - Written informed consent - Single kidney transplant recipient (i.e. no combined organ transplants) - Treated with belatacept - At least 1 year after transplantation or after initiation of belatacept - Stable renal function - Blood biomarkers indicate immune quiescence - No history of biopsy-proven acute rejection - Absence of donor-specific antibodies (at any MFI) - No history of BK viremia in current allograft

	- Spot urine protein/creatinine ratio < 0.5 g/g
Maximum duration of follow-up per participant	24 months after inclusion
Protocol version	Version 1.4 (10/25/2019)
Version and date of protocol amendments	N/A

2. Background

Belatacept is a selective costimulation blocker that is a non-nephrotoxic alternative to the calcineurin inhibitors cyclosporine and tacrolimus. In renal transplantation, belatacept has demonstrated similar graft and patient survival compared with cyclosporine, but is associated with superior renal function and lower rates of donor specific antibody (DSA) development.[1,2]

Like calcineurin inhibitors, belatacept is generally prescribed as part of an immunosuppressive regimen that includes either mycophenolic acid (MMF) or and mTOR inhibitor (mTORi) and steroids. Clinical experience suggests that in a significant proportion of belatacept-treated patients, MMF, mTORi and/or steroids can be reduced or even stopped, likely because they have reached a state of partial immunological tolerance. However, true personalization of immunosuppressive therapy, i.e. delivering the minimum amount of immunosuppression necessary to prevent kidney graft rejection for any given patient, is not yet possible because of a lack of reliable tests to monitor the degree of alloimmune reactivity in a kidney graft. So-called protocol biopsies are useful in this respect, but are invasive and suffer from sampling bias and inter-observer variability.

Several non-invasive immune monitoring tools have recently been developed that may aid in the personalization of immunosuppressive therapy. Two in particular are the focus of the current study. First, the presence of donor-derived cell-free DNA (dd-cfDNA) in the blood of kidney transplant recipients has been demonstrated to reflect graft injury.[3–6] Very high values are a strong predictor of antibody-mediated rejection and low values (<1%) exclude active rejection with a negative predictive value of up to 98%.[7] Second, a blood-based gene expression array (Trugraf™) can predict subclinical rejection in stable allografts with a negative predictive value of 78-88%.[8]

Whether patients with both dd-cfDNA levels < 1% and a Trugraf profile compatible with a state of immunological quiescence can safely undergo a reduction in the intensity of their immunosuppressive regimen has not been explored. In the current study, this hypothesis will be tested in belatacept-treated patients because they have a relatively high a priori likelihood of being in a state of immunological quiescence, which is also reflected by low rejection rates (<1%) after the first posttransplant year.[9]

3. Trial objectives and Design

3.1 Trial objectives

- To determine the utility of novel blood-based immune monitoring tools (Allosure and Trugraf) to facilitate belatacept monotherapy.

- To determine the percent of belatacept-treated renal transplant patients that can be safely converted to belatacept monotherapy.

3.2 Trial design

Prospective single-center, single-arm interventional pilot study

3.3 Primary endpoint at 24 months

- Occurrence of biopsy-proven acute rejection

3.4 Secondary endpoints at 24 months

- Change in eGFR (CKD-EPI)
- Appearance of proteinuria
- Appearance of de novo donor-specific antibodies
- Overall and death-censored graft survival

4. Selection and withdrawal of subjects

4.1 Inclusion criteria

- Age minimum 18 years
- Written informed consent
- Single kidney transplant recipient (i.e. no combined organ transplants)
- Treated with belatacept
- At least 1 year after transplantation or after initiation of belatacept
- Stable renal function (eGFR > 40 ml/min continuously during previous 6 months)
- Blood biomarkers indicate immune quiescence (for Allosure this corresponds to dd-cfDNA < 1%; for Trugraf this corresponds to “TX” signature)
- No history of BK viremia in current allograft

4.2 Exclusion criteria

- History of biopsy-proven acute rejection
- Presence of donor-specific antibodies (at any MFI)
- Spot urine protein/creatinine ratio > 0.5 g/g

4.3 Selection of participants

Belatacept-treated single kidney transplant recipients will be recruited from the outpatient clinic. Eligible patients are preselected through review of medical records. They are informed of the possibility to participate by a physician (the co-investigator, who is not their own nephrologist) at the time of an outpatient clinic. They will be given a copy of the informed consent and asked to call the co-investigator if they are interested in participating.

4.4 Withdrawal of subjects

Subjects will be withdrawn from the trial in case of:

- Refusal of the subject to continue
- For any other reason deemed necessary by the investigator and in the interest of the subject.

In the following circumstances, subjects will continue to be monitored in the study but immunosuppression will not be weaned further:

- Either one of the immune monitoring tests are positive at any point (Allosure > 1% or Trugraf signature compatible with rejection)
- Occurrence of biopsy-proven acute rejection (Banff 2017 criteria)
- Occurrence of new proteinuria > 0.5 g/g creatinine, confirmed on repeat urine sample after 2-4 weeks
- Occurrence of de novo donor-specific antibodies at any MFI
- Decrease in eGFR > 20% from baseline without alternative explanation, confirmed on repeat urine sample after 2-4 weeks

5. Trial Procedures

5.1 By visit

For all belatacept-treated patients, monthly visits in the outpatient clinic, where belatacept infusions are administered, are part of routine clinical care. Trial visits will coincide with these infusion visits, occurring monthly for the first year after inclusion. At study visits, subjects are questioned and examined by a physician and blood samples are drawn (see below). For patients who receive monthly belatacept infusions at home, they will have blood samples (clinical and research specimens) drawn at their local Quest laboratory and the study physician will call them to assess for adverse events. If any adverse events are noted, a telemedicine or in-person visit with a transplant nephrologist will be scheduled at the discretion of the investigator.

Reduction of immunosuppression occurs in a stepwise fashion, whereby each reduction is contingent on several factors:

- Renal function is stable, defined as eGFR no more than 20% lower compared to value at inclusion
- Both the Allosure and Trugraf tests indicate a state of immune quiescence. For the Allosure, this corresponds to a dd-cfDNA concentration of < 1%; for the Trugraf test, this corresponds to a “TX” signature.
- No appearance of any de novo DSA (at any MFI) at any previous point during study
- No proteinuria > 0.5 g/g creatinine on spot urine sample

If, at any point, a patient does not fulfill all of the abovementioned criteria, immunosuppression will not be (further) reduced. In such cases, the principal investigator will decide whether to continue with the reduced-intensity immunosuppressive regimen or revert back to conventional triple therapy (belatacept + mycophenolate/mTORi + steroids).

Criteria for performing a kidney biopsy are:

- Unexplained decrease in eGFR > 40% from baseline value
- Occurrence of new proteinuria > 1.0 g/g creatinine on 2 separate random urine samples

The principal investigator can decide to perform a kidney biopsy in patients who do not strictly meet the abovementioned criteria, if the probability of rejection is felt to be high, i.e. a combination of abnormalities such as new DSA and proteinuria < 1 g/g, or a very high (>3%) Allosure result combined with a mild (20%) decrease in eGFR.

In case of a discrepancy between Allosure and Trugraf results (i.e. Allosure < 1% but Trugraf positive for rejection), immunosuppression will not be weaned further.

Study timeline

Time point	**Blood sample including immune monitoring**	**Urine protein**	**DSA monitoring**	**Action if stable renal function, immune quiescence, no de novo DSA, no proteinuria > 0.5 g/g**
Inclusion	Yes	Yes	Yes	
Month 1	Yes	Yes		50 % reduction of MMF/mTORi dose
Month 2	Yes			
Month 3	Yes		Yes	
Month 4	Yes	Yes		Stop MMF/mTORi
Month 5	Yes			
Month 6	Yes		Yes	
Month 7	Yes	Yes		Reduction in prednisone to alternating 5mg – 2.5mg
Month 8	Yes			Reduction in prednisone to 2.5mg
Month 9	Yes		Yes	Reduction in prednisone to 2.5mg – 0mg alternating
Month 10	Yes			Stop steroids
Month 11	Yes			
Month 12	Yes	Yes	Yes	

From months 13 through 24, subjects will resume routine (standard of care) clinical monitoring, namely monthly laboratory analyses when they get their belatacept infusion. In addition to this, proteinuria and DSA will be checked at 24 months (which is not standard of care).

5.2 Laboratory testing and sample shipping

The following laboratory tests are performed at each study visit during the first 12 months:

- Routine laboratory analyses, including complete blood count, basic metabolic panel, total protein, fasting glucose. These analyses will be performed by the Central Laboratory of Massachusetts General Hospital or at the patient's local Quest laboratory.
- Two 10 ml Streck Cell-Free DNA BCT® whole blood tubes are drawn and stored at 7°C pending shipment. Streck tubes are shipped at ambient temperature (between 6° and 37°C) to CareDx using the shipping material and instructions provided by CareDx.
- Two 2.5ml Paxgene® whole blood tubes are drawn and stored at -20°C pending shipment. These tubes are shipped on ice (-20°C) to Transplant Genomics.
- One 10 ml EDTA and one 10 ml SST tube will be collected, centrifuged at 4°C for 10 minutes at 3000 RPM. The resulting 5 ml of EDTA plasma and 5 ml of SST plasma will be stored at -80°C pending possible future analysis (i.e. biobanked).

Donor-specific antibody screening occurs at 1, 4, 7, 10, 12 and 24 months. These analyses will be performed by the HLA laboratory of Massachusetts General Hospital.

6. Statistics

Normality will be tested using the Shapiro-Wilk test. Average eGFR and proteinuria at baseline and 24 months will compared using paired samples t test. A 2-sided p-value < 0.05 will be considered statistically significant. All analyses will be performed using IBM SPSS Statistics version 23 (IBM, New York, NY). Figures will be generated using Graphpad Prism version 6 (Graphpad Prism, La Jolla, CA).

7. Direct access to source data and document

The investigator and the institution will permit trial-related monitoring, audits, EC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents (e.g. subjects' case sheets, blood test reports, X-ray reports, histology reports etc).

8. Ethics and regulatory approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of GCP and in accordance with all applicable regulatory requirements. This protocol and related documents will be submitted for review to the Ethics Committee.

The Study can and will be conducted only on the basis of prior informed consent by the Subjects, or their legal representatives, to participate in the Study. The Participating Site will obtain a signed informed consent form (ICF) for all subjects prior to their enrollment and participation in the Study in compliance with all applicable laws, regulations and the approval of the (local) Ethics Committee, if required. The Participating Site will retain such ICFs in accordance with the requirements of all applicable regulatory agencies and laws.

The Investigator and the Participating Site will treat all information and data relating to the Study disclosed to Investigator in this Study as confidential and will not disclose such information to any third parties or use such information for any purpose other than the performance of the Study. The

collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data.

9. Data Handling and Management

The principal investigator is responsible for monitoring efficacy and safety every time a patient presents to the transplant clinic, which in principle is monthly for the duration of the study. An adverse event tracking log will be kept and unanticipated adverse events will be reported to Partners' IRB within 5 working days, in accordance with PHRC guidelines for adverse event reporting.

The PI is responsible for monitoring the validity and integrity of the data and adherence to the IRB-approved protocol.

Most of the information collected on subjects throughout this study is stored in the Electronic Medical Record (Epic) as part of routine clinical care. This includes vital parameters, laboratory results (including Allosure results, which are reported in Epic), reported adverse events and clinical findings on monthly outpatient visits. Relevant findings are extracted to an anonymized spreadsheet. Only the (co-)principal investigators will have access to the file that links patient names to anonymized identifiers. Paper informed consent documents are stored securely in the Department of Nephrology.

10. Financial Aspects

The study is financed by the companies CareDx and Transplant Genomics.

11. References

1. Durrbach A, Pestana JM, Pearson T *et al.* A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT Study). *Am. J. Transplant.* 2010; **10**: 547–557.

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3. Bloom RD, Bromberg JS, Poggio ED *et al.* Cell-Free DNA and Active Rejection in Kidney Allografts. *J. Am. Soc. Nephrol.* 2017; **28**: 2221–2232.

4. Whitlam JB, Ling L, Skene A *et al.* Diagnostic application of kidney allograft-derived absolute cell-free DNA levels during transplant dysfunction. *Am. J. Transplant* 2019; **19**: 1037–1049.

5. Huang E, Sethi S, Peng A *et al.* Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am. J. Transplant* 2019; **19**: 1663–1670.

6. Gielis EM, Ledeganck KJ, Dendooven A *et al.* The use of plasma donor-derived, cell-free DNA to monitor acute rejection after kidney transplantation. *Nephrol. Dial. Transplant* 2019.

7. Oellerich M, Shipkova M, Asendorf T *et al.* Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study. *Am. J. Transplant* 2019.

8. Friedewald JJ, Kurian SM, Heilman RL *et al.* Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant. *Am. J. Transplant* 2018.