의학박사 학위논문

한국 장기이식 코호트 (KOTRY) 에서 신이식의 예후 분석 및 항체매개 거부반응의 영장류 혈관이식 모델 개발

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2020 년 8 월

서울대학교 대학원
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이 논문을 의학박사 학위논문으로 제출함

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정종철의 박사학위논문을 인준함

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Abstract

Outcome analysis of renal allograft in Korean Organ Transplantation Registry (KOTRY) and the development of a non-human primate vascular graft model of antibody mediated rejection

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Background

Antibody mediated rejection (ABMR) is a significant risk factor for the long-term kidney allograft survival and a hurdle to the highly-sensitized transplantation. Large scale cohort or registry is needed to study clinical transplantation with adequate statistical power. Eplet of human leukocyte antigen is important molecular target to the development of ABMR. ABMR in xenotransplantation are being overcome by the genetic manipulation of donor pig. However, immune sensitization from natural antibody or induced antibody is still important challenge to xenotransplantation. One hurdle to study ABMR in non-human primate xenotransplantation model is the technical complexity, which is being improved by porcine artery patch graft model.

Methods

To study clinical predictors of early post-transplant outcome and clinical usefulness of eplet mismatch, patients included in Korean Organ Transplantation Registry (KOTRY) were used. Kidney transplant recipients who have been transplanted from 2014 to 2018 were enrolled. As variable selection methods, least absolute shrinkage and selection operator (LASSO) and backward stepwise elimination were used.

Dominance analysis was used to rank relative importance of selected variables. Eplet mismatch were imputed by using human leukocyte antigen (HLA) 4 digits transformation based on HLA haplotype distribution. Fractional polynomial was used for the non-linear modeling of eplet mismatches to acute rejection. Also, pathogenesis of ABMR were studied by using xenotransplantation model. As a repeated xenotransplantation model, porcine arterial grafts from GalT knockout pig were transplanted to cynomolgous monkeys under the triple immunosuppressants, anti-CD154 monoclonal antibody and stratification on the anti-thymocyte globulin.

Results

Among 4,839 kidney transplant recipients from KOTRY, overall patient survival rates were 98.4%, 97.8%, and 97.6% at 1, 3, and 5 years, respectively. Death-censored graft survival rates were 98.4%, 97.0%, and 96.9% at 1, 3, and 5 years, respectively. Biopsy-proven acute rejection free survival rates were 90.3%, 87.6%, and 87.3% at 1, 3, and 5 years, respectively. Acute T-cell mediated rejection free survival rates were 92.8%, 91.0%, and 90.6% at 1, 3, and 5 years, respectively. Acute antibody mediated rejection free survival rates were 96.5%, 95.2%, and 95.2% at 1, 3, and 5 years, respectively. In the KOTRY study population, the most dominant predictors to acute rejection within 1 year were donor age, and the mismatch number of HLA. Dominant factors to antibody mediated rejection were desensitization, followed by ATG induction, HLA mismatch numbers. Eplet mismatches were significant independent risk factors to acute rejection even in the low HLA locus mismatch subgroups, which was most prominent in the HLA class II eplet to the association of biopsy-proven T-cell mediated rejection. Compared to the previous porcine patch graft model, this porcine arterial graft model, functional monitoring (auscultation, Doppler) and safe graft removal were possible, which enabled unique sensitization model in xenotransplantation. Elevated serum interleukin 6, enhanced

complement dependent cytotoxicity, elevated tissue factor expression in harvested xenograft, and vigorous rejection histology were observed.

Conclusion

In this study, I found dominant predictors for acute rejection by using KOTRY data, and validated clinical usefulness of eplet mismatches. In repeated xenotransplantation model, increased ABMR were associated with enhanced complement dependent cytotoxicity, elevated level of peripheral IL-6 and prominent tissue factor expression at the xenograft.

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keywords: Organ transplantation registry, antibody mediated rejection, non-human primate study, eplet mismatches

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1. Introduction

In transplantation field, nationwide or international transplantation registries has provided many valuable data resource and led the development of clinical science of transplantation. [1–3] The Korean Organ Transplantation Registry (KOTRY) group had been operating observational cohort of organ transplantation since 2012. Including myself, In 2014, the authors reported first nationwide retrospective data summary of 4,500 kidney transplantation cases which have received operation between 2009 and 2012. [4] Based on that project, prospective observational cohort of 5 different organ transplantation (kidney, liver, heart, lung and pancreas) started in 2014 with the same name as KOTRY. [5] KOTRY is composed of 5 solid organ transplantation cohorts, including those of kidney, liver, heart, lung, and pancreas transplants. KOTRY is expected to answer the following fundamental questions:

1. What is the primary indication for solid organ transplantation in the Korean population?
2. How severe is the comorbidity burden of solid organ transplantation?
3. What are the immediate post-surgical risks of solid organ transplantation?
4. What is the long-term course of solid organ transplantation?
5. What is the most common cause of death after solid organ transplantation?
6. What is the most common cause of allograft failure?
7. What is the prevalence of induction and maintenance immunosuppression?
8. What is the prevalence of post-transplant comorbidities?
9. What are the genetic factors associated with the deterioration of allograft function?
10. What are the biomarkers that predict the deterioration of allograft function?
11. What are the short- and long-term courses of living donors?

Among these questions, understanding the mechanism of transplanted organ rejection is critical, because it is associated with the early outcome and long-term prognosis of clinical transplantation. To understand the impact of clinical predictors in the real-world kidney transplantation, and to further raise study questions in terms of molecular mechanisms, three-way strategies were adopted: (1) I deployed the exploration study of clinical predictors to early post-transplant outcomes including acute rejections by developing clinical kidney transplantation cohort (KOTRY & Asian society transplantation registry (ASTREG)) and analyzing its early outcomes, (2) I investigated the role of molecular discrepancies of human leukocyte antigen by adopting the concept of eplet mismatches and, (3) I investigated the immunological phenomenon and involved mechanism of antibody-mediated rejection combined with xenogenic coagulation by using novel porcine vascular conduit retransplantation model.

In terms of statistical modelling used in exploratory study, most of clinical epidemiological studies have long used inferential methods which is based on knowledge of expert and predefined hypothesis. On the contrary, data-driven approach does not depend on prior hypothesis, which is usually used to build a prediction or prognostic model. Prognostic models in kidney transplantation is an active area of research, however, it was scarce to compare relative importance or weight of clinical predictors to post-transplant outcomes. [6,7,16,17,8–15] In the present study, I tried to compare relative importance of clinical predictors based on data-driven approach.

Human leukocyte antigen is target antigen in transplantation. Development of molecular and structural biology have led new findings in this molecule, which have been exploded in recent decades. With the advent of immunosuppressant, HLA mismatches are not anymore contraindication in solid organ transplantation. However, it is still significant risk factors for long term graft survival and acute

rejection in spite of modern immunosuppression.[18] Antibody mediated rejection and accompanying donor specific antibody (DSA) is current research topic because it interfere long term graft survival.[19,20] The risk factors for the development de novo DSA are class II HLA mismatching, early T cell-mediated rejection, sensitization status, inadequate immunosuppression, or patient nonadherence. [21–27]

As the antigenicity determining site of HLA has been understood, the concept of molecular mismatch has been developed. Eplets are one of those achievements, which is defined as small configurations of amino acid residues that play dominant roles in HLA epitopes reactive with antibodies. [28] Compared to single amino acid polymorphism as a basic unit of antigen mismatch, an eplet represent the smallest functional unit of an epitope-paratope interface, and are under assumption that it react with the central complementary determining regions of the antibody and locates in the surface of HLA molecules.[29] Clinical outcomes with the molecular mismatches have been reported, however, most of the associations were interpreted as the process of chronic allograft rejection or gradual development of de novo DSA. It is relatively scarce to study the association of early post-transplant outcomes with molecular mismatches. I investigated whether eplet mismatches is associated with early post-transplantation outcome, and gives additional precision value to HLA genotyping by using KOTRY data.

Advancement of genetic manipulation to donor pig kidney and better understanding of immunologic response in xenotransplantation setting are the key drivers for the development of xenotransplantation. Depletion of carbohydrate surface antigen and insertion of human complement regulatory proteins have made long term survival of transplanted graft.[30] Recently, triple knock out and 9 human gene modified pig are prepared, which is expected to extend transplanted organ survival even further.[31] However, proper patient selection is still challenging, and presence of natural antibody or induced antibody from previous exposure to

xenogenic materials might be another challenge to successful xenotransplantation.[32] Only few studies have been done to the sensitized xenotransplantation. [33,34] One of the barrier to sensitization study in xenotransplantation was the complexity of surgical skill to functioning organ transplantation, whereas feasible skin transplantation have limitation to observe humoral immune response. Because solid organ transplantation in xenotransplantation had resulted in vigorous acute rejection, study for antibody mediated rejection and sensitization was difficult. Hence, a pig to non-human primate xenotransplantation model using porcine artery graft was developed during this study as a modified version of artery patch graft, [35] which enables us to harvest after single episode of transplantation and to observe histological changes. ATG was administered to compare whether T cell depletion might affect to the development of induced antibody and maintained transplanted xenograft until 4 weeks to allow antigen exposure during acquired immunity development period.

2. Material and Methods

2.1 Development of Clinical Kidney Transplantation Registry (KOTRY and ASTREG)

First, nationwide solid organ transplantation cohort (Korean Organ Transplantation Registry, KOTRY) were developed to continuously capture the clinical status of kidney transplantation patients in South Korea. The experience of developing transplant registry was expanded to the development of Asian Society Transplantation Registry (ASTREG). For the development of ASTREG, here I briefly describe only the difference compared to KOTRY, because of the similarity of design concept of both registry.

Study Organization

The KOTRY consists of 59 participating centers (30 centers for kidney, 15 for liver, 4 for heart, 5 for lung, 5 for pancreas), a central coordination unit, and a medical research coordinating center (MRCC). The organizational structures include the organ-specific committee, executive committee, and steering committee. A central coordination unit leads the study process, checks enrollment status weekly, and gives feedback to the participating centers. The MRCC is in charge of data validation and statistical consultation. The Korean National Research Institute of Health (KNIH) developed and offered a global web-based electronic data capturing system, named iCReaT. KNIH also participates in the quality assurance of the collected data, regular surveillance of study conductance process, and the management and improvement of the electronic data capturing system. Bio-specimen collection, storage, and quality control are done under contract with

LabGenomics, and part of the deposited biosamples are transferred to KNIH for backup and future collaboration. All of the activities are managed by the KOTRY Foundation (http://www.kotry.org/).

For ASTREG, any kidney transplantation center in Asia can freely use the ASTREG-H platform and contact the ASTREG office after registration as a participating center. Currently, six individual Asian kidney transplantation centers use the ASTREG-H platform, including centers in the Philippines, Mongolia, Myanmar, and South Korea.

Exclusion criteria, Enrollment and Informed Consent

Recipients younger than 19 years are excluded. Except simultaneous pancreas-kidney co-transplantation, those undergoing simultaneous multi-organ transplantation are excluded to ensure the homogeneity of graft-related outcome. However, sequential organ transplantations are not excluded. For liver transplantation, there is no exclusion criteria for age. For the KOTRY, both the donor and recipient are required to register at KOTRY prior to transplantation for living donor organ transplantation. The medical records of eligible individuals are reviewed after receiving their informed consent. Blood samples are taken for DNA and serum/plasma storage before transplantation. In deceased donor organ transplantation, informed consent is taken from the recipient. Under the strengthened data protection laws of Korea, the social security identification number cannot be collected during the KOTRY. However, for outcome matching with the Korean national statistical office data or the centralized health insurance claims data, KOTRY receives an optional informed consent for the use of collected data for study of secondary outcomes. To achieve the best standardized process, the opinions of

each individual institutional review board had been acquired, then a standardized protocol and standardized consent format were submitted. This study was approved by the institutional review boards of all participating centers, and was performed in accordance with the Declaration of Helsinki and the Declaration of Istanbul.

For the ASTREG, data on kidney transplantation can be collected by using the ASTREG-H platform. As an online data collection format, ASTREG-H does not use any specific exclusion criteria for data entry. However, the current data format is oriented toward solitary kidney transplantation, not toward simultaneous kidney pancreas co-transplantation or other solid-organ transplantations. ASTREG provides an online secure and friendly data entry platform that enables each participating center to enter and download their data by using a secure registered ID. The ASTREG-H platform provides automated data verification as a built-in data cleansing system, and the data manager produces data queries to each participating center for data errors in a deidentified manner. ASTREG anonymizes the data to take care of patient confidentiality and privacy issues. The Gachon University Gil Medical Center institutional review boards approved the whole platform system provision (IRB No. GAIRB2019-098), and the waiver of informed consent was approved.

Study Design and Collected Variables

The KOTRY collects solid organ transplantation data to analyze epidemiological trends, graft-related outcomes, and patient mortality. In total, data on 5,014 variables are collected, which are summarized in Tables 1–3. The kidney aspect involves a total of 950 variables, which are comprised of 12 domains of recipient data, 3 domains of donor baseline data, and 4 domains of living donor follow-up data. The liver aspect involves 523 variables in total, which consist of 13

domains of recipient data, 4 domains of donor baseline data, and 3 domains of living donor follow-up. The heart aspect involves 886 variables with 13 domains of recipient data, and 3 domains of donor baseline data. The lung aspect involves 1,495 variables with 22 domains of recipient data, and 3 domains of donor baseline data. The pancreas aspect involves 1,160 variables with 16 domains of recipient data, 4 domains of donor baseline data, and 3 domains of living donor follow-up. Each domain was constructed as a single sheet in electronic case-report format (CRF) on a web-based system (iCReaT). Longitudinal data collection is based on a regular annual interval. For the collection of early comorbidities and adverse outcome, different time points were selected according to each organ's clinical characteristics.

ASTREG-H collects data on pre-transplant clinical and laboratory profiles and post-transplant outcomes. The kidney component collects 227 items across five different domains (Table 4). One domain is for baseline donor characteristics, and the remaining four domains are for recipient data, including recipient baseline characteristics, details about immunosuppression, post-transplant event (irregular interval outcome), and post-transplant annual evaluation. The regular annual evaluation provides a longitudinal panel format, which enables a multilevel longitudinal data analysis. The post-transplant event record is based on the date of outcome occurrence, which enables a time-to-event analysis. Patient death and graft failure are the major outcomes. Graft failure is defined as sustained (>3 months) dependency on dialysis. A pathology report is another important outcome, which is a structured format that follows the BANFF classification scoring system. Few examples of the definitions of other important major post-transplant outcomes are as follows: Cardiovascular disease is defined as cardiovascular death, myocardial infarction, ischemic heart disease with relevant clinical evidence (accompanied by therapeutic intervention or objective findings), and new-onset congestive heart failure requiring hospital admission. Cerebrovascular accident is defined as non-

traumatic hemorrhagic or ischemic brain disease diagnosed using computed tomography or magnetic resonance image. Tuberculosis is defined as a clinically active disease evidenced by typical chest radiographic imaging, microbiological confirmation, or treatment with anti-tuberculosis drugs.

To account for the time-varying nature of post-transplantation comorbidity and to deal with repeated events, post-transplantation comorbidity at every follow-up visit were collected, which allows the analysis of comorbidity duration, and the effects of new-onset comorbidities and their duration on post-transplant outcomes. For example, the duration of transient new-onset diabetes after transplantation (NODAT) or repeated incidence of cardiovascular disease can be collected. Follow-up records will be tracked up to the patients' deaths. However, graft-related variables, including rejection, graft function, and general laboratory profiles, will be tracked until graft loss. To minimize follow-up loss, newsletters regarding registration status and follow-up performance are periodically sent to each participating center and a transfer system is used. If a patient underwent transplantation in center A, and was then followed by center B that also participated in KOTRY, the transfer system allows center B to input that patient's data. To increase the follow-up rate of living donors, the KOTRY emphasizes the importance of follow-up of living donors to each participating center's physicians and surgeons.

Biosamples in KOTRY

For the KOTRY, DNA samples from each donor and recipient are collected prior to organ transplantation. In kidney, heart, lung, and pancreas transplantation, sera are collected from recipients at baseline, prior to transplantation, and again at 1- and 3-years after organ transplantation. Baseline samples are collected in liver transplantation recipients. From 2017, additional plasma samples from the recipients

are collected prior to kidney transplantation, and again at 1- and 3-years post-kidney transplant.

Study Outcomes

The primary outcomes are graft failure and patient death. In kidney transplantation, graft failure is defined as sustained (more than 3 months) dependency on dialysis. In liver, heart, and lung transplantation, graft failure is defined as patient death or re-transplantation. Pancreas graft failure is defined as insulin dependence or death with a full or partially functioning graft.

Pathology data collected included acute or chronic rejection and other diagnoses, such as virus infection and calcineurin inhibitor toxicity. Definitions of the major post-transplantation outcomes are as follows: cardiovascular disease is defined as cardiovascular death, myocardial infarction, ischemic heart disease with relevant clinical evidence (accompanied by therapeutic intervention or objective findings), new-onset congestive heart failure requiring hospital admission and arrhythmia. Stroke includes non-traumatic hemorrhagic or ischemic brain disease confirmed by computed tomography or magnetic resonance image. Tuberculosis is defined as clinically active disease, as evidenced by typical chest radiography imaging, microbiological confirmation, or treatment with anti-tuberculosis drugs. Causes of death are classified into cardiovascular, sudden cardiac death, infection, malignancy, liver disease, accident, suicide, and others.

Living donor outcomes are collected for living liver or kidney transplantation cases. Death, cause of death, and surgical morbidities are collected in both liver and kidney transplantations. Newly developed diseases, including diabetes, hypertension, and urinary stones, are collected in living kidney transplantation donors.

Data validation

Quadruple layers of data validation are available. First, a pre-defined automated data validation system is used at data input, to prevent simple errors. Automated data validation system checks are implemented for essential data elements, to minimize missing variables, and have pre-defined allowed data ranges, to reduce extreme outliers due to simple input error. Additionally, an automated data validation system guide is used to prevent entering of values inconsistent with other variables, by opening or blocking data fields in screens following a logical test of pre-entered data values. Second, manual data validation is performed quarterly by the MRCC by feedback to each participating center. Third, during the outcome adjudication meeting, the distribution of major outcomes is discussed, and outlier values are sent to each investigator. Finally, annual auditing are conducted for all participating centers, to survey their status, including ethical study conductance, adherence to the standardized study protocol, and direct comparison of randomly selected data with the original medical record. These processes are conducted using the Registries for Evaluating Patient Outcomes tool by the Agency for Healthcare Research Quality (AHRQ).

Building statistical analysis files and response to the data request

A statistical analysis file is built thrice a year, following a quarterly data cleansing process. When the participating center requests their own data, the last validated statistical analysis file is sent to the requesting center. A feedback time of 4 hours was aimed at, in parallel with the standard operating protocol of Scientific Registry of Transplant Recipients (SRTR).[3] To request all centers' data, items of the

requested variable are released as a de-identified set (at patient- and center-level) after approval of the organ committee in KOTRY, following review of the study proposal. Each center has access to the main database located in KNIH, and can download their own dataset; however, this is not recommended due to network traffic and incompleteness of data validation. Currently, KOTRY focuses on the ease of data cleansing through an attached online automatic plotting system, and on giving more informative feedback to the participating center, and finally on enforcing information technology-security issues.

Statistical considerations

Descriptive data analysis will be conducted for baseline characteristics. To study outcomes, time–to-event analysis will be primarily used. Life-table methods or Kaplan-Meier curves will be used to represent allograft or patients' survival, and time to major outcomes (cardiovascular disease, cancer, infection, acute rejection, etc.). For the competing nature of outcome events (e.g., patient death vs. cancer occurrence), competing risk models will be adopted for regression modeling.[36] The multilevel characteristics of data were adjusted using a shared-frailty model,[37] in which adjustment should be made for time-dependent confounders or different data hierarchies. To encompass the wide variability of allograft functional decline, the Bayesian smoother will be used.[38] Longitudinal allograft functional changes and associated factors will be analyzed using a mixed linear model. Since the format of follow-up data is a repeated panel structure, the marginal structural model with time-varying confounder adjustment can be applied.[39]

Statistical Power

From 2017, new annual enrollments are estimated as 1,200 for kidney, 700 for liver, 100 for heart, and 30 for lung and pancreas transplantation, respectively. In kidney transplantation, the previous Retro-KOTRY collected the data of 4,987 kidney recipients, and the effort is ongoing to collect the missing information (approximately 1,200 kidney recipient's data) from the end of the previous Retro-KOTRY enrollment and the launch of the prospective KOTRY-kidney. With the assumption of attaining the patient enrollment plan, Table 5 shows the minimum hazard ratios (HRs) detectable at a given prevalence level of risk factors by 2019, using exponential models based on the 20-year patient and graft survival for solid organ transplants from the Organ Procurement and Transplantation Network. The KOTRY-kidney cohort is estimated to detect a relative risk of 1.05 and 1.06 for graft survival and patient survival, respectively, with a 50% prevalent risk factor, at 5% alpha error and 20% beta error in an analysis using a Cox regression model (Table 5). Similarly, the KOTRY-liver, heart, lung, and pancreas cohorts will be able to detect HRs of 1.11, 1.32, 1.87, and 1.82, respectively, for graft survival.

Representativeness

In 2015, the total numbers of organ-transplant centers and KOTRY-participating centers were as follows: for kidney, 30 of 66 centers participated in KOTRY; for liver, 15 of 44; for heart, 4 of 13; for lung, 5 of 7; for pancreas, 5 of 9. As large-volume centers joined KOTRY, the numbers of organ transplantations performed in KOTRY-participating centers were predominantly as follows: for kidney, 1565 of 1891 (82.8%); for liver, 1073 of 1392 (77.1%); for heart, 127 of 145 (87.6%); for lung, 61 of 64 (95.3%); for pancreas, 51 of 59 (86.4%).

2.2 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

Next, I investigated the early outcomes in KOTRY kidney transplant recipients, and dominant predictors for the early outcomes and acute rejection.

Study objective, design, covariables, and statistical approach

For this study, dataset of kidney transplant recipients who received kidney transplantation KT from 2014 to 2018 were used. Total 4,839 KT recipients were analyzed. Mean duration of follow up was 26.0 ± 15.5 months. To derive best prediction model for post-transplantation outcome (patient survival, graft survival, acute rejection, post-transplant eGFR) from baseline (pre-transplant) covariables, diverse variable selection approach were tried depending on the availability of existing methods to the character of variables. For the continuous measures, Leaps and Bound algorithm determined by Akaike's information criteria was used for variable selection. [40,41] For the time to event outcomes and binary outcomes, least absolute shrinkage and selection operator (LASSO) or backward stepwise selection were used. A total 20 covariate candidates for prediction model construction were as follows: recipient age, donor age, recipient sex, donor sex, recipient's history of diabetes, recipients' history of cardiovascular disease, recipient's history of cancer, pre-transplant systolic blood pressure of recipient, pre-transplant body mass index of recipient, donor's diabetes history, donor's hypertension history, waiting times to kidney transplantation, pre-transplant systolic blood pressure of donors, pre-transplant body mass index of donor, deceased donor, total numbers of HLA mismatches, desensitization, ATG as induction agent, smoking history of donors and recipients. When all covariables were entered to the prediction model for the death

censored graft loss, c-statistics was 0.692, which was comparable to the previous prediction studies.[12]

After model has been built, dominance analysis were applied to rank relative importance of each selected variables to target outcome.[42,43] Because dominance analysis can be applied to generalized linear model, application of the methods were conducted only for continuous or binary outcome such as patient/graft survival status at 1 year, acute rejection within 1 year, or post-transplant eGFR at 1 year. Continuous data are presented as mean with standard deviation. Categorical data are presented as count with percent. Cox regression for time to event data was done under the proportional hazard assumption. Statistical analyses were performed using Stata software (version 16; StataCorp LP, College Station, TX) and R (version 3.6.3; R Foundation, Vienna, Austria).

Study Ethics

The study protocol was approved by the Seoul National University Hospital institutional review board (IRB No:H-1902-138-1014). Data analysis was done with de-identified datasets. Patient privacy was preserved in all instances, and the study methods complied with the tenets of the Declaration of Helsinki.

2.3 Significance of eplet mismatch in rejection

Here, I further investigated the molecular representation of human leukocyte antigen mismatch as more precise target of transplantation rejection. I adopted the concept of eplet mistmach.

Study population and eplet estimation

Kidney transplantation donor-recipients pairs of KOTRY were study population. Until now, KOTRY has two separated phase of data collection, the first one was retrospective data collection of 2009 – 2012 kidney transplant patients, and the other one is prospective data collection from 2014. Design and methods and summary data of each phase of KOTRY were described in detail in the previous reports. [4,44] In both dataset, common components were pretransplant evaluation including all HLA genotype (2 digits) results, immunologic risks, induction and maintenance immunosuppressants, biopsy-proven acute rejection, graft function measured as eGFR, graft and patient survival. Details of biopsy reports were available in prospective KOTRY. For this study, dataset of kidney transplant recipients who received KT from 2009 to 2012 (retrospective data) and from 2014 to 2017 (prospective data) were used. Total 7,448 KT donor-recipients pairs were used for eplet estimation.

Previously validated multistep HLA imputation process were conducted to derive 4 digits HLA genotype from 2 digits genotype [45], which is a method based on HLA haplotype frequencies data set for target population. Imputation of HLA-A, -B, -DRB1, and -DQ alleles (4-digit specificity) were done by using Korean HLA haplotype distribution in bone marrow donors to adjust HLA distribution in Korean population. For class I eplet estimation, from total 7,448 patients, 6,834 (91.8%) patients' 4 digits 1st haplotype were successfully called. Among the 6,834 1st haplotype-called patients, 2,857 (41.8%) patients' 4 digits counter-phase haplotype were successfully called. Other 3,977 patients' haplotype were combined by using the mixture of the most frequent allele in each locus. For class II eplet estimation, 6,859 (92.1%) patients' 4 digits 1st haplotype were successfully called. Among the them, 3,012 (43.9%) patients' 4 digits counter-phase haplotype were successfully

called. Other 3,847 patients' haplotype were combined as the same way in class I eplet estimation. If the rare 2 digits genotype were not typed as 4 digits in the distribution reference database, those were considered as failure of imputation, and excluded from data analysis. Finally, 5,871 (78.8%) completely called pairs were used for analysis. The presence of individual eplet and numbers of eplet mismatches for each recipient and donor pair at HLA class I (HLA-A,-B) and class II (HLA-DR,-DQ) loci was imputed by HLAMatchmaker (Version 2.1).

Study Objective and Design

I tested whether eplet mismatches was associated with post-transplant graft outcomes. I performed multivariable analysis and adjustment. I tested whether eplet mismatch gives additional prediction value by the area under ROC curve comparison.[46]

Study outcome, exposure, mediator, and covariables

Post-transplant acute rejection (overall, biopsy-proven total, biopsy-proven cellular, biopsy-proven antibody-mediated) and eGFR were the main outcome. For the secondary outcome, allograft survival, interstitial fibrosis and tubular atrophy at biopsy within post-transplant 1 year were used. As study exposure, number of total eplet mismatches, number of class I or class II eplet mismatches were used, which was calculated described above. In our study, acute rejection was defined as composite outcome of clinical rejection (rejection treatment without kidney biopsy results) and biopsy proven rejection. Pathology reports were based on the reading of pathologist in local center. Data entry format of KOTRY necessitates the entry of individual component of Banff scoring.

Study Ethics, Covariables and Statistical model

The study protocol was approved by the Seoul National University Hospital institutional review board (No:H-1902-138-1014). Deidentified dataset was used, and patient privacy was preserved in all instances. The study was conducted under the Declaration of Helsinki. Missing rates of included covariables in KOTRY datasets were under 0.05%, which enables complete data analysis in the most of our analysis. Continuous data are presented as mean with standard deviation. Categorical data are presented as count with percent. Non-linearity was assumed to the number of eplet mismatches, which were conducted by applying fractional polynomial term to variable of interest. Time to event analysis was conducted by Cox regression under proportional hazard assumption. To compare model's predictability, I constructed multivariable logistic regression models to within 1 years outcome of interest (total rejection within 1 yr, biopsy proven acute rejection within 1 yr, acute T-cell mediated rejection within 1 yr, acute antibody-mediated rejection within 1 yr). As covariables, ten covariables were included in the multivariable logistic regression models: recipient age, recipient sex, donor age, donor sex, deceased donor, ATG induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Statistical significance of eplet mismatches were checked in graphic presentation with 95% confidence interval of predicted coefficients. Performance of overall prediction model was compared by Youden's index of area under ROC curve. All statistical analyses were performed using Stata software (version 16; StataCorp LP, College Station, TX).

2.4 Non-human primate model of antibody mediated rejection

I studied mechanism of antibody mediated rejection in the sensitized setting by using non-human primate xenotransplantation model.

Animals

Cynomolgous monkeys (Macaca fascicularis, M:F = 2:2, 4-6 years old) were used as recipients. Monkeys were obtained from Xenia (Seoul, Korea) and the nonhuman primate center of Korea Institute of Toxicology (Jeongeup, Korea). Genetically modified pigs (n=2, 10 - 20 kgs) which lacks alpha-Gal epitope (GalTKO) were used as donors to provide artery graft. [47] Maintenance care have been offered inside the animal care facility of Seoul National University Hospital. All procedures and medication were approved by the Seoul National University Institutional Animal Care and Use Committee. (IACUC No-15-0218) Experiments were performed under the guidelines in the National Institute of Health guide for the Care and Use of Laboratory Animals.

Surgical procedure of artery graft transplantation and removal

U shaped artery graft patch were produced from GalT-KO porcine aorta. GalT-KO pigs were sacrificed at 36 – 60 weeks after birth. Surgical procedures were as follows: Briefly, femoral artery and vein of recipients were connected by porcine artery graft similar as an arteriovenous shunt. After 1st xenotransplant experiment, all xenograft were removed by surgical exploration of inguinal area. After the periods of immunosuppressant weaning (more than 6 months), 2nd GalT-KO porcine artery graft xenotransplantation were conducted (n=4). All transplantation operation were successful without any significant bleeding complications, localized edema, or

occlusion of graft from immediate thrombus formation. The patency of xenograft were checked by Doppler ultrasound, manual inspection and auscultation. (Figure 1)

Immunosuppression and medical care

The experimental protocol is shown in Figure 2. Immunosuppression was based on the CD40-154 axis blockade (anti-CD154 mAb, 20mg/kg, Genexin, Seongnam, Korea) on Day -1, 0, 3, 7, 10, 14, 21. Cobra venom factor (0.05mg/kg, Quidel, San Diego, CA, USA) were given on Day -1, 0, 1 to suppress post-op immediate coagulation. Oral aspirin (50mg/day), low molecular weight heparin (1mg/kg.day s.c.), cefazolin (10mg/kg.day), and omeprazole (10mg/day) were administered as a maintenance medical care. Clinically applicable triple immunosuppressants (tacrolimus, steroid and mycophenolate) were applied (daily tacrolimus 1mg/kg, methylprednisolone 2mg/kg, and mycophenolate 40mg/kg). To evaluate the impact of anti-thymocyte globulin (ATG) (Genzyme, Cambridge, MA, USA) to the repopulation of memory cell, ATG were given to two recipient animals (ATG group, 5mg/kg/day x 4 days on Day -2, -1, 0, 1) among four animals.

Histology, immunohistochemical stain and immunofluorescence staining

Porcine aortic xenograft were removed at post transplantation day 28 or at the time of necropsy. When animals were living, procedures were conducted under general anesthesia. Xenograft were removed as a whole to preserve the structure of conduit and were fixed 10% formalin and embedded in paraffin blocks for hematoxylin and eosin staining. For immunohistochemical stain, sections (5 micrometer) were labeled with primary antibodies for CD68 (1:200, Invitrogen, Cat no: MA5-13324, CA, USA) and myeloperoxidase (MPO) (1:1000, Abcam, Cat no:

ab9535, Cambridge, UK) and secondary antibody. Monkey spleen was used as positive control. The stained slide were photographed using an Olympus inverted microscope. (Olympus Imaging America, CA, USA) For immunofluorescence staining, deparaffinized sections of xenograft specimen were probed with primary antibodies. (anti-CD-68 and anti-MPO; same as previous, anti-Tissue factor; American Diagnostic, Cat no: 4508 CJ, NY, USA)

Chemical laboratory parameters

SNUH large animal central laboratory provides daily clinical practice laboratory results.

Cytokine analysis

Serum samples from transplanted monkeys were tested for Tumor necrosis factor-alpha, IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1). Luminex-based standard multiplex panel and magnetic beads were used. All assays were done under the provider's manual. (Invitrogen, ProcartaPlex, EXP040-49031-801, CA, USA).

Statistical analysis

Fisher's exact test, Student t-tests were used for the difference comparisons, as appropriately. Significance was defined as $P < 0.05$. GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) were used for the data visualization. Statistical testes were done by using Stata 15 (Statacorp, College Station, TX, USA)

3. Results

3.1 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

Baseline characteristics

In Table 6, baseline characteristics of kidney transplant recipients are described. Mean age of kidney transplant recipients were 49.1 ± 11.5 years old. In deceased donor KT, mean age of recipients was higher (51.7 ± 10.6, $p < 0.001$). Female recipients were 40.6%. More male recipients received deceased donor kidney. Mean body mass index was 23.1 ± 3.6 kg/m^2, mean systolic blood pressure before kidney transplant was 140.1 ± 34.6 mmHg. Proportion of current smoker was 8.6%. As comorbidities, diabetes was in 29.8% and hypertension in 89.7% of recipients. Proportion of cardiovascular disease was 6.1%, which was higher in deceased donor kidney transplant recipients. History of malignancy was present in 6.6%. The most common cause of ESRD was chronic glomerulonephritis (33.5%) followed by diabetic nephropathy (23.5%). Hemodialysis was the most frequently used dialysis modality before transplantation (70.9%). Preemptive kidney transplantation was 24.0% among living donor KT. Mean waiting time for deceased donor KT was 67.1 ± 37.2 months. Retransplantation was in 7.7%. Mean numbers of HLA mismatch was 3.4 ± 1.8. As induction agent, Basiliximab was used in 78.7% of total KT and ATG was used in 31.9% of DDKT. Tacrolimus was the main calcineurin inhibitor (95.7%). Early steroid withdrawal was done in 2.0% of patients.

Donor characteristics

Donor data was described as cases. (Table 7) Mean age of donor cases was 46.9 ± 13.0 years old. Female was more prevalent in living donor, and male was more prevalent in deceased donor. Diabetic donors was 11.9% in deceased donors, and 1.1% in living donors. Donors with hypertension were 24.4% in DDKT and 9.5% in LDKT. Mean BMI of donors was 23.8 and mean pretransplant SBP of donors was 122.4 mmHg. Proportion of smokers was 17.3% in LDKT. Mean cold ischemic time was 289mins in deceased donor. Continuous renal replacement therapy was applied to 6.7% of deceased donors. Extracorporeal membrane oxygenator was applied to 2.7% of deceased donors.

Patient survival and cause of death

Overall patients survival rate were 98.4%, 97.8%, 97.6% at 1,3,5 years respectively. Among living donor kidney transplantation recipients, patient survival were at 1,3,5 years were 99.3%, 99.1%, 98.9%, respectively. Among deceased donor kidney transplantation, patient survival rate were at 1,3,5 years were 97.0%, 95.9%, 95.6% respectively. (Figure 3) The most common cause of death were infection (47.6%) followed by cardiovascular disease (11.9%), the latter occurred exclusively in deceased donor kidney transplantation. (Table 8)

Death-censored graft survival and cause of graft failure

Death-censored graft survival rate were 98.4%, 97.0%, 96.9% at 1,3,5 years respectively. Among living donor kidney transplantation recipients, death-censored graft survival rate were 99.0%, 98.3%, 97.6% at 1,3,5 years respectively. Among deceased donor kidney transplantation, death-censored graft survival rate were 97.4%, 96.1%, 95.9% at 1,3,5 years respectively. Rejection (43.5%) was the most

common cause of graft loss. Primary graft failure was in 11.1% of graft failure. BK virus nephropathy was 3rd common cause (5.6%). (Table 9)

Acute rejection, indication of kidney biopsy and pathology outcomes

Acute rejection free survival rates were 82.4%, 77.0%, and 76.2% at 1, 3, and 5 years, respectively. Among living donor kidney transplant recipients, acute rejection free survival rates were 82.3%, 78.5%, and 76.1% at 1, 3, and 5 years, respectively. Among deceased donor kidney transplants, acute rejection free survival rates were 81.7%, 77.1%, and 76.3% at 1, 3, and 5 years, respectively (Figure 4A). Biopsy-proven acute rejection free survival rates were 90.3%, 87.6%, and 87.3% at 1, 3, and 5 years, respectively. Among living donor kidney transplant recipients, biopsy-proven acute rejection free survival rates were 90.4%, 87.3%, and 87.0% at 1, 3, and 5 years, respectively. Among deceased donor kidney transplant recipients, biopsy-proven acute rejection free survival rates were 90.2%, 88.0%, and 87.7% at 1, 3, and 5 years, respectively (Figure 4B).

Total 2,769 kidney biopsies were performed. Among them, 58.7% were protocol biopsies. (Table 10) The most common indication of kidney biopsy was increased creatinine. (37.5%) Among for-cause biopsies, acute T cell mediated rejection were 26.8%, acute antibody mediated rejection were 14.1%, and borderline rejection were 21.1%. Recurrent glomerulonephritis were 9.2% and BK virus associated nephropathy were 7.7%. If protocol biopsy are included, the proportion of biopsy findings declined, however, the proportion of borderline rejection were not declined. (Table 11) Biopsy-proven acute T-cell mediated rejection free survival rates were 92.8%, 91.0%, and 90.6% at 1, 3, and 5 years, respectively. (Figure 5A). Biopsy proven acute antibody mediated rejection free survival rates were 96.5%, 95.2%, and 95.2% at 1, 3, and 5 years, respectively (Figure 5B).

Predictors to patient survival and dominance analysis

To explore predictors to patient survival, cross-validated LASSO were applied, which resulted all of variables were included at the optimum lambda. I interpret this due to sufficient n to predictors (not p > n condition), where LASSO might not show its strength in variable selection. (Table 12, Table 17, Figure 6) Traditional backward stepwise selection showed reduced predictors from 20 variables to 15 variables. (Table 13) To compare relative importance of predictors, I chose 1 year patient survival as outcome, and applied dominant analysis method. Deceased donor kidney transplantation was the most dominant predictor to 1 year patient death, followed by recipient age, cardiovascular disease history of recipients, duration of dialysis, diabetes of recipients. Interpretation of maintenance immunosuppressant should be cautious because possibility of primary graft failure.

Dominant predictors to graft survival and acute rejection

Dominant predictors for death-censored 1 year graft survival were standard deceased donor kidney transplant, desensitization, donor hypertension, systolic blood pressure of recipients, diabetic recipients. Dominant factor for acute rejection within 1 year were determined by preselected acute rejection predictors (Table 18). Dominant factors to acute rejection were donor age, followed by HLA mismatch numbers, desensitization, female recipients, body mass index of recipients. (Table 19) Dominant factors for antibody mediated rejection within 1 year were determined by preselected predictors (Table 20). Dominant factors to antibody mediated rejection were desensitization, followed by ATG induction, HLA mismatch numbers,

recipient age, and deceased donor. (Table 21) Among re-transplantation recipients, dominant factors for acute rejection within 1 year were deceased donor kidney, donor hypertension, and HLA mismatch numbers. (Table 22) The most dominant predictors to post-transplant 1 year graft eGFR were donor age, followed by acute rejection within 1 yr, BKVAN within 1 yr, female donor, and recipient BMI. (Table 23)

3.2 Significance of eplet mismatch in rejection

Baseline characteristics

Mean age of study population was 46.8 years old. Proportion of deceased donor kidney transplantation was 38%. Female was 42%. Mean HLA mismatch numbers was 3.2 ± 1.7. Mean HLA mismatch numbers in class I was 2.2 ± 1.2, 1.1 ± 0.7 in class II (DR only). The proportion of retransplantation, desensitization, and abo incompatible kidney transplantation were 7%, 18%, and 12%, respectively. Mean cold ischemic time was 1.9 ± 2.3 hrs in deceased donor kidney transplantation. Overall acute rejection including clinical rejection occurred in 16% during follow up period, and biopsy-proven acute rejection occurred in 9% of study population. Compared to 2009-2012 cohorts, newer cohorts showed higher proportion of desensitization, abo incompatibility, overall rejection and biopsy proven acute rejection. Other clinical characteristics are described in Table 24.

Eplet distribution in study population

Mean eplet class I difference was 10.6 ± 6.8, and class II difference was 24.1 ± 17.6. (Table 25) Figure 7 shows the distribution of eplet mismatch. In HLA

zero mismatch subgroup, estimated class I eplet show little deviance from zero eplet mismatch, however, in class II eplet mismatch showed overt increment than serotype mismatch, which could be explained by the inclusion of DQ mismatch in eplet estimation. Except HLA serotype zero mismatch, other estimated eplet mismatch showed Gaussian distribution.

Association of eplet mismatches to acute rejection

Table 26 shows the results of univariate analysis of predictors to acute rejection. Each eplet mismatches were significant predictors to acute rejection. Reduced hazard ratio is due to large eplet mismatch numbers compared to 1 or 2 mismatches in HLA serotype. I tested whether there are any non-linearity in eplet mismatches to the prediction of acute rejection by applying fractional polynomial term in eplet mismatch numbers. In Figure 8, there are downward curvature over 85 eplet mismatches, and it is explained by the downward curvature in non-antibody verified eplet mismatch. However, total eplet mismatches showed statistical significant increased risk to zero eplet mismatches. I investigated the nonlinear association of eplet mismatches to biopsy proven acute rejection only, to test its effect is more precisely explained in those proven outcomes. (Figure 9) Still, eplet mismatches showed increased risks in total eplet, and non-antibody verified eplets. However, eplet mismatches did not show any superior predictability to HLA serotype mismatches when adjusted other multiple covariables, and I could not find any strong non-linear pattern in the association of rejection, and biopsy proven rejection with eplet mismatch numbers.

Association of eplet mismatches to acute rejection in low HLA genotype mismatches

I tried to validate previous finding that eplet mismatches has significant meaning in low HLA-serotype mismatch subpopulation. Figure 10 showed that eplet mismatches show significant risks in low HLA mismatch groups (0 – 2 HLA mismatches), and interestingly, its increased risk is strongly associated in biopsy-proven acute T cell mediated rejection. When class I eplet and class II eplet was analyzed, similar pattern to total eplet mismatches to biopsy-proven acute T cell mediated rejection was shown in class II total eplet mismatches. However, replacement of HLA serotype mismatch to eplet mismatch in multivariable prediction model did not show any statistical improvement in AUC or IDI. (Figure 11)

Studies of individual eplet locus

Finally, I tried to find any significant individual eplet predicting acute rejection. I tested multiple t-tests, however, no significant p-value were achieved. I explored top 10 (least p-value) eplets, and discovered that they were located near the groove of MHC molecule. (Table 27 and 28) (Figure 12)

3.3 Non-human primate model of antibody mediated rejection

Clinical Course of Experiment Group

GalTKO porcine artery xenograft were transplanted into recipient monkeys (n=4). Xenografts were maintained with the described immunosuppressants by post-operative 4 weeks. At day 28, xenografts were removed. Maintenance

immunosuppressant were weaned after xenograft removal. Tacrolimus and mycophenolate were ceased at the day of graft removal, and prednisolone were gradually tapered down until post-transplant 7 weeks. The 2nd transplantation were conducted at least after 5 months from the removal of 1st xenograft (150 days, and 300 days after first transplantation). Group allocation of ATG vs control were the same as first xenotransplantation. Post-operative immediate assessment of xenograft showed that all graft were patent. Three of 4 recipients survived healthy until the 2nd removal of xenograft. One recipient in non-ATG group (R23-10) died at the day 22 of 2nd transplantation. The patency of graft have been maintained until the removal of xenograft or the day before of mortality.

Coagulation profile

Thrombocytopenia was not prominent in 1st transplantation, and there was no significant difference between ATG treatment vs control group. However, in the 2nd transplantation, the non-ATG group recipient R23-10 experienced with lethal thrombocytopenia. (Figure 13A ~ 13D) In R23-10, development of thrombocytopenia was accompanied by systemic inflammation represented as increased CRP level. Similar to previous report [48], systemic inflammation preceded thrombocytopenia. Deterioration of clinical course in R23-10 was quite abrupt, leading to death on the course of pondering euthanasia and giving antibiotics. Blood culture tests were conducted post-mortem, which did not show any growth of microorganism. Except deceased recipient, both 1st and 2nd transplantation showed similar pattern of increment of systemic inflammation along the experimental course and there was no significant difference between ATG treatment vs control group.

To test whether those intra-graft coagulation reaction is represented by systemic markers, Fibrinogen and Anti-thrombin III were checked. (Figure 13E ~ 13J) Post-

operative early fibrinogen consumption was found in the 2nd transplant of recipient of ATG non-treated group. However, it recovered within 1 week and there was not any significant bleeding or ischemic complications. Systemic measurement of fibrinogen did not reveal any significant difference in deceased recipient. Anti-thrombin III dropped to almost 60% in deceased recipient R23-10, which represents the severity of intravascular coagulation responses. However, change of anti-thrombin III level did not precede mortality event, and the similar level change was also shown in another surviving recipient.

Histopathology and immunohistochemical staining

Histopathology of 1st and 2nd xenograft were compared. (Figure 14) In low power light microscope, the 2nd transplanted graft showed more distorted gross morphology of vascular graft. Also in the 2nd transplanted graft, more inflammatory cells infiltrate in tunica adventitia, which is filled with many blood plug. Loss of endothelial cell in tunica intima and loss of nuclei in tunica media were also observed in 1st xenograft, however, those findings were much more prominent in 2nd xenograft. The administration of ATG did not affect the histology in 1st xenograft nor 2nd xenograft. In the 2nd xenograft, endothelial lining was completely lost regardless of ATG treatment. Large intraluminal thrombus indicates the severe inflammatory reaction in those graft.

Immunohistochemical staining reveals prominent infiltration of CD68+ cells and MPO+ cells. The feature was comparable both in ATG (-) group and ATG (+) group. Anti-tissue factor antibody were stained strongly in first and second transplant. Tissue factor stain was prominent inside the xenograft along the tunica media to tunica adventitia. Luminal area showed relatively weak expression of tissue factor. Tissue factor expression were prominent in the 2nd transplantation group,

which indicated much strong coagulation response in the 2nd transplantation group. (Figure 15)

Cytokine & lymphocyte population

As expected, lymphocyte depletion was achieved by using ATG administration. Total lymphocyte count decreased under the $1x10^3$ cells/μL and was maintained until the weaning of immunosuppression. Unlike the depleted lymphocytes, monocytes and neutrophils showed no significant difference between ATG administration or not. (Figure 16) Although the circulating monocyte was not different between ATG usage. Infiltrating CD-68+ cells were more prominent in ATG (-) groups. When peripheral blood circulating cytokine level was analyzed, MCP-1 level was significantly high at the 1 week of xenotransplantation. At the second transplantation, IL-6 level were prominently higher in the ATG (-) groups than ATG (+) group. Both MCP-1 and IL-6 level were generally higher than one in 1^{st} xenotransplantation, and the phenomenon that showed reduced level of MCP-1 in the ATG (+) group at the 1^{st} xenotransplantation was attenuated at the 2^{nd} xenotransplantation experiment. (Figure 17)

Total IgG and IgM level were measured. During the period when xenograft exposed to the recipient, there was no significant trend of total antibody level elevation or depletion. However, when we check the donor specific complement dependent cytotoxicity, much strong cytotoxicity reaction were observed at the serum from 2^{nd} transplantation. Interestingly, after the removal of second transplant xenograft, one animal from ATG receiving group showed decrement of total IgG level, which coincides the reduced cytotoxic reaction in the donor specific cytotoxicity assay. (Figure 18)

4. Discussion

4.1 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

In the present study, I reported the baseline characteristics and early outcome of Korean Organ Transplantation Registry (KOTRY). Baseline predictors to early outcomes were explored, and dominant factors to patient and graft outcomes were reported. Dominant factors to patient survival were found as predictors associated with recipient's age or recipient's comorbidities. To graft survival, dominant factors were proper immunosuppression, and donor kidney function. It was interesting to see that donor age was found as the most dominant factor to acute rejection. Donor age and donor sex were dominant factors to the graft function at 1 year.

When the KOTRY launched, annual transplantation numbers were 1,400. At the design stage of KOTRY, annual enrollment of 1,200 cases were aimed to cover more than 80% of total kidney transplantation in South Korea. However, recent rapid increment of kidney transplantation numbers have made KOTRY covers about 50-60% of total kidney transplantation in South Korea. Still, KOTRY projects is the largest multi-center cohorts in this country. In KOTRY, clinical details which claim data cannot capture are important resources to future research. Another strength of KOTRY is it's role as a biobank. Prospective sample collection will be invaluable research resources.

The most common cause of ESRD in South Korea is diabetic nephropathy [49], which is reflected as the high proportion of diabetes in KOTRY. High proportion of glomerulonephritis as cause of ESRD could represent selection criteria of comorbidities for kidney transplantation. Another important feature of Korean kidney transplantation is high proportion of living donor kidney transplantation.

Among living donor kidney transplantation, 24% were preemptive kidney transplantation. Long waiting is another feature of Korean kidney transplantation, of which reduction is important future task. Aside from standard triple maintenance immunosuppressants, ATG induction was observed variation in immunosuppressant. It was of note to see the proportion of steroid withdrawal was 2%. Cold ischemic time is short in Korea due to its concentrated population structure. The most common cause of death was infection, followed by cardiovascular disease. These cause of death is compatible with the predictors selected in data-driven approach, because the recipient age and history of cardiovascular disease were selected as dominant predictors to 1 year mortality.

Recent investigations of donor safety have concerned higher lifetime ESRD risk in young donors.[50] In terms of graft survival of recipient side, it is interesting to see selected predictors were donor characteristics such as donor age, donor hypertension, and donor diabetes. However, extension of this finding to long term risk predictors needs caution, because non-modifiable donor factor could be exaggerated in early transplantation outcomes. When we think about donor safety, marginal kidney function would also affect donor's long term outcome, therefore this data is an evidence to the importance of proper donor selection.

It was interesting to see that donor age was the most dominant factor to acute rejection. There were several publication to see the significance of donor age as the risk factors of acute rejection, however, to the best of our knowledge, this is the first study to find that donor age is the most dominant factor to the acute rejection in a quantitative comparison. HLA incompatibility was the 2nd dominant predictors to acute rejection. This finding could be an epidemiological evidence that support the importance of passenger leukocyte and its memory, or endothelial cell damage and PAMP expression. Desensitization was selected as important predictors to acute rejection, which implies although mitigation of immunological risk was performed

by desensitization, residual risk still persist. Future studies are anticipated to investigate the details about desensitization.

Dominant predictors to 1 year post-transplant recipient's eGFR were donor age, recipient BMI, HLA mismatches. Because donor age and HLA mismatches were both dominant predictors to acute rejection, I could prove that both predictors were mediating acute rejection to post-transplant graft eGFR. The importance of donor kidney-recipient weight gap was well known factor to post-transplant eGFR [51,52]. In this study, its importance to predict post-transplant eGFR was high.

The limitation of study are as follows: First, this project have enrolled about 50% of total kidney transplantation in South Korea. Informed consent is requirement to observational cohort, therefore information bias might exist. For example, recipients with poor compliance could refuse study enrollment, and urgent transplantation performed during weekends or late night might not be enrolled in this project. However, when early outcome was compared to previous reports which covered over 92% of total kidney transplantation, there was no statistical difference in early outcomes. Second, dominance of predictors was based on variable selection in traditional stepwise regression, which is not completely independent on the randomness of entering variables. I tried to overcome this limitation by applying regularized regression methods (LASSO), which was unsuccessful due to large numbers of cases compared to selected predictors. However, I think this quantitative comparison of relative importance of variables is noble to transplantation field. Provided model can produce better AUC than EPTS, which assures that the finding is significant.

In summary, I described clinical characteristics of patient enrolled in KOTRY during recent 5 years, and presented dominant predictors to early post-transplantation outcomes by comparing relative contribution to outcome prediction. The dominant predictors to recipient mortality within 1 years were deceased donor,

steroid usage, recipient age, and recipient cardiovascular disease history. The dominant predictors to death censored graft loss within 1 year were immunosuppressant usage, deceased donor. The dominant predictors to acute rejection within 1 year were donor age, and HLA mismatches. Finally, donor age, donor sex, recipient BMI were the dominant predictors to post-transplant 1 year recipient's eGFR.

4.2 Significance of eplet mismatch in rejection

In this study, I investigated the association of eplet mismatch with acute rejection in two sets of Korean kidney transplantation cohorts. Eplet mismatch was not a superior predictor to HLA serotype when it was added to multivariable clinical variables. However, eplet mismatches was significant risk factors in low MHC mismatch group, which is the external validation for previous study results. Interestingly, in the present study, class II eplet mismatches were strongly associated with acute T cell mediated rejection.

Several approaches have been used to investigate the association of molecular mismatch to the clinical phenotype in organ transplantation. Differences in single amino acid and its position information were associated with delayed graft function and allograft survival. [53,54] Electrostatic mismatch concept based on surface electrostatic potential differences between HLA molecules revealed that this amino acid mismatch approach predicts de novo alloimmunization against HLA-A,B,DRB, and DQB. [55–57] Eplet mismatches proposed by Rene Duquesnoy was the most popular method to be used. Eplet mismatches have been reported to be associated with development of de novo DSA, transplant glomerulopathy, antibody mediated rejection, graft survival, and acute T-cell mediated rejection including borderline phenotype. [58–63] Finally, consideration of indirect presentation of class I MHC

peptide onto class II MHC in addition to eplet mismatches have been suggested, and also was associated with the development of dnDSA.[64,65] Each method has own unique interpretation and limitations, however, in terms of predictability to the development of dnDSA, it was reported that there was no significant differences between methods.[66]

Previous studies reported good association of eplet mismatches with the development of de novo DSA. Because of non-invasive nature, monitoring of de novo DSA has strengths with the completeness of measurements and serial measurements. Previous studies determined the cutoff points to categorize alloimmune risks by using ROC curve to the development of dnDSA.[58,61] In this study, I tried to find the cutoff points to categorize alloimmune risks also, whereas the target outcome were acute rejection, biopsy proven acute rejection, acute T cell mediated rejection, or acute antibody mediated rejection in the present study. Therefore, the cutoff points of eplet mismatch numbers were higher than the numbers derived from previous studies which used dnDSA as target outcome.

Compared to the development of dnDSA, association of biopsy proven cellular rejection was scarce, which could be explained by invasive nature of measurement, administration of induction agents, T cell as main target of modern maintenance immunosuppression. Therefore, association with acute rejection was reported in large scale registry study, [67] of which the present study function as external validation although there is differences in the proportion of deceased donor kidney transplantation, ethnicity, and ABO incompatibility. Acute antibody mediated rejection was not associated with eplet mismatches in this study. I interpret this phenomenon derives from the relatively short duration of current study, and gradual progression of transplant glomerulopathy might not be properly captured at 1st biopsy. In future study, a proper analysis for repeated biopsy samples are warranted. Strong association of class II eplet mismatch with T cell mediated rejection in low HLA

mismatch pairs is a new finding in this study. Eplet mismatches was proposed to represent the interaction between MHC molecule and antibody, therefore it focused on the surface amino acid residue, and structural information as intact molecule. However, it is well known phenomenon that class I or II MHC peptide can be digested inside recipient APC, then can be presented to immune responder cell. (indirect presentation) Donor MHC fragmented peptide presented on recipient MHC can induce an activation of helper T cells, which can offer helper signal to effector T cells or B cell activation. Although the association was not clearly shown as in the present study, one of the early studies of eplet mismatches also reported the preceding T-cell mediated rejection was associated with the development of dnDSA according to eplet mismatches, and cellular rejection including borderline phenotype was reported to be associated with DR/DQ eplet mismatches.[21,68]

The limitation of study as follows: First, eplet determination is based on imputation by HLA haplotype distribution. Second, measurement of de novo DSA were scarce and excluded from analysis. Third, follow up duration was too short to delineate chronic manifestation such as transplant glomerulopathy, the development of dnDSA, or allograft survival. Fourth, pathology reporting was dependent on pathologist from individual center.

In summary, eplet mismatches in class II MHC was found as significant risk factors to biopsy proven acute T cell mediated rejection in low degree HLA mismatches (1 or 2 mismatches).

4.3. Non-human primate model of antibody mediated rejection

In this study, GalT-KO porcine vascular conduit xenotransplantation to cynomolgous monkeys were conducted. Conventional triple immunosuppressant with anti-CD-154 monoclonal antibody and cobra venom factors were applied as immunosuppressants. Additionally, experiment groups were divided by the presence

of ATG usage. Although the ATG successfully depleted lymphocyte, there was no significant difference of cellular infiltration or tissue factor expression inside the graft. However, circulating IL-6 level and platelet consumption in the second transplantation was elevated in the ATG non-use group, suggesting partial role of T cell depletion for attenuating systemic inflammation in the second transplantation.

Vascular conduit was used as the model of xenotransplantation in the present study. Previous studies used pig artery patch model, which has strengths of technical easiness and ability to test humoral immunological response. Vascular conduit model has similar strength of technical feasibility and exposure to humoral immune system. In addition, this conduit model can offer the chance of functional monitoring (auscultation, doppler) and safe graft removal, which enables unique sensitization model in xenotransplantation. In this experiment, all monkeys were alive during 4 weeks of first vascular xenograft transplantation, and the rejected xenograft were successfully removed. After those 4 weeks exposure to porcine vascular conduit could elicit very strong sensitization which was confirmed by strong complement dependent cytotoxicity assay and vigorous rejection confirmed by histology in 2nd transplanted xenograft.

In this experimental model, whether T-cell depletion by ATG could affect the development of sensitization in the 2nd xenotransplantation were tried to be delineated. GalTKO pig and old world monkey which express Neu5Gc antigen in their cell surface were used. In this system, the two major proposed carbohydrate xenoantigen (alpha 1,3-gal, Neu5Gc) were compatible. Therefore, overt hyperacute rejection due to profound preformed natural antibodies could be avoided and the importance of non-Gal antibodies were tested. After the removal of first rejected xenograft, 2nd transplantation were conducted after more than 6 months, which led sufficient time to develop induced memory and recovery from the effect of immunosuppressants. Although more vigorous rejection and enhanced complement

dependent cytotoxicity of sensitized serum in 2nd transplantation were observed, any biochemical difference across T cell depletion were found. However, one monkey in ATG-non treated group had expired during 2nd transplantation course.

T-cell help to antibody development is thought to be mediated through follicular helper T cell. In secondary lymphoid organs, primed follicular helper T cell can engage with B cells in the T-B border, and can prime B cells to differentiate into either plasma cells or germinal center B cells which subsequently produce high affinity antibodies.[6970] Enhanced complement dependent cytotoxicity of recipient serum in the 2nd transplantation clearly shows the affinity maturation. The lymphocyte depletion in the present study was successful by ATG, however, the susceptibility of follicular helper T cell to ATG have been reported as mixed results [71,72], which is one probable explanation of similar rejection phenomenon across ATG usage. Analysis of circulating follicular helper T cell is undergoing. Another explanation for non-difference between ATG vs non-ATG group is the resistance of memory T cells to ATG. Memory T cells proliferates quickly when encountered target antigen, expresses qualitatively enhanced antigen responsiveness, does not need costimulation signal to be activated, and are not restricted to lymphoid organs. [73] T cell depletion can make empty space where homeostatic proliferation of T cells could happen, which is advantageous condition for memory T cell to proliferate in a more fast way.[74] Xenoantigen from pig might be thought as new antigen to recipient monkeys, however large animals who was grown in non-SPF conditions have presensitized memory T cells compared to SPF mice due to heterologous immunity. The presence of sensitized T cells at pre-transplant stages were proven to be associated with transplanted graft rejection.[75,76] Following results of immunophenotyping for the circulating peripheral blood cells are needed. Another explanation of inefficacy of ATG is the presence of anti-CD154 monoclonal antibody which is a potent costimulatory blocker. Because CD40 ligand is offered by follicular

helper T cell, superimposed ATG depletion might have not added further effects on the already blocked costimulation signal.

In recent xenotransplantation studies, long-term survival is achieved by multi-faceted approach, which includes intervention of innate inflammation response (IL-6R antibody, Anti-TNF alpha receptor blockade) [77,78], regulation of complement propagation (human thrombomodulin, human EPCR, hCD55, membrane cofactor protein (CD46))[79,80], and depletion of B cells. (Rituximab) In this study, rituximab was not used intentionally to study antibody formation after xenoantigen sensitization. Minimal blockade of innate immunity enabled to study innate immunity and coagulation phenomenon. Consumptive coagulopathy is a manifestation of severe inflammation. Immune-thrombosis is a recent active research area. In this study, there was no definite measured differences of coagulopathy between ATG administration or re-transplantation. However, more vigorous rejection in 2nd transplantation, and a platelet consumption with mortality during 2nd transplantation suggest more enhanced immune-thrombosis might have occurred in 2nd transplantation, which is evidence by elevated tissue factor expression in 2nd xenograft.

Elevated IL-6 was observed in 2nd transplantation. IL-6 is known to be excreted from various cell sources including neutrophil, macrophage, or activated endothelial cells.[81] Accompanying this phenomenon, at the histology level macrophage infiltration or neutrophil infiltration to xenograft were more prominent in the 2nd transplantation, and the extent of damage in vascular xenograft were also more prominent in the 2nd transplantation. Because the histological phenotype of 2nd xenograft resembles chronic rejection, earlier graft recovery and histological assessment might have revealed more comparable active rejection phenomenon.

In terms of second transplantation, one might think it is too far future in xenotransplantation. However, in the scope of sensitization, it is very close topic to

contemporaneous situation. With the advancement of genetic manipulation of donor pig, xenotransplantation are at the gate of clinical application, where careful patient selection is critical. The result of present study might be interpreted in that context. Xenogenic material exposure (i.e. cardiac tissue valves) could affect induced antibody levels. Heterologous immunity might explain the various degree of natural antibodies, however, functional difference and consequential immunologic reponses are not well defined in xenotransplantation. In recent study, significant difference of endothelial cell activation between natural antibody and induced antibody was reported.[82] The present study shows more vigorous rejection phenomenon in sensitized setting, however, the results of present study examines overall immunologic phenomenon, not restricted in endothelial cell activation. To evaluate endothelial cell response to sensitized serum is another important topic[83], and I expect future investigation would come out.

There are several limitation of this study as follows: Though the non-human primate was used as experimental object, small numbers are limitation. Cobra venom factor and anti CD-154 monoclonal antibody is not clinically applicable drugs. Vascular conduit graft is a feasible model to study histology and sensitization, however, its applicability is limited because it is a model system rather than real target organ.

In summary, a repeated GalT knockout porcine artery transplantation model to non-human primate were developed. More severe rejection phenotype in 2nd transplantation was accompanied by circulating elevated IL-6 and tissue factors expression in the affected graft under the CD154-40 costimulation blockade.

5. Conclusion

To understand the impact of clinical predictors in the real-world kidney transplantation to study the underlying mechanism of graft failure and rejection, kidney transplantation cohort (KOTRY and ASTREG) were developed and were used for analysis. Study of KOTRY revealed that donor age and HLA mismatch were the dominant predictors for acute rejection. Detailed analysis of HLA was further done by adopting eplet mismatch concept, which revealed that class II eplet mismatches were a significant risk factor to the development of acute T-cell mediated rejection. Repeated vascular artery xenotransplantation of GalT-knockout pig to cynomolgous monkeys revealed that at the second transplantation, vigorous rejection was accompanied by the elevated IL-6 and tissue factor expression in the CD154-40 cosimulation blockade. These findings collectively suggest the importance of donor antigenicity in relation to recipient's sensitization status, the necessity to control overall inflammation not only sole B or sole T cell, and the importance of interplay between distinct immune pathways including interplay of innate and adaptive immunity or interplay between B-cell and T-cell, and interplay between preformed antibody to the coagulation pathway. These findings generate the hypothesis whether target the linker cell or linker system between different immune pathways is effective to the control of immunological rejection of kidney transplantation, which could be a new therapeutic option in controlling multi-faceted rejection in high-risk transplantation settings.

Table 1. KOTRY data collection formats for organ recipients: common variables in all organ transplantation

Categories	Variables	Collection timing		
		B	<1y	A
Demographics	Age, gender, ethnicity, date of transplantation, cause of organ failure, number of transplantation	O		
Comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including diabetes, hypertension, cardiovascular disease, malignancies and selected medication	O		
Laboratory assessment	CBC, routine chemistry, uric acid, lipid panel, urinalysis	O	O	O
Immunologic assessment	ABO/HLA typing, crossmatch, PRA, DSA	O		
Viral markers	HBsAg, anti-HBsAb, anti-HCV Ab, anti-CMV Ab, anti-EBV Ab, anti-HIV Ab	O		

Immunosuppressants	Induction and maintenance immunosuppressants, concentration of immunosuppressants (calcineurin inhibitors and mTOR inhibitors)	O	O	O
Immediate complications	Surgical complications		O	
Discharge data	Date of discharge, functioning parameter of transplanted organ		O	
Post-transplant outcomes	Allograft rejection, graft failure, infection, patient death		O	O
Post-transplant comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including new-onset diabetes, hypertension, cardiovascular disease, tuberculosis, fracture and malignancies		O	O
Biosamples	DNA	O		
	Serum and plasma	O		O*

Abbreviations: <1yr, post-transplant visits within 1 year; A, annual visit; B, baseline visit; CBC, complete blood count; CMV, cytomegalovirus; DSA, donor specific antibody; EBV, Epstein-Barr virus; HBsAg, hepatitis B virus surface antigen; anti-HBsAb, anti-hepatitis B virus surface antibody; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin; PRA, panel reactive antibody

Post-transplant visits within 1 year are differently set among each organ. In kidney transplantation, visits are set at 6 month, in liver and heart transplantation, at 1 and 6 month, in pancreas transplantation, at 3 and 6 month, and in lung transplantation, at 3, 6, and 9 month. Baseline recipients' DNAs are collected in all organ transplantation. Post-transplant sera are collected at 1- and 3- years after transplantation in kidney, heart, lung and pancreas transplantation. In kidney transplantation, post-transplant plasma is additionally collected at 1- and 3- years after transplantation.

Table 2. KOTRY data collection formats for organ donors: common variables in all organ transplantation

Categories	Variables	Collection timing	
		B	A
Demographics	Age, gender, relationship to recipients, ethnicity	O	
Comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including diabetes, hypertension, cardiovascular disease, and malignancies	O	
Deceased donor profile	Deceased donor profile (cause of brain death, inotropics management, vital-supporting devices, cold ischemic time)	O	
Laboratory assessment	CBC, routine chemistry, uric acid, lipid panel, ABO typing, urinalysis	O	O
Immunologic assessment	ABO/HLA typing	O	
Viral markers	HBsAg, anti-HBsAb, anti-HCV Ab, anti-CMV Ab, and anti-EBV Ab	O	
Living donor outcome	Post-operative surgical comorbidities, death, ESRD		O
Biosamples	DNA	O	

Abbreviations: A, annual visit; B, baseline visit; CBC, complete blood count; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ESRD, end stage renal disease; HBsAg, hepatitis B virus surface antigen; anti-HBsAb, anti-hepatitis B virus surface antibody; HCV, hepatitis C virus; HDL, high-density lipoprotein ; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin inhibitor

Baseline donors' DNAs are collected in all organ transplantation.

Table 3. Organ-specific information of Korean Organ Transplantation Registry

Organ	At baseline	At follow-ups
Kidney		Allograft biopsy based on Banff reports, living donor outcome (ESRD, urinary stone, hypertension)
Liver	Child-Pugh score, MELD/PELD score, donor-recipient liver volumetry, treatment history of hepatocellular carcinoma, surgical type of liver transplantation	Post-transplant rehabilitation status, recurrence of HBV or HCV, living donor outcome (hepatic morbidity)
Heart	Usage of cardiac assisting device and ventilator, intraoperative cardiopulmonary bypass usage	Serum cardiac markers (NT-proBNP, troponin I and T) at discharge, echocardiography
Lung	Latent tuberculosis infection (TST, IGRA), bone mineral density, lung size measure (donor & recipient), arterial blood gas analysis, donor bronchoscopic exam	Primary graft dysfunction, 6 minutes walking test, pulmonary function test (spirometry), post-transplant functioning status (tracheostomy, home O2, BiPAP)

Pancreas	C-peptide, anti-GAD antibody, HbA1c, surgical technique (drainage type, portal vein extension, arterial Y graft, artery and vein anastomosis type)	Insulin, C-peptide, HbA1c

Abbreviations: BiPAP, bi-level positive airway pressure; BNP, blood natriuretic peptide; ESRD, end stage renal disease; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; IGRA, interferon gamma releasing assay; MELD, model for end-stage liver disease; PELD, pediatric end -stage liver disease; TST, tuberculin skin test

Table 4. Representative items included in ASTREG-H

Domains	Variables
Baseline recipient characteristics	Age, sex, ethnicity, number of kidney transplant, smoking history, cause of end stage renal disease, previous history of renal replacement therapy, date of end stage renal disease diagnosis, comorbidities (diabetes, hypertension, cardiovascular disease, cerebrovascular attack, peripheral arterial disease, malignancy), height, weight, blood pressure, serostatus (CMV, HBsAg, HBsAb, HCV, EBV, HIV), ABO blood type
Baseline donor characteristics	Age, sex, ethnicity, donor relationship with recipients, deceased donor, comorbidities (diabetes, hypertension, malignancy), serostatus (CMV, HBsAg, HBsAb, HCV, EBV, HIV), ABO blood type, measured glomerular filtration rate in living donors, cause of brain death in deceased donor, serum creatinine before donation, complications after kidney donation
Immunologic parameters	Human leukocyte antigen mismatch (A,B,DR), crossmatch, panel reactive antibody profiles, baseline donor specific antibodies, baseline ABO titer, desensitization

	regimen, induction agent, maintenance immunosuppressants, trough level of calcineurin inhibitors
Post-transplant event of recipients (irregular outcome)	Delayed graft function, surgical complications, acute rejection, report of every kidney biopsy, vascular disease, infection, malignancy
Post-transplant annual surveillance of recipients (regular annual evaluation)	Height, weight, blood pressure, serum creatinine, parathyroid hormone, cholesterol, development of donor specific antibodies, plasma BK virus titer

Abbreviations: ASTREG, Asian Society of Transplantation Registry; CMV, cytomegalovirus; HBsAg, hepatitis B virus surface antigen; HBsAb, Antibody to the hepatitis B virus surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; EBV, Epstein-Barr virus

Table 5. Minimum detectable increase in relative risk of graft survival, patient survival and acute rejection from Korean Organ Transplantation Registry (KOTRY)

Organ (Expected enrollment number by 2019)	Survival Outcomes	Estimated Number of outcomes by 2019 based on observed events*	Detectable statistically significant minimum of hazard ratios		
			Risk Factor with 10% Prevalence	Risk Factor with 20% Prevalence	Risk factor with 50% Prevalence
Kidney Transplant (12,000)	Graft	1,379	1.09	1.07	1.06
	Patient	736	1.09	1.07	1.05
Liver Transplant (3,900)	Graft	839	1.18	1.13	1.11
	Patient	636	1.17	1.13	1.10
Heart Transplant (570)	Graft	130	1.51	1.38	1.32
	Patient	119	1.50	1.38	1.31

Lung Transplant (150)	Graft	48	2.34	1.98	1.87
	Patient	51	2.37	2.00	1.89
Pancreas Transplant (150)	Graft	41	2.25	1.92	1.82
	Patient	25	2.09	1.81	1.72

Table 6. Baseline clinical characteristics of the kidney transplant recipients of Korean Organ Transplantation Registry (2014 – 2018)

Variables	Total (n=4,839)	Living (n=3,039)	Deceased (n=1,800)	P
Age, yrs	49.1 ± 11.5	47.6 ± 11.7	51.7 ± 10.6	<0.001
Female sex	1,965 (40.6)	1,265 (41.6)	700 (38.9)	0.061
Body mass index, kg/m^2	23.1 ± 3.6	23.2 ± 3.7	23.0 ± 3.3	0.187
SBP, mmHg	140.1 ± 34.6	137.4 ± 39.8	144.7 ± 22.3	<0.001
DBP, mmHg	84.6 ± 32.1	84.7 ± 39.2	84.4 ± 13.3	0.002
Smoking				<0.001
Never	3,670 (75.8)	2,287 (75.3)	1,383 (76.8)	
Current	414 (8.6)	235 (7.7)	179 (9.9)	
Former	702 (14.5)	490 (16.1)	212 (11.8)	
Unknown	53 (1.1)	27 (0.9)	26 (1.4)	
Comorbidities				
Diabetes	1,442 (29.8)	913 (30.0)	529 (29.4)	0.631
Hypertension	4,340 (89.7)	2,727 (89.7)	1,613 (89.6)	0.976
Cardiovascular disease	294 (6.1)	158 (5.2)	136 (7.6)	<0.001
Malignancies	317 (6.6)	183 (6.0)	134 (7.4)	0.115

Cause of end stage renal disease				<0.001
Diabetic nephropathy	1,135 (23.5)	708 (23.3)	427 (23.7)	
Hypertension	763 (15.8)	413 (13.6)	350 (19.4)	
Glomerulonephritis	1,610 (33.3)	1,067 (35.1)	543 (30.2)	
ADPKD	231 (4.8)	148 (4.9)	83 (4.6)	
Other	150 (3.1)	95 (3.1)	55 (3.1)	
Unknown	950 (19.6)	608 (20)	342 (19)	
Dialysis before transplantation				<0.001
Hemodialysis	3,429 (70.9)	2,009 (66.1)	1,420 (78.9)	
Peritoneal dialysis	619 (12.8)	241 (7.9)	378 (21)	
Kidney transplant	59 (1.2)	59 (1.9)		
Preemptive	732 (15.1)	730 (24.0)	2 (0.1)	
Duration of waitlist, mos	55.6 ± 41.6	8.1 ± 18.2	67.1 ± 37.2	<0.001
2nd Kidney transplantation	375 (7.7)	216 (7.1)	159 (8.8)	0.137
Desensitization	1,106 (22.9)	1,064 (35.0)	42 (2.3)	<0.001
HLA mismatch numbers	3.4 ± 1.8	3.3 ± 1.7	3.4 ± 1.9	0.021
Induction agent				<0.001

Anti-thymocyte globulin	1,005 (20.9)	434 (14.4)	571 (31.9)	
Basiliximab	3,780 (78.7)	2,577 (85.5)	1,203 (67.2)	
No induction	21 (0.4)	4 (0.1)	17 (0.9)	
Calcineurin inhibitor				<0.001
Tacrolimus	4,631 (95.7)	2,872 (94.5)	1,759 (97.7)	
Cyclosporin A	153 (3.2)	132 (4.3)	21 (1.2)	
No Calcineurin inhibitors	55 (1.1)	35 (1.2)	20 (1.1)	
mTOR inhibitor				0.283
Sirolimus or everolimus	53 (1.1)	37 (1.2)	16 (0.9)	
Steroid				0.194
Yes	4,739 (97.9)	2,983 (98.2)	1,756 (97.6)	
No	99 (2.0)	56 (1.8)	43 (2.3)	

Abbreviations) ADPKD, autosomal dominant polycystic kidney disease; DBP, diastolic blood pressure; SBP, systolic blood pressure

Table 7. Baseline clinical characteristics of the kidney transplant donors of Korean Organ Transplantation Registry (2014 – 2018)

Variables	Total (n=4,838)	Living (n=3,039)	Deceased (n=1,799)	P
Age, yrs	46.9 ± 13	46.1 ± 11.8	48.4 ± 14.8	<0.001
Female sex	2,245 (46.4)	1,709 (56.2)	536 (29.8)	<0.001
Comorbidities				
Diabetes	248 (5.1)	34 (1.1)	214 (11.9)	<0.001
Hypertension	728 (15.0)	288 (9.5)	440 (24.4)	<0.001
Body mass index, kg/m^2	23.8 ± 3.4	24.2 ± 3.2	23.2 ± 3.7	<0.001
SBP, mmHg	122.4 ± 17.2	122.2 ± 13.9	122.7 ± 21.8	0.418
DBP, mmHg	75.3 ± 12.7	76.3 ± 10.0	73.6 ± 16.2	<0.001
Smoking				<0.001
Never	3,105 (64.2)	2,229 (73.4)	876 (48.7)	
Current	1,203 (24.9)	525 (17.3)	678 (37.7)	
Former	313 (6.5)	243 (8)	70 (3.9)	
Unknown	218 (4.5)	42 (1.4)	176 (9.8)	
Cold ischemic time, mins	140.2 ± 138.0	61.9 ± 41.8	289.0 ± 134.6	<0.001

CRRT	110 (6.7)	0	110 (6.7)
ECMO	45 (2.7)	0	45 (2.7)

Abbreviations) CRRT, continuous renal replacement therapy; DBP, diastolic blood pressure; ECMO, extracorporeal membrane oxygenation; SBP, systolic blood pressure

Table 8. Causes of Death of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=84)	Living (n=23)	Deceased (n=61)
Cardiovascular	10 (11.9%)	0 (0%)	10 (16.4%)
Infection	40 (47.6%)	11 (47.8%)	29 (47.5%)
Malignancy	4 (4.8%)	0 (0%)	4 (6.6%)
Sudden cardiac death	3 (3.6%)	3 (13.0%)	0 (0%)
Liver disease	1 (1.2%)	0 (0%)	1 (1.6%)
Others	16 (19.0%)	5 (21.7%)	11 (18.0%)
Unknown	10 (11.9%)	4 (17.4%)	6 (9.8%)

Table 9. Causes of graft loss of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=108)	Living (n=50)	Deceased (n=58)
Rejection	47 (43.5%)	24 (48%)	23 (39.7%)
BK virus nephropathy	6 (5.6%)	3 (6%)	3 (5.2%)
Glomerulonephritis	4 (3.7%)	0 (0%)	4 (6.9%)
Non-compliance	4 (3.7%)	3 (6%)	1 (1.7%)
Early surgical complication	3 (2.8%)	2 (4%)	1 (1.7%)
Primary graft failure	12 (11.1%)	5 (10%)	7 (12.1%)
Others	16 (14.8 %)	6 (12%)	10 (17.2%)
Unknown	16 (14.8%)	7 (14%)	9 (15.5%)

Table 10. Causes of Biopsies of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=2,769)	Living (n=1,617)	Deceased (n=1,152)
Increased creatinine	1,039 (37.5%)	579 (35.8%)	460 (39.9%)
Increased proteinuria	51 (1.8%)	21 (1.3%)	30 (2.6%)
Protocol biopsy	1,625 (58.7%)	987 (61.0%)	638 (55.4%)
Others	54 (2.0%)	30 (1.9%)	24 (2.1%)

Table 11. Result of kidney allograft biopsy (all kidney biopsy)

Variables	Including protocol biopsies			Only indication biopsies		
	Total (n=2,769)	Living (n=1,617)	Deceased (n=1,152)	Total (n=1,144)	Living (n=630)	Deceased (n=514)
Borderline change	552 (19.9%)	332 (20.5%)	220 (19.1%)	241 (21.1%)	128 (20.3%)	113 (22.0%)
Acute T-cell mediated rejection	437 (15.8%)	268 (16.6%)	169 (14.7%)	306 (26.8%)	192 (30.5%)	114 (22.2%)
Acute antibody mediated rejection	203 (7.3%)	124 (7.7%)	79 (6.9%)	161 (14.1%)	94 (14.9%)	67 (13.0%)

Chronic active T cell mediated rejection	28 (1.0%)	14 (0.9%)	14 (1.2%)	27 (2.4%)	13 (2.1%)	14 (2.7%)
Chronic active antibody mediated rejection	29 (1.1%)	20 (1.2%)	9 (0.8%)	25 (2.2%)	17 (2.7%)	8 (1.6%)
Interstitial fibrosis and tubular atrophy	358 (12.9%)	177 (11.0%)	181 (15.7%)	188 (16.4%)	93 (14.8%)	95 (18.5%)
BK nephropathy	99 (3.6%)	54 (3.3%)	45 (3.9%)	88 (7.7%)	47 (7.5%)	41 (8.0%)
Glomerulonephritis	146 (5.3%)	75 (4.6%)	71 (6.2%)	105 (9.2%)	53 (8.4%)	52 (10.1%)
Calcineurin inhibitor toxicity	164 (5.9%)	79 (4.9%)	85 (7.4%)	90 (7.9%)	47 (7.5%)	43 (8.4%)

Others	587 (21.2%)	310 (19.2%)	277 (24.1%)	326 (28.5%)	193 (30.6%)	135 (5.9%)

- Multiple selections are allowed

Table 12. Comparison of predictors to death of patient estimated by least absolute shrinkage and selection operator (LASSO) and multivariable Cox regression

Variables	Selective Inference by LASSO variable selection			Multivariable Cox regression		
	Coefficient	P-value	Post-selection interval	Coefficient	P-value	95% C.I.
Age (Recipients)	0.434	0.070	0.707 – 0.724	0.434	0.010	0.103 – 0.764
Age (Donors)	-0.035	0.705	1.407 – 0.273	-0.035	0.786	-0.287 – 0.217
Sex (Recipients)	-0.358	0.247	0.522 - -0.070	-0.358	0.248	-0.966 – 0.249
Sex (Donors)	-0.571	0.069	0.074 – 0.668	-0.571	0.069	-1.185 – 0.043
DM history (Recipients)	0.729	0.010	1.197 – 1.003	0.729	0.010	0.175 – 1.283
CVD history (Recipients)	0.888	0.001	1.344 – 1.278	0.888	0.001	0.349 – 1.427

Cancer history (Recipients)	0.816	0.016	1.437 – 1.341	0.816	0.016	0.151 – 1.480
SBP (Recipients)	-0.054	0.660	0.793 – 0.914	-0.054	0.660	-0.295 – 0.187
BMI (Recipients)	-0.050	0.727	1.126 – 7.583	-0.05	0.727	-0.327 – 0.228
DM history (Donors)	0.557	0.209	1.146 – 1.241	0.557	0.126	-0.156 – 1.269
HTN history (Donors)	-0.067	0.801	4.810 – 0.840	-0.067	0.838	-0.707 – 0.574
Dialysis duration	0.235	0.053	0.447 – 1.007	0.235	0.053	-0.003 – 0.472
SBP (Donors)	0.051	0.632	0.204 – 0.652	0.051	0.631	-0.158 – 0.261
BMI (Donors)	-0.166	0.198	0.170 - -0.011	-0.166	0.198	-0.419 – 0.087
Deceased donor	1.311	0.018	2.177 – 1.965	1.311	0.001	0.504 – 2.117
HLA mismatch numbers	0.313	0.067	0.538 – 0.577	0.313	0.023	0.043 – 0.583
Desensitization	1.047	0.006	1.739 – 1.792	1.047	0.006	0.299 – 1.794

ATG induction	0.086	0.761	0.435 – 0.488	0.086	0.760	-0.467 – 0.640
Ever smoker (recipients)	0.225	0.447	0.700 - -0.180	0.225	0.446	-0.353 – 0.802
Ever smoker (donors)	-0.292	0.300	0.582 – 0.595	-0.292	0.299	-0.842 – 0.258

Abbreviations) ATG, anti-thymocyte globulin; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HLA, human leukocyte antigen; SBP, systolic blood pressure;

Table 13. Selected predictors to patient death by stepwise backward selection

Variables	Coefficient	95% C.I.	P
Recipient age, yrs	0.037	0.011 - 0.063	0.005
Donor BMI, kg/m^2	-0.059	-0.129 - 0.011	0.098
Female recipient	-0.530	-1.066 - 0.005	0.052
Female donor	-0.339	-0.853 - 0.175	0.196
Diabetes (recipient)	0.673	0.164 - 1.181	0.009
Cardiovascular disease (recipient)	0.831	0.322 - 1.340	0.001
Cancer (recipient)	0.672	0.023 - 1.321	0.042
Desensitization	0.903	0.199 - 1.608	0.012
HLA mismatch numbers	0.176	0.034 - 0.317	0.015
RRT duration, months	0.005	0.001 - 0.008	0.006
Deceased donor	1.179	0.476 - 1.882	0.001

Abbreviations) BMI, body mass index; HLA, human leukocyte antigen; RRT, renal replacement therapy

Table 14. Selected predictors to 1 year patient death and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	1.202	0.420 - 1.984	0.003	0.578	1
Age (recipients), yrs	0.043	0.013 - 0.073	0.004	0.498	2
Cardiovascular disease (recipients)	0.876	0.296 - 1.455	0.003	0.269	3
Duration of renal replacement therapy, months	0.006	0.002 - 0.009	0.005	0.341	4
Diabetes (recipients)	0.713	0.139 - 1.286	0.015	0.323	5
Diabetes (donors)	0.545	-0.203 - 1.294	0.153	0.121	6
Body mass index (donors), kg/m^2	-0.079	-0.158 - 0.001	0.052	-0.264	7

Female recipients	-0.543	-1.145 - 0.059	0.077	-0.268	8
HLA mismatch numbers	0.120	-0.037 - 0.276	0.134	0.210	9
Desensitization	0.934	0.131 - 1.737	0.023	0.394	10
Systolic blood pressure (donors), mmHg	-0.009	-0.022 - 0.005	0.198	-0.150	11

Abbreviations) HLA, human leukocyte antigen

Table 15. Selected predictors to death-censored graft loss by stepwise backward selection

Variables	Coefficient	95% C.I.	P
BMI (recipient), kg/m^2	0.067	0.013 - 0.121	0.015
Age (donor), yrs	0.015	-0.002 - 0.032	0.081
HLA mismatch numbers	0.092	-0.028 - 0.212	0.132
Female donor	0.299	-0.126 - 0.724	0.168
Donor diabetes	-1.004	-2.181 - 0.172	0.094
Desensitization	0.691	0.163 - 1.220	0.010
Donor systolic blood pressure, mmHg	-0.014	-0.025 - -0.002	0.020
Deceased donor	0.915	0.412 - 1.418	<0.001

Abbreviations) BMI, body mass index; HLA, human leukocyte antigen

Table 16. Selected predictors to 1 year death-censored graft loss and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	1.442	0.781 - 2.103	<0.001	0.694	1
Desensitization	1.129	0.441 - 1.817	0.001	0.476	2
Donor hypertension	0.691	0.091 - 1.291	0.024	0.249	3
Systolic blood pressure (recipients), mmHg	-0.013	-0.026 - -0.001	0.039	-0.272	4
Diabetes (recipients)	0.441	-0.088 - 0.971	0.102	0.200	5
Diabetes (donors)	-1.363	-2.825 - 0.099	0.068	-0.304	6
Body mass index (recipients), kg/m^2	0.070	0.001 - 0.139	0.048	0.247	7
Systolic blood pressure (donors), mmHg	-0.013	-0.027 - 0.001	0.067	-0.228	8

Cancer (recipients)	0.535	-0.272 - 1.341	0.194	0.132	9

Table 17. Comparison of predictors to acute rejection estimated by least absolute shrinkage and selection operator (LASSO) and multivariable Cox regression

Variables	Selective Inference by LASSO variable selection			Multivariable Cox regression		
	Coefficient	P-value	Post-selection interval	Coefficient	P-value	95% C.I.
Age (Recipients)	-0.101	0.008	-0.164 - -0.035	-0.101	0.008	-0.176 - -0.027
Age (Donors)	0.214	<0.001	0.147 – 0.300	0.214	<0.001	0.136 – 0.293
Sex (Recipients)	-0.310	<0.001	-0.446 - -0.173	-0.310	<0.001	-0.471 - -0.149
Sex (Donors)	0.103	0.116	-0.091 – 0.919	0.103	0.230	-0.065 – 0.270
DM history (Recipients)	-0.086	0.316	-0.224 – 0.188	-0.086	0.314	-0.254 – 0.081
CVD history (Recipients)	-0.150	0.232	-0.353 – 0.195	-0.150	0.231	-0.395 – 0.095

Cancer history (Recipients)	0.261	0.052	-0.007 – 0.482	0.261	0.052	-0.002 – 0.523
SBP (Recipients)	-0.044	0.227	-0.104 – 0.056	-0.044	0.226	-0.116 – 0.027
BMI (Recipients)	0.080	0.161	-0.056 – 0.139	0.080	0.028	0.009 – 0.152
DM history (Donors)	-0.339	0.056	-0.629 – 0.016	-0.339	0.055	-0.685 – 0.008
HTN history (Donors)	0.041	0.691	-0.811 – 0.181	0.041	0.689	-0.160 – 0.242
Dialysis duration	-0.067	0.129	-0.144 – 0.035	-0.067	0.128	-0.154 – 0.019
SBP (Donors)	-0.010	0.759	-0.072 – 0.368	-0.010	0.778	-0.080 – 0.060
BMI (Donors)	-0.005	0.901	-0.029 – 0.888	-0.005	0.900	-0.077 – 0.068
Deceased donor	0.249	0.053	-0.007 – 0.632	0.249	0.017	0.044 – 0.454
HLA mismatch numbers	0.132	<0.001	0.070 – 0.193	0.132	<0.001	0.060 – 0.205

Desensitization	0.359	<0.001	0.213 – 0.505	0.359	<0.001	0.187 – 0.532
ATG induction	0.082	0.353	-0.221 – 0.226	0.082	0.356	-0.092 – 0.256
Ever smoker (recipients)	-0.158	0.081	-0.321 – 0.032	-0.158	0.079	-0.335 – 0.019
Ever smoker (donors)	-0.013	0.883	-0.085 – 1.791	-0.013	0.883	-0.184 – 0.159

Abbreviations) ATG, anti-thymocyte globulin; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HLA, human leukocyte antigen; SBP, systolic blood pressure;

Table 18. Selected predictors to acute rejection by stepwise backward selection

Variables	Hazard Ratio	95% C.I.	P
Age (Recipients)	0.990	0.984 – 0.996	0.001
Age (Donors)	1.016	1.011 – 1.022	<0.001
Sex (Recipients)	0.762	0.661 – 0.877	<0.001
Desensitization	1.493	1.269 – 1.756	<0.001
Deceased donor	1.212	1.033 – 1.423	0.019
Mycophenolate mofetil	0.639	0.540 – 0.756	<0.001
HLA mismatch numbers	1.084	1.042 – 1.128	<0.001
SBP (Recipients)	0.997	0.994 – 1.001	0.129
Body mass index (Recipients)	1.017	0.998 – 1.037	0.073
DM history (Donors)	0.732	0.527 – 1.018	0.064
Steroid	1.451	0.854 – 2.466	0.169

Abbreviations) HLA, human leukocyte antigen; SBP, systolic blood pressure

Table 19. Selected predictors to acute rejection with post-transplant 1 year and dominance

Variables	Coeffieicnt	95% C.I.	P	Standardized Beta	Rank
Donor age, yrs	0.019	0.012 - 0.025	<0.001	0.235	1
HLA mismatch numbers	0.092	0.043 - 0.141	<0.001	0.163	2
Desensitization	0.443	0.239 - 0.647	<0.001	0.188	3
Female recipients	-0.368	-0.556 - -0.181	<0.001	-0.181	4
Body mass index (recipients), kg/m^2	0.023	-0.001 - 0.046	0.063	0.080	5
Diabetes mellitus (donors)	-0.523	-0.939 - -0.106	0.014	-0.116	6
Recipient age, yrs	-0.009	-0.016 - -0.001	0.021	-0.100	7
Systolic blood pressure (recipients), mmHg	-0.003	-0.007 - 0.001	0.125	-0.067	8
Deceased donor	0.196	-0.003 - 0.394	0.053	0.094	9

Ever smoker (recipients)	-0.162	-0.370 - 0.046	0.127	-0.070	10

Abbreviations) HLA, human leukocyte antigen

Table 20. Selected predictors to antibody mediated rejection by stepwise backward selection

Variables	Coefficients	95% C.I.	P
Age (Recipients)	-0.020	-0.033 - -0.007	0.003
Age (Donors)	0.011	-0.003 – 0.024	0.114
ATG induction	0.385	0.048 – 0.723	0.025
Female donor	0.271	-0.092 – 0.634	0.144
SBP (Recipients)	-0.007	-0.016 – 0.002	0.131
Ever smoker (donors)	0.404	0.037 – 0.771	0.031
Desensitization	0.441	0.035 – 0.848	0.033
Deceased donor	1.143	0.784 – 1.502	<0.001
Donor hypertension	-0.404	-0.897 – 0.090	0.109
HLA mismatch numbers	0.144	0.052 – 0.237	0.002

Abbreviations) ATG, anti-thymocyte globulin; HLA, human leukocyte antigen; SBP, systolic blood pressu

Table 21. Selected predictors to antibody mediated rejection with post-transplant 1 year and dominance

Variables	Coeffieicnt	95% C.I.	P	Standardized Beta	Rank
Desensitization	1.284	0.868 – 1.699	<0.001	0.542	1
ATG induction	0.547	0.167 – 0.926	0.005	0.217	2
HLA mismatch numbers	0.118	0.013 – 0.224	0.027	0.208	3
Age, yrs	-0.019	-0.034 - -0.004	0.015	-0.218	4
Deceased donor	0.394	-0.070 – 0.859	0.096	0.190	5
Donor hypertension	-0.451	-1.015 – 0.112	0.116	-0.162	6
Ever smoker (donors)	0.273	-0.084 – 0.630	0.134	0.131	7
Donor age, yrs	0.010	-0.005 – 0.025	0.182	0.128	8

Abbreviations) ATG, anti-thymocyte globulin; HLA, human leukocyte antigen

Table 22. Selected predictors to acute rejection with post-transplant 1 year and dominance in re-transplantation patients

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	0.923	0.143 – 1.704	0.020	0.456	1
Donor hypertension	0.569	-0.186 – 1.323	0.140	0.214	2
HLA mismatch numbers	0.147	-0.034 – 0.328	0.112	0.264	3
ATG induction	0.484	-0.169 – 1.137	0.146	0.236	4
Desensitization	0.741	-0.034 – 1.517	0.061	0.348	5
DM history (Recipients)	-0.788	-1.792 – 0.216	0.124	-0.302	6
Mycophenolate mofetil	-0.583	-1.345 – 0.179	0.134	-0.225	7

Abbreviations) ATG, anti-thymocyte globulin; DM, diabetes mellitus; HLA, human leukocyte antigen

Table 23. Selected predictors to post-transplant 1 year estimated glomerular filtration rate and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Donor age, yrs	-0.590	-0.639 - -0.540	<0.001	-7.515	1
Acute rejection within 1 yr	-10.122	-11.701 - -8.543	<0.001	-3.890	2
BKVAN within 1 yr	-23.426	-28.413 - -18.438	<0.001	-2.834	3
Female donor	-2.305	-3.761 - -0.850	0.002	-1.151	4
Body mass index (recipients), kg/m^2	-0.471	-0.649 - -0.292	<0.001	-1.664	5
Diabetes mellitus (donors)	-2.777	-5.633 - 0.080	0.057	-0.615	6
Ever smoking (donors)	1.808	0.342 - 3.273	0.016	0.866	7
Body mass index (donors), kg/m^2	0.396	0.206 - 0.585	<0.001	1.303	8
Deceased donor	-2.470	-4.171 - -0.768	0.004	-1.189	9

Systolic blood pressure (recipients), mmHg	0.063	0.032 - 0.093	<0.001	1.275	10
Systolic blood pressure (donors), mmHg	-0.043	-0.079 - -0.008	0.017	-0.746	11
Age (recipients), yrs	-0.042	-0.096 - 0.012	0.125	-0.487	12
Female recipients	0.762	-0.513 - 2.037	0.241	0.375	13
Duration of renal replacement therapy, months	0.011	-0.001 - 0.023	0.068	0.669	14
Desensitization	-1.474	-3.031 - 0.083	0.064	-0.616	15

Abbreviations) BKVAN, BK virus associated nephropathy

Table 24. Baseline clinical characteristics of the study population

Variables	Total (n=5,871)	Cohort 1 (n=2,806)	Cohort 2 (n=3,065)	P
Age, yrs	46.8 ± 11.8	48.7 ± 11.6	45.0 ± 11.7	<0.001
Female sex	2,440 (42)	1,148 (41)	1,292 (42)	0.335
Deceased donor	2,236 (38)	1,098 (39)	1,138 (37)	0.122
HLA mismatch (total)	3.2 ± 1.7	3.2 ± 1.8	3.2 ± 1.7	0.382
Class I mismatch	2.2 ± 1.2	2.2 ± 1.2	2.1 ± 1.2	0.400
Class II mismatch	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	0.496
Eplet mismatch, class I	10.6 ± 6.8	10.5 ± 6.7	10.6 ± 6.9	0.801
Eplet mismatch, class II	24.1 ± 17.6	23.7 ± 17.2	24.3 ± 17.6	0.197
Retransplantation	432 (7)	212 (8)	220 (7)	0.580
Desensitization	1,063 (18)	626 (22)	437 (14)	<0.001
ABO incompatible KT	693 (12)	425 (15)	268 (9)	<0.001
Cold ischemic time (hrs)	710 (12)	548 (20)	162 (5)	<0.001
Total acute rejection	1.9 ± 2.3	1.9 ± 2.2	1.9 ± 2.3	0.838
Biopsy-proven acute rejection	520 (9)	335 (12)	185 (6)	<0.001

Cause of end stage renal disease				<0.001
Diabetic nephropathy	1,176 (20)	622 (22)	554 (18)	
Hypertension	909 (15)	461 (16)	448 (15)	
Glomerulonephritis	1,988 (34)	950 (34)	1,038 (34)	
Others	260 (4)	233 (8)	27 (1)	
Unknown	1,538 (26)	540 (19)	998 (33)	
Diabetes (recipient)	1,496 (25)	787 (28)	709 (23)	<0.001
Cardiovascular disease (recipients)	562 (10)	296 (11)	266 (9)	0.016
Donor age, yrs	44.8 ± 12.9	46.6 ± 12.8	43.1 ± 12.8	<0.001
Donor sex	2,611 (44)	1,278 (46)	1,333 (44)	0.121
Donor diabetes	220 (4)	146 (5)	74 (3)	<0.001
Donor hypertension	710 (13)	419 (15)	291 (10)	<0.001

Abbreviations) ADPKD, autosomal dominant polycystic kidney disease; DBP, diastolic blood pressure; SBP, systolic blood pressure

Table 25. Estimated eplet of the study population

Variables	Total (n=5,871)	Cohort 1 (n=2,806)	Cohort 2 (n=3,065)	P
Eplet mismatch class I	10.6 ± 6.8	10.5 ± 6.7	10.6 ± 6.9	0.801
Antibody verified eplet	5.9 ± 4.0	5.9 ± 4.0	6.0 ± 4.1	0.656
Other eplet	4.6 ± 3.2	4.6 ± 3.2	4.6 ± 3.2	0.980
Eplet mismatch class II	24.1 ± 17.6	23.7 ± 17.2	24.3 ± 17.6	0.197
DR	10.6 ± 8.6	10.6 ± 8.5	10.6 ± 8.7	0.986
Antibody verified DR	4.3 ± 3.9	4.3 ± 3.8	4.2 ± 3.9	0.202
Other DR	6.3 ± 5.3	6.3 ± 5.4	6.4 ± 5.3	0.341
DQ	13.5 ± 10.6	13.2 ± 10.5	13.7 ± 10.7	0.036
Antibody verified DQ	5.3 ± 4.9	5.0 ± 4.8	5.5 ± 5.0	<0.001
Other DQ	8.2 ± 6.4	8.1 ± 6.4	8.2 ± 6.5	0.701

Table 26. Association of HLA eplet mismatches with acute rejection

Variables	Unadjusted Hazard Ratio	95% C.I.	P	Adjusted hazard ratio	95% C.I.	P
HLA mismatches						
HLA-A	1.182	1.050 – 1.331	0.006	0.965	0.806 – 1.155	0.695
HLA-B	1.389	1.222 – 1.578	<0.001	1.169	0.973 – 1.406	0.096
HLA-DR	1.382	1.222 – 1.563	<0.001	1.240	1.020 – 1.509	0.031
Eplet mismatches						
Class I	1.024	1.011 – 1.037	<0.001	1.007	0.985 – 1.029	0.523
Class II	1.001	1.004 – 1.014	<0.001	N/A	N/A	N/A
DR	1.018	1.009 – 1.028	<0.001	1.003	0.989 – 1.017	0.685
DQ	1.012	1.004 – 1.020	0.003	0.997	0.986 – 1.008	0.546

Recipient age (10yrs)	0.956	0.889 – 1.029	0.231	0.895	0.829 – 0.965	0.004
Recipient female sex	0.853	0.714 – 1.018	0.079	0.856	0.715 – 1.024	0.088
Donor age (10yrs)	1.241	1.156 – 1.332	<0.001	1.229	1.142 – 1.323	<0.001
Deceased donor	1.124	0.943 – 1.339	0.192	1.048	0.875 – 1.256	0.611

Table 27. Identification of individual eplet to biopsy proven acute rejection

Eplets	Non-rejection (n=2,471)	Rejection (n=335)	Total (n=2,806)	P
Class I				
113-76ED	141 (5.7)	30 (9.0)	171 (6.1)	0.242
120-143S	221 (8.9)	42 (12.5)	263 (9.4)	0.297
121-147L	221 (8.9)	42 (12.5)	263 (9.4)	0.297
19-180E	323 (13.1)	57 (17.0)	380 (13.5)	0.341
109-71KA	95 (3.8)	20 (6.0)	115 (4.1)	0.351
4-65QIA	350 (14.2)	60 (17.9)	410 (14.6)	0.385
107-66KA	318 (12.9)	54 (16.1)	372 (13.3)	0.428
5-69AA	411 (16.6)	68 (20.3)	479 (17.1)	0.430
108-66IS	378 (15.3)	63 (18.8)	441 (15.7)	0.432

6-69TNT	116 (4.7)	22 (6.6)	138 (4.9)	0.453
Class II				
DR-2-11-STS	381 (15.4)	75 (22.4)	456 (16.3)	0.124
DQB-6-37YA	187 (7.6)	41 (12.2)	228 (8.1)	0.145
DR-8-77N	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-10-98Q	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-1-13SE	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-17-71K	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-7-73A	0 (0)	1 (0.3)	1 (0.03)	0.160
DR-8-77T	0 (0)	1 (0.3)	1 (0.03)	0.160
DQB-14-86G	226 (9.1)	47 (14.0)	273 (9.7)	0.164
DR-9-	226 (9.1)	47 (14.0)	273 (9.7)	0.164

Table 28. Characteristics of suggested individual eplet

Eplets	Antibody verified	Ellipro score	Luminex Allele of Epitope
Class I			
76ED	No	High	B*27:03, B*27:05, B*37:01, B*47:01
143S	Yes	High	B*40:01, B*48:01, B*81:01, C*17:01
147L	No	High	B*40:01, B*48:01, B*81:01, C*07:01, C*07:02, C*07:04, C*17:01
180E	Yes	High	B*07:02, B*07:03, B*08:01, B*40:01, B*41:01, B*41:02, B*42:01, B*48:01, B*81:01
71KA	No	Low	B*27:03, B*27:05, B*27:08, B*73:01
65QIA	Yes	Intermediate	B*07:02, B*27:03, B*27:05, B*27:08, B*42:01, B*54:01, B*55:01, B*56:01, B*67:01, B*73:01, B*81:01, B*82:01, B*82:02

66KA	No	Intermediate	A*02:01, A*02:02, A*02:03, A*02:05, A*02:06, A*23:01, A*23:02, A*24:02, A*24:03, A*34:01
69AA	Yes	Intermediate	B*07:02, B*15:16, B*27:03, B*27:05, B*27:08, B*42:01, B*54:01, B*55:01, B*56:01, B*57:01, B*57:03, B*58:01, B*67:01, B*73:01, B*81:01, B*82:01, B*82:02
66IS	No	Low	B*13:01, B*13:02, B*15:01, B*15:02, B*15:03, B*15:12, B*15:13, B*18:01, B*37:01, B*40:01, B*40:02, B*40:05, B*40:06, B*41:01, B*41:02, B*44:02, B*44:03, B*45:01, B*47:01, B*48:01, B*49:01, B*50:01, B*52:01
69TNT	Yes	Intermediate	B*07:03, B*08:01, B*13:01, B*13:02, B*14:01, B*14:02, B*14:05, B*14:06, B*15:01, B*15:02, B*15:03, B*15:10, B*15:11, B*15:12, B*15:13, B*15:18, B*18:01, B*35:01, B*35:08, B*37:01, B*38:01, B*39:01, B*39:05, B*40:01, B*40:02, B*40:05, B*40:06, B*41:01, B*41:02, B*44:02, B*44:03, B*45:01, B*47:01, B*48:01, B*49:01, B*50:01, B*51:01, B*51:02, B*52:01, B*53:01, B*59:01, B*78:01

Class II

DR-2-11-STS	Yes	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02, DRB1*14:03, DRB1*14:54
DQB-6-37YA	No	DQB1*03:01, DQB1*03:02, DQB1*03:03, DQB1*03:19, DQB1*04:01, DQB1*04:02, DQB1*06:02, DQB1*06:03, DQB1*06:04, DQB1*06:09
DR-8-77N	Yes	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-10-98Q	Yes	DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-1-13SE	No	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02,

		DRB1*14:03, DRB1*14:54, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-17-71K	No	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*04:01, DRB1*13:03, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-7-73A	Yes	DRB1*01:01, DRB1*01:02, DRB1*01:03, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05, DRB1*08:01, DRB1*08:02, DRB1*09:01, DRB1*09:02, DRB1*10:01, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*12:01, DRB1*12:02, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02, DRB1*14:03, DRB1*14:04, DRB1*14:54, DRB1*15:01, DRB1*15:02, DRB1*15:03, DRB1*16:01, DRB1*16:02, DRB4*01:01, DRB4*01:03, DRB5*01:01, DRB5*02:02
DR-8-77T	Yes	DRB1*01:01, DRB1*01:02, DRB1*01:03, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05,

		DRB1*07:01,	DRB1*08:01,	DRB1*08:02,	DRB1*09:01,
		DRB1*09:02,	DRB1*10:01,	DRB1*11:01,	DRB1*11:03,
		DRB1*11:04,	DRB1*12:01,	DRB1*12:02,	DRB1*13:01,
		DRB1*13:02,	DRB1*13:03,	DRB1*13:05,	DRB1*14:01,
		DRB1*14:02,	DRB1*14:03,	DRB1*14:04,	DRB1*14:54,
		DRB1*15:01,	DRB1*15:02,	DRB1*15:03,	DRB1*16:01,
		DRB1*16:02,	DRB4*01:01,	DRB4*01:03,	DRB5*01:01,
		DRB5*02:02			
DQB-14-86G	No	DQB1*06:04, DQB1*06:09			
DQB130Q-	No	DQB1*06:04, DQB1*06:09			

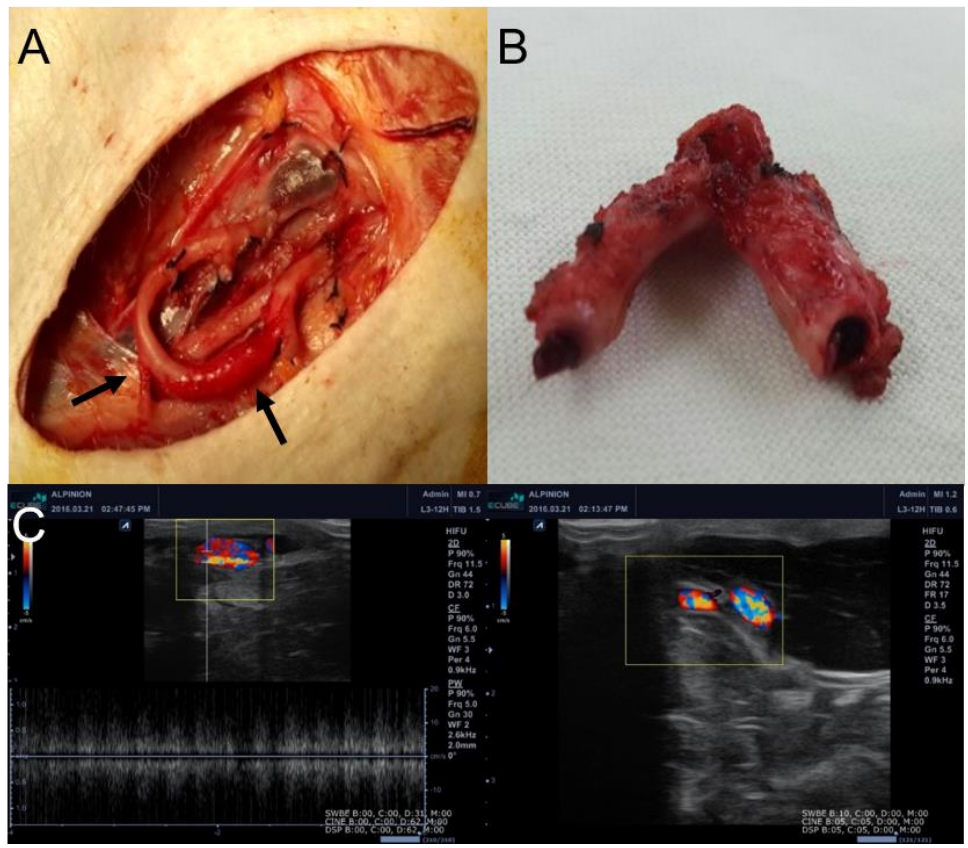


Figure 1. Alpha-galactosyltransferase knock out (GTKO) porcine vascular transplantation to Cynomolgus monkey

(A) Porcine artery graft anastomosed to femoral artery and femoral vein of cynomolgous monkey (B) Excised porcine artery graft after 4 weeks of transplantation periods. Note intraluminal thrombus. (C) Doppler ultrasonographical assessment of transplanted graft.

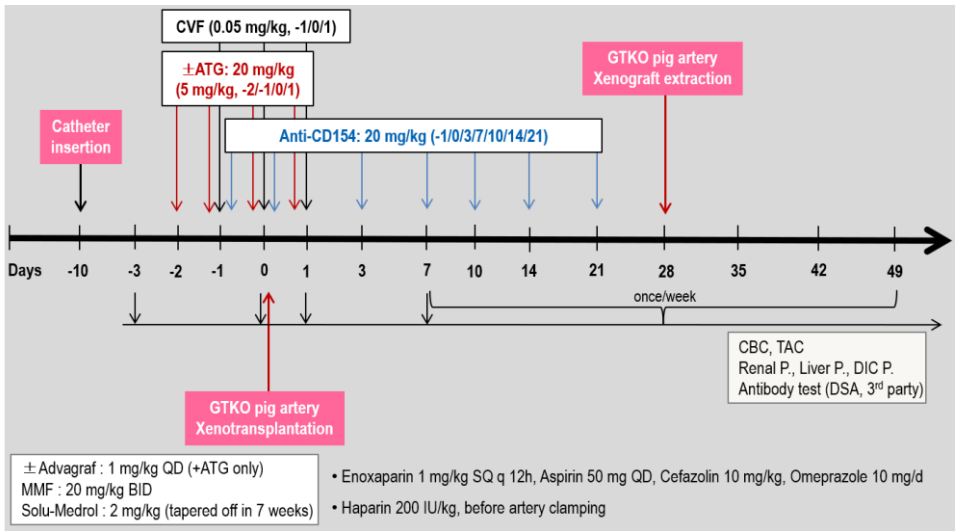
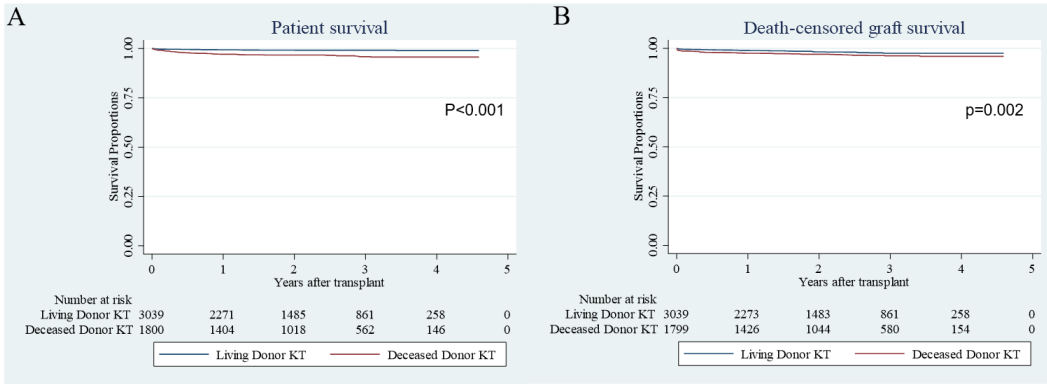


Figure 2. Immunosuppressive regimens of the GTKO pig artery transplantation in Cynomolgus monkey

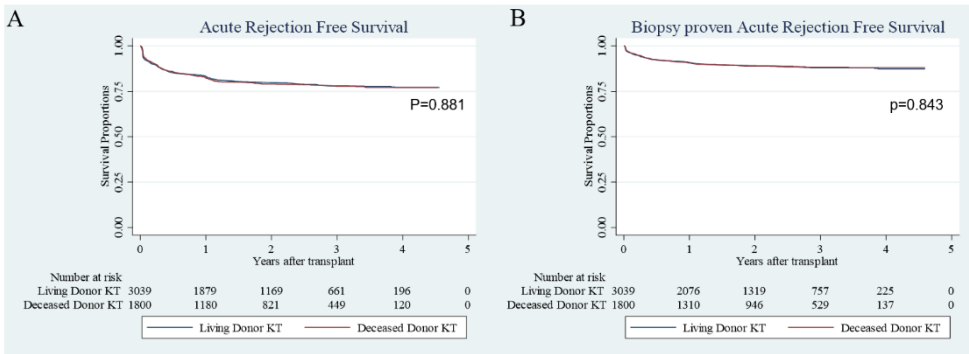


	1yr	2yr	3yr	4yr	5yr
Total	98.4 (98.0 – 98.8)	98.2 (97.8 – 98.6)	97.8 (97.3 – 98.3)	97.6 (97.0 – 98.1)	97.6 (97.0 -98.1)
LDKT	99.3 (98.5 – 99.2)	99.2 (97.6 – 98.7)	99.1 (96.8 – 98.2)	98.9 (96.8 – 98.2)	98.9 (96.8 – 98.2)
DDKT	97.0 (96.0 – 97.8)	96.6 (95.6 – 97.4)	95.9 (94.7 – 96.8)	95.6 (94.2 – 96.6)	95.6 (94.2 – 96.6)

	1yr	2yr	3yr	4yr	5yr
Total	98.4 (98.0 – 98.7)	97.8 (97.3 – 98.2)	97.0 (96.4 – 97.6)	96.9 (96.2 – 97.5)	96.9 (96.2 -97.5)
LDKT	99.0 (98.5 – 99.2)	98.3 (97.6 – 98.7)	97.6 (96.8 – 98.2)	97.6 (96.8 – 98.2)	97.6 (96.8 – 98.2)
DDKT	97.4 (96.5 – 98.1)	97.0 (96.0 – 97.7)	96.1 (94.9 – 97.0)	95.9 (94.5 – 96.9)	95.9 (94.5 – 96.9)

Figure 3. Patient and death-censored graft survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of patient survival (B) Kaplan-Meier curve of death-censored graft survival

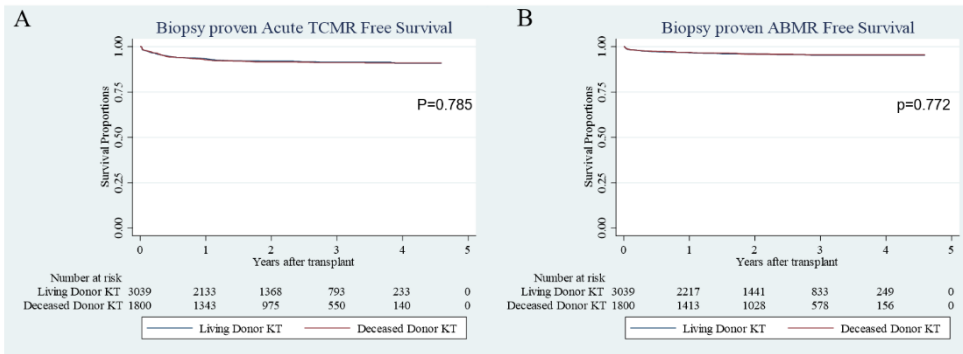


	1yr	2yr	3yr	4yr	5yr
Total	82.4 (98.0 – 98.8)	78.4 (97.8 – 98.6)	77.0 (97.3 – 98.3)	76.2 (97.0 – 98.1)	76.2 (97.0 -98.1)
LDKT	82.3 (80.8 – 83.7)	78.5 (76.9 – 80.1)	76.8 (75.0 – 78.5)	76.1 (74.2 – 77.9)	76.1 (74.2 – 77.9)
DDKT	81.7 (79.7 – 83.4)	78.2 (76.1 – 80.1)	77.1 (74.9 – 79.2)	76.3 (73.9 – 78.5)	76.3 (73.9 – 78.5)

	1yr	2yr	3yr	4yr	5yr
Total	90.3 (89.4 – 91.2)	88.5 (87.5 – 89.5)	87.6 (86.5 – 88.6)	87.3 (86.1 – 88.3)	87.3 (86.1 -88.3)
LDKT	90.4 (89.2 – 91.5)	88.5 (87.2 – 89.7)	87.3 (85.9 – 88.6)	87.0 (85.4 – 88.4)	87.0 (85.4 – 88.4)
DDKT	90.2 (88.7 – 91.6)	88.6 (86.9 – 90.0)	88.0 (86.2 – 89.5)	87.7 (85.8 – 89.3)	87.7 (85.8 – 89.3)

Figure 4. Acute rejection free- and biopsy-proven acute rejection free- survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of acute rejection-free survival (B) Kaplan-Meier curve of biopsy-proven acute rejection-free survival



	1yr	2yr	3yr	4yr	5yr
Total	92.8 (92.0 – 93.5)	91.4 (90.5 – 92.2)	91.0 (90.0 – 91.8)	90.6 (89.6 – 91.6)	90.6 (89.6 - 91.6)
LDKT	93.0 (91.9 – 93.9)	91.5 (90.3 – 92.5)	91.0 (89.7 – 92.1)	90.6 (89.3 – 91.8)	90.6 (89.3 – 91.8)
DDKT	92.5 (91.1 – 93.7)	91.3 (89.8 – 92.6)	90.9 (89.4 – 92.3)	90.7 (89.0 – 92.1)	90.7 (89.0 – 92.1)

	1yr	2yr	3yr	4yr	5yr
Total	96.5 (95.9 – 97.0)	95.7 (95.1 – 96.3)	95.2 (94.4 – 95.8)	95.2 (94.4 – 95.8)	95.2 (94.4 -95.8)
LDKT	96.4 (95.6 – 97.1)	95.7 (94.9 – 96.5)	95.1 (94.1 – 95.9)	95.1 (94.1 – 95.9)	95.1 (94.1 – 95.9)
DDKT	96.7 (95.7 – 97.5)	95.8 (94.6 – 96.7)	95.4 (94.2 – 96.4)	95.4 (94.2 – 96.4)	95.4 (94.2 – 96.4)

Figure 5. Acute T-cell mediated rejection free- and acute antibody mediated rejection free- survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of acute T-cell mediated rejection-free survival (B) Kaplan-Meier curve of acute antibody mediated rejection-free survival

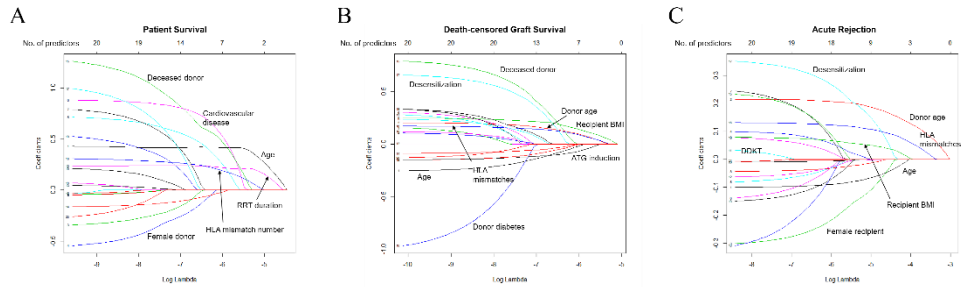


Figure 6. Variable selection and coefficient pathways in least absolute shrinkage and selection operator (LASSO) method for patient survival, death-censored graft survival, and acute rejection

(A) Coefficient path plot to patient survival (B) Coefficient path plot to death-censored graft survival (C) Coefficient path plot to acute rejection

Upper x-axis indicates included numbers of predictors in regularized LASSO models at certain log lambda values. Numeric lables indicate each predictors as follows: 1, recipient age; 2, donor age; 3, female recipient; 4, female donor; 5, diabetic recipient; 6, history of cardiovascular disease in recipient; 7, history of cancer in recipient; 8, systolic blood pressure in recipient; 9, body mass index in recipient; 10, donor diabetes; 11, donor hypertension; 12, duration of renal replacement therapy; 13, systolic blood pressure in donor; 14, body mass index in donor; 15, deceased donor; 16, HLA mismatch numbers; 17, desensitization; 18, anti-thymocyte globulin induction; 19, ever-smoker (recipient); 20, ever-smoker (donor)

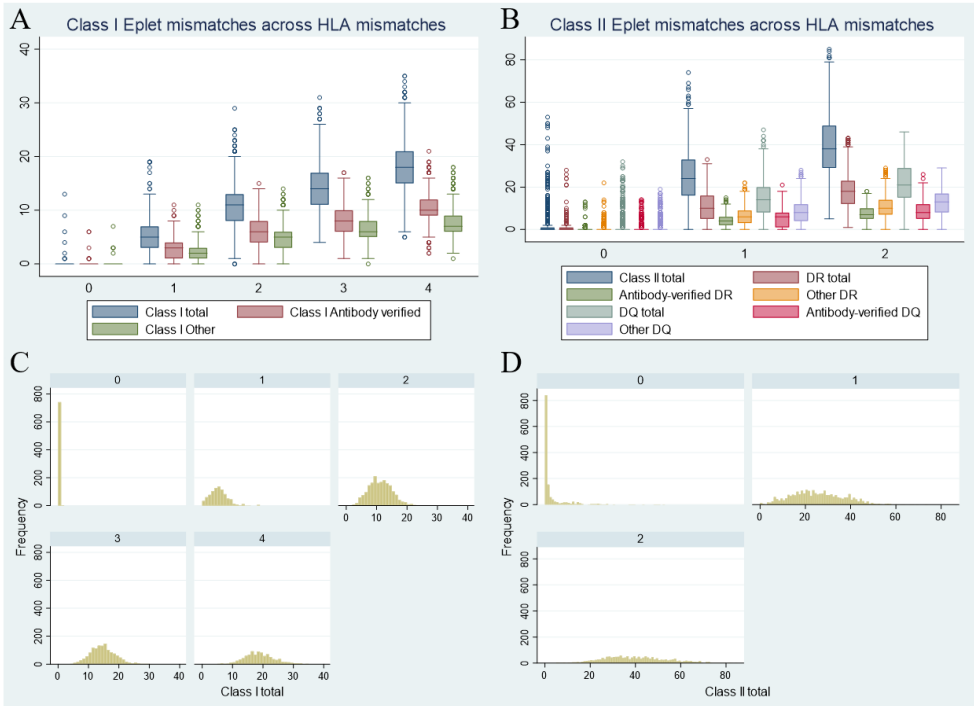


Figure 7. Distribution of eplet mismatches across human leukocyte antigen mismatches

(A) Box and Whisker plot of class I eplet mismatches across HLA class I mismatches (B) Box and Whisker plot of class II eplet mismatches across HLA class II mismatches (C) Histogram of eplet mismatch distribution across class I HLA mismatches (D) Histogram of eplet mistmach distribution across class II HLA mismatches

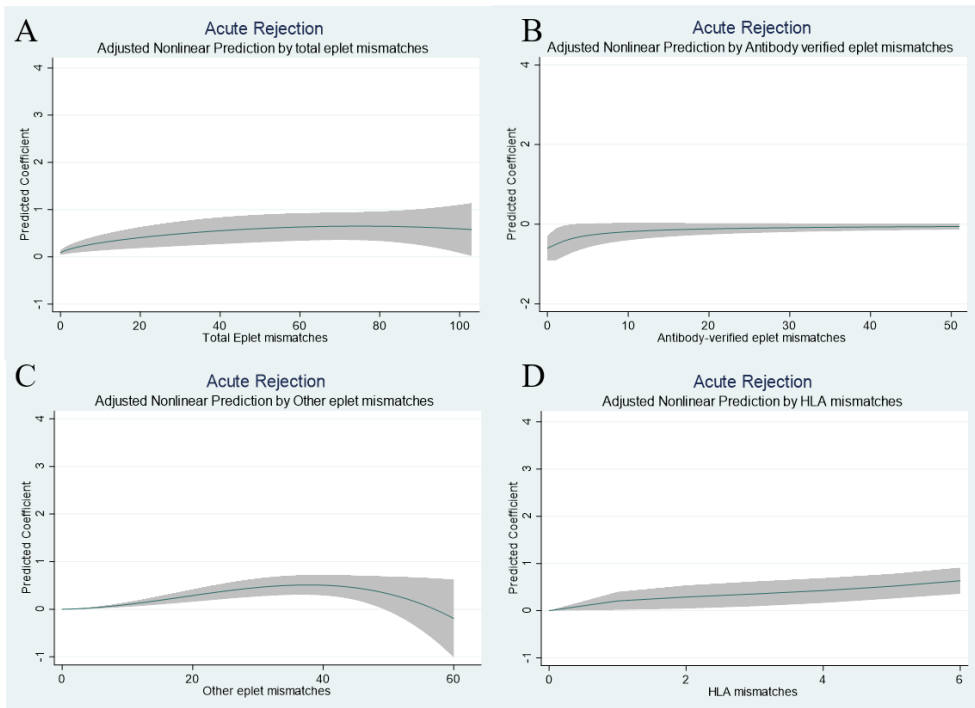


Figure 8. Adjusted risks of eplet mismatches or HLA mismatches to overall rejection (A) Non-linear risk of total eplet mismatch to acute rejection (B) Non-linear risk of antibody-verified eplet mismatch to acute rejection (C) Non-linear risk of non-antibody-verified eplet mismatch to acute rejection (D) Non-linear risk of HLA conventional genotype mismatch to acute rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta coefficient of target variable. Grey area indicate 95% confidence interval of

estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

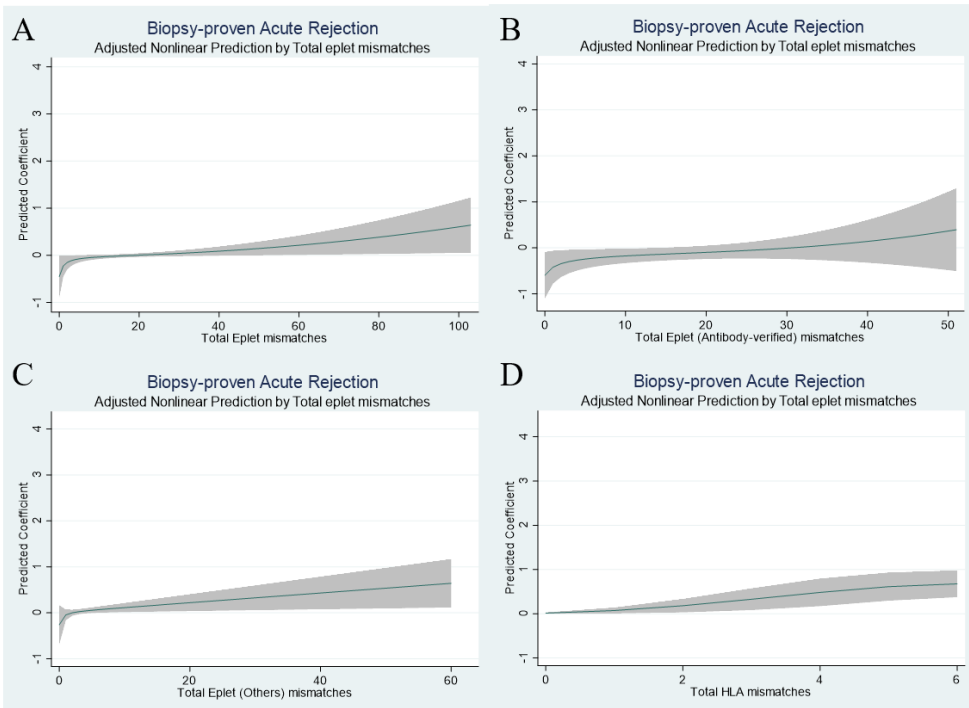


Figure 9. Adjusted risks of eplet mismatches or HLA mismatches to biopsy-proven rejection

(A) Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (B) Non-linear risk of antibody-verified eplet mismatch to biopsy-proven acute rejection (C) Non-linear risk of non-antibody-verified eplet mismatch to biopsy-proven acute rejection (D) Non-linear risk of HLA conventional genotype mismatch to biopsy-proven acute rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta

coefficient of target variable. Grey area indicate 95% confidence interval of estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

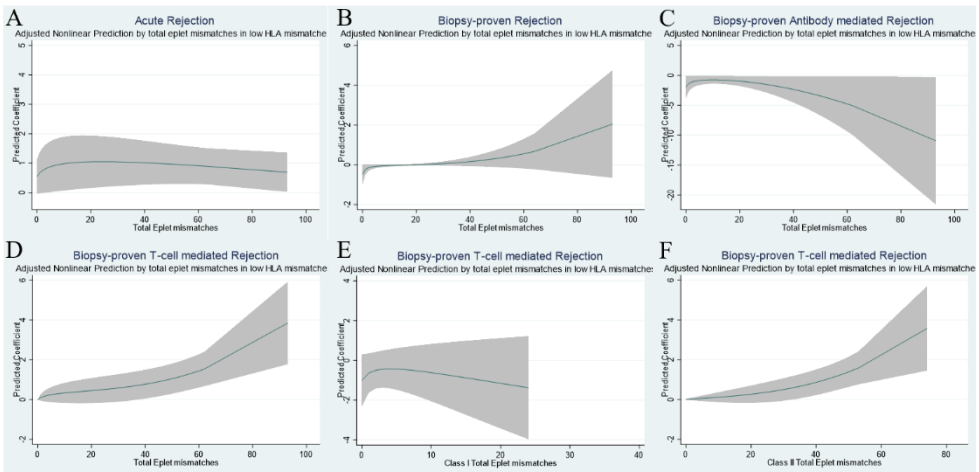


Figure 10. Adjusted risks of eplet mismatches to various rejection outcomes in low HLA mismatch settings (HLA mismatches < 3)

(A) Non-linear risk of total eplet mismatch to acute rejection (B) Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (C) Non-linear risk of total eplet mismatch to biopsy-proven antibody mediated rejection (D) Non-linear risk of total eplet mismatches to biopsy-proven acute T-cell mediated rejection (E) Non-linear risk of class I total eplet mistamches to biopsy-proven acute T-cell mediated rejection (F) Non-linear risk of class II total eplet mismatches to biopsy-proven acute T-cell mediated rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta coefficient of target variable. Grey area indicate 95% confidence interval of estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

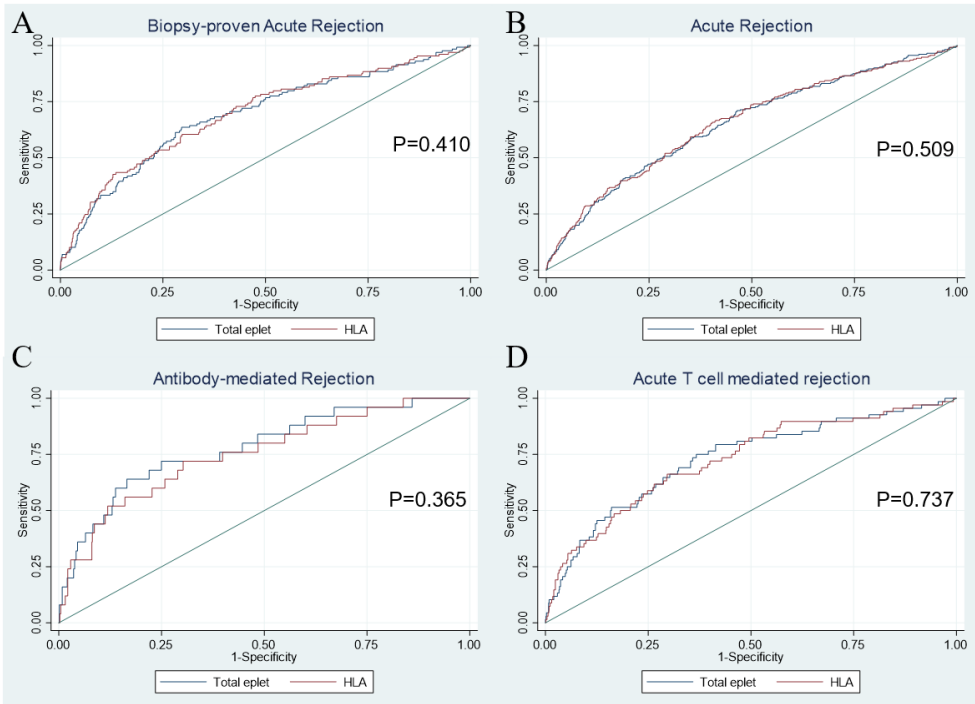


Figure 11. Comparisons of ROC curves of eplet mistmaches and human leukocyte antigen mismatches in low HLA mismatch settings (HLA mismatches < 3)

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension.

(A) Area under curve of prediction model Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (B) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute rejection (C) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute antibody-mediated rejection (D) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute T-cell mediated rejection

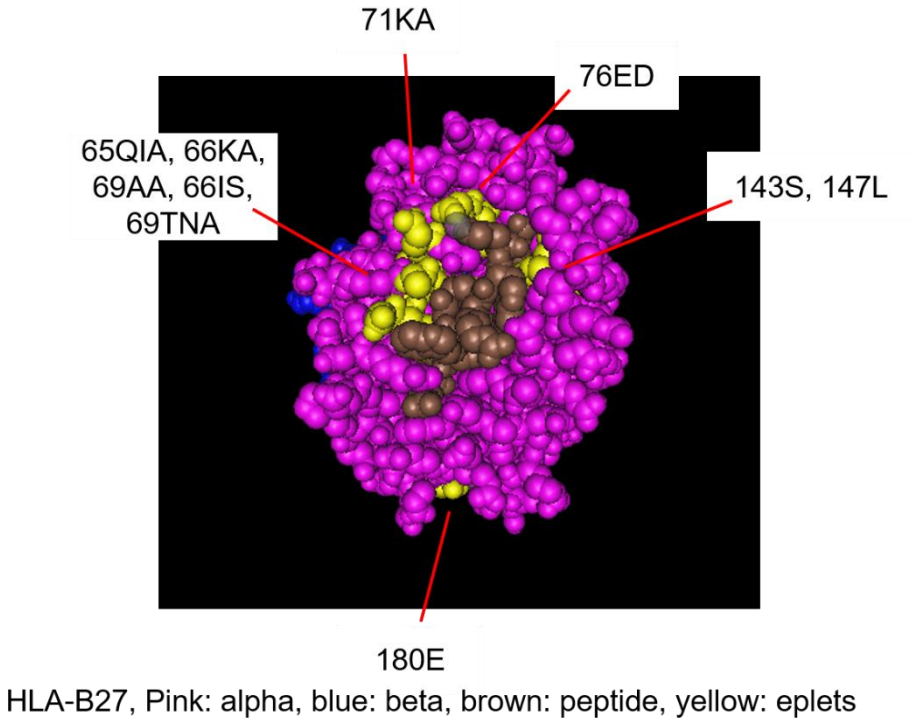


Figure 12. Graphical presentation of candidate eplets on three-dimensional HLA class I molecule (HLA-B27)

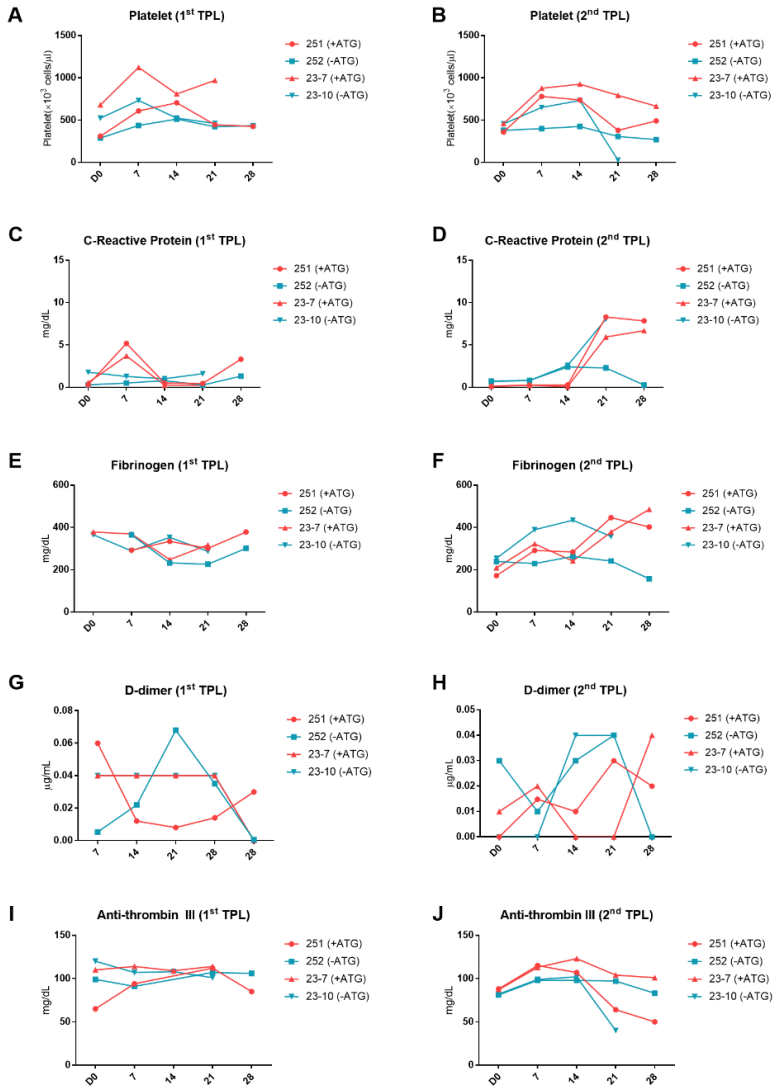


Figure 13. Platelet level and coagulation profiles after GTKO pig artery transplantation in Cynomolgus monkey

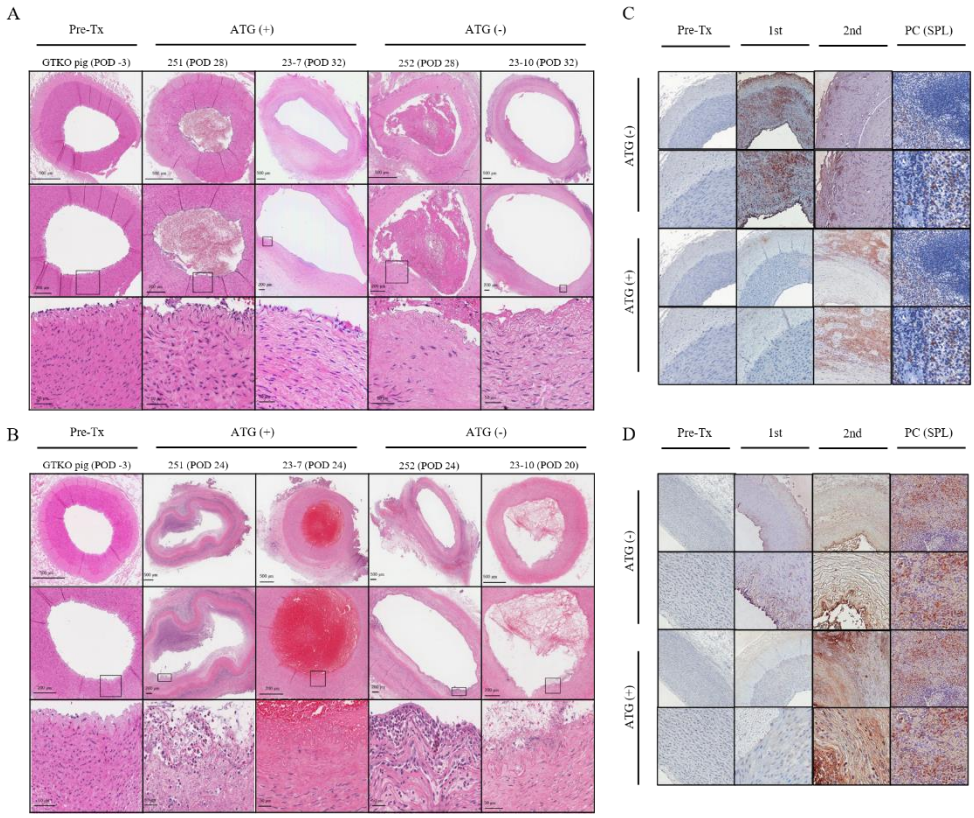


Figure 14. Histology and immunohistochemical stain of porcine vascular graft

(A) H&E stain of excised porcine vascular graft in 1st transplantation (B) H&E stain of excised porcine vascular graft in 2nd transplantation. Compared to 1st transplantation, more vigorous phenotype are observed (C) Immunohistochemical stain of anti-CD68 in 1st and 2nd transplantation (D) Immunohistochemical stain of anti-myeloperoxidase in 1st and 2nd transplantation

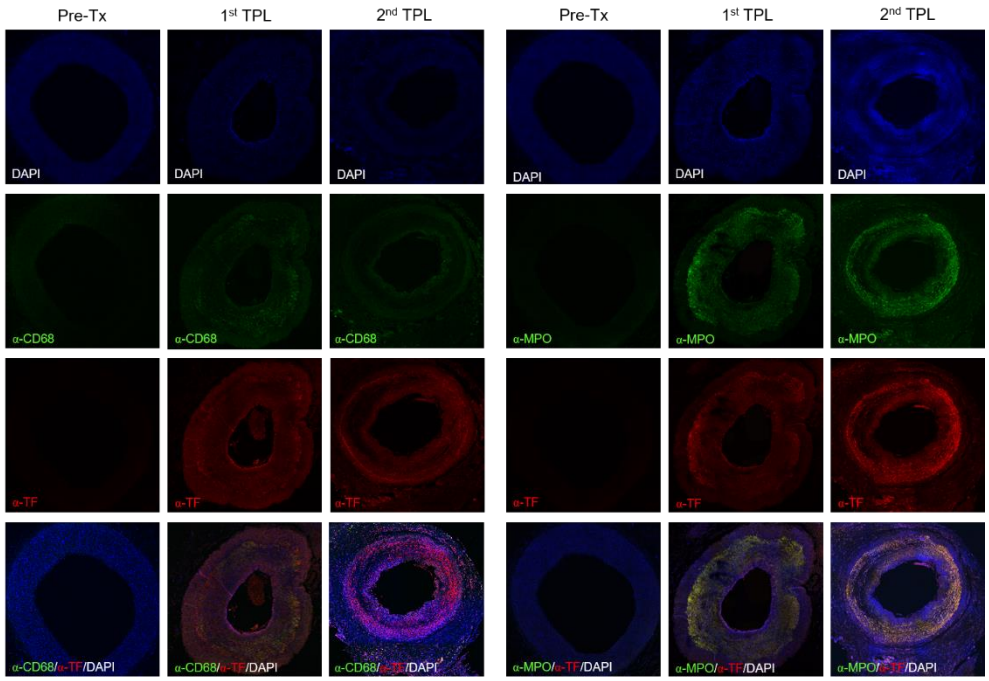


Figure 15. Immunofluorescence assay of CD68, myeloperoxidase, and tissue factor among 1st and 2nd xenograft.

Strong expression of myeloperoxidase and tissue factor are observed in tunica media and adventitia of excised porcine artery conduit xenograft

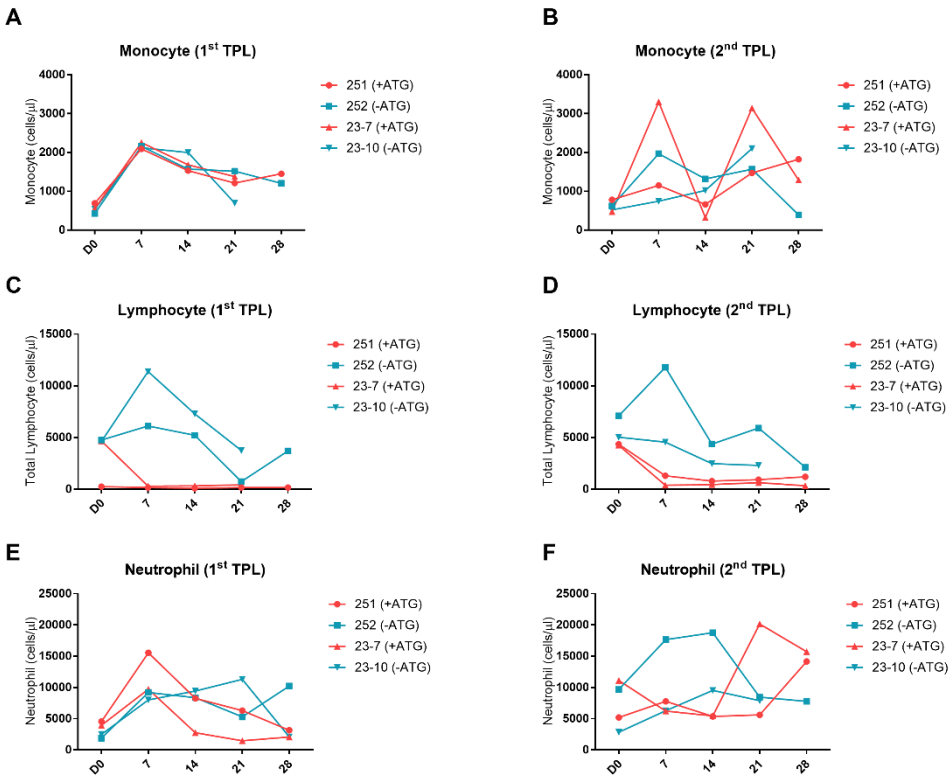


Figure 16. Peripheral circulating cell monitoring of GTKO pig artery transplantation in Cynomolgus monkey

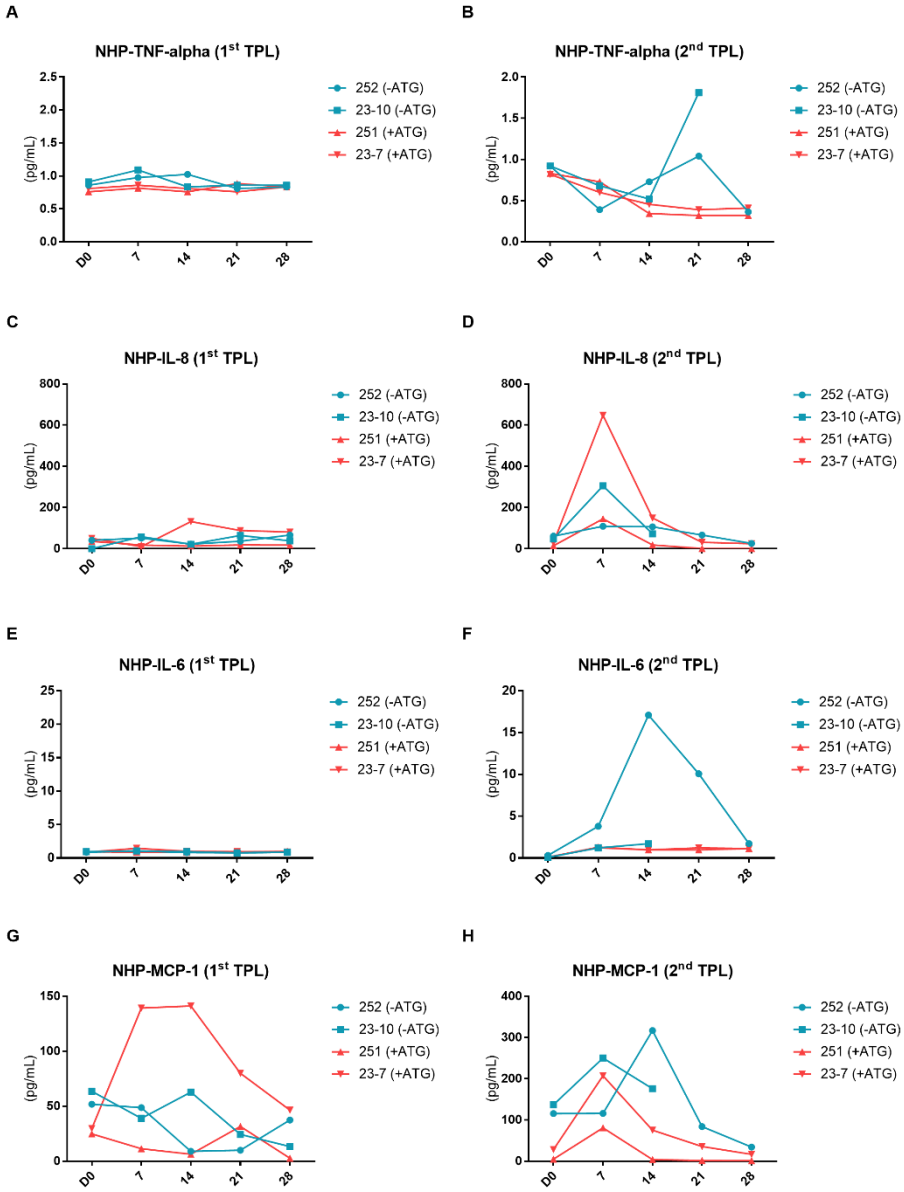


Figure 17. Longitudinal cytokine follow up of GTKO pig artery transplantation in Cynomolgus monkey

Statistical significance were achieved at day 14 of IL-6 in 2nd transplantation (P-value < 0.001) , and at at day 7 of IL-8 in 2nd transplantation. (P=0.017)

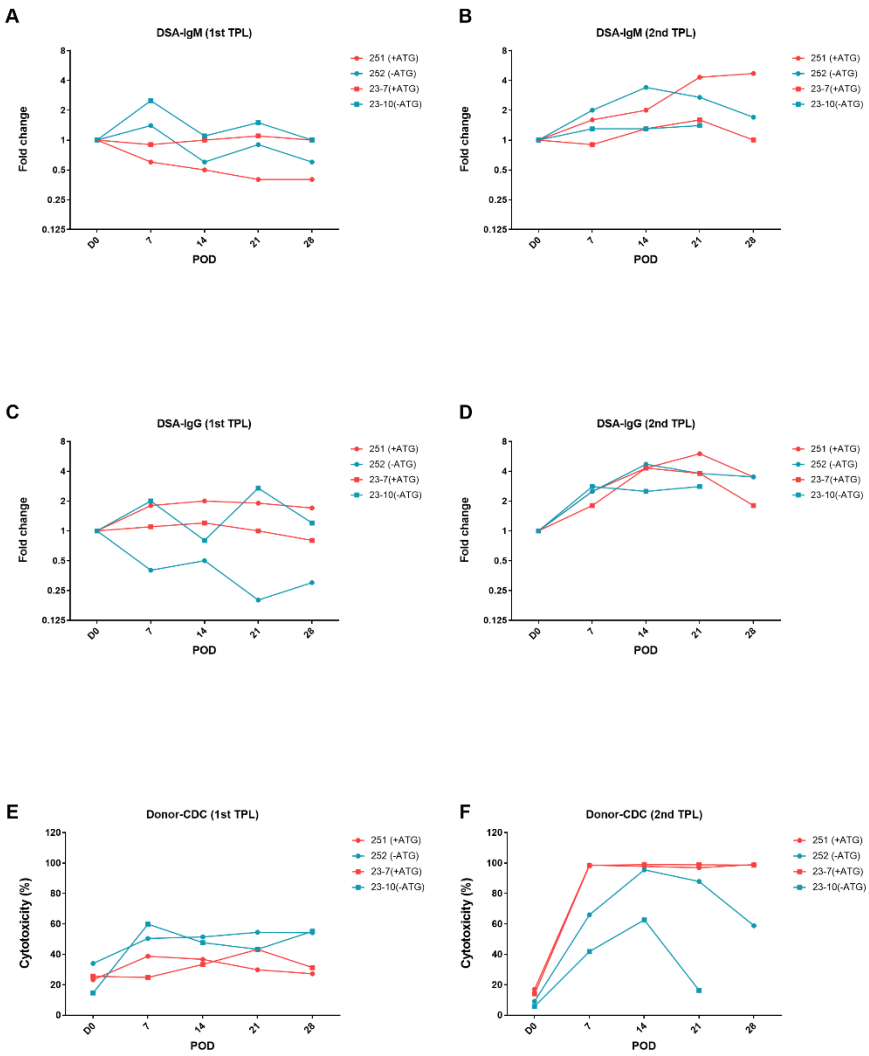


Figure 18. Trend of immunoglobulin M, immunoglobulin G and complement dependent cytotoxicity

Complement dependent cytotoxicity was strongly enhanced at the second transplantation.

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한국 장기이식 코호트 (KOTRY) 에서 신이식의 예후 분석 및 항체매개 거부반응의 영장류 혈관이식 모델 개발

배경

항체 매개 거부 반응은 동종 이식의 측면에서 장기 이식신 실패의 유의한 위험 요인이며, 고도 감작 이식에서의 극복해야할 대상이다. 한편, 신장이식의 경과를 통계학적으로 충분한 검정력을 가지고 파악하기에는 다기관 연구, 레지스트리 구축이 필요하다. 주조직항원의 에플렛은 항체매개 거부반응의 중요한 타겟 분자이다. 이종 이식에서 항체 매개 거부반응은 공여자 돼지의 유전자 조작을 통하여 극복되고 있으나, 자연 항체 및 유도 항체에 의한 면역의 감작 현상들은 여전히 중요한 도전과제이다. 영장류 이식 모델에서 항체 매개 거부반응을 연구하는데 한 가지 장애요인은 모델 형성의 기술적 난이도인데, 이는 현재 돼지 동맥 패치 모델을 통해서 개선되고 있다.

방법

이식 후 초기 성적의 예측 인자 연구 및 에플렛의 임상적 효용성 연구를 위해 한국 장기이식 코호트 (KOTRY) 의 자료가 이용되었다. 2014 년부터 2018 년까지 신장이식을 받은 환자가 등록되었다. 변수 선택법으로 라소 (LASSO), 후진 소거법을 이용하였고, 우세분석을 활용하여 선택 변수간의 상대적인 중요도를 서열화하여 우세 요인을 도출하였다. 에플렛 불일치는 HLA 하플로타입 분포의 매칭을 통하여여 HLA 4 자리수로 변경하여 추정하였다. 에플렛 불일치의 급성 거부반응과의 상관관계 모델링으로는 비선형 모형으로 분해 다항식 모형을 활용하였다. 항체매개성 거부반응의 기전 연구를 위하여 이종 이식

모형이 이용되었다. 이종재이식 모형으로 Gal 유전자 적중 돼지의 동맥편을 항-CD154 항체 및 면역억제제 3 제 요법 하에서 항-치모글로불린 유무에 따라 마카카 원숭이에 이식한 이종혈관이식 모형을 활용하였다.

결과

총 4,839 명의 한국장기이식코호트 신장 이식 수여자의 자료에서, 환자의 생존율은 각각 1 년째 98.4%, 3 년째 97.8%, 5 년째 97.6% 였다. 사망중도절단 이식신 생존율은 각각 1 년째 98.4%, 3 년째 97.0%, 5 년째 96.9% 였다. 생검으로 확인된 급성 거부반응이 없는 생존율은 각각 1 년째 90.3%, 3 년째 87.6%, 5 년째 87.3% 였다. T 세포 매개성 급성 거부반응이 없는 생존율은 각각 1 년째 92.8%, 3 년째 91.0%, 5 년째 90.6%였다. 급성 항체 매개성 거부반응이 없는 생존율은 각각 1 년째 96.5%, 3 년째 95.2%, 5 년째 95.2% 였다. 한국 장기이식 코호트에서 도출된 1 년 이내 초기 거부반응의 가장 우세한 예후 인자로서는 공여자 연령, HLA 불일치 개수였다. 1 년 이내 급성 항체매개성 거부반응의 가장 우세한 예후 인자로는 탈감작 여부, ATG 유도 요법, HLA 불일치 개수 였다. 60 개 이상의 에플렛 불일치는 HLA 유전자위 불일치가 2 개 이하로 적은 세부 그룹의 경우에 독립적인 급성 거부반응의 예측 인자였고, Class II 에플렛 불일치가 생검으로 확인된 급성 T 세포 매개 거부반응의 두드러진 유의한 예측인자였다. 돼지 동맥 패치 모델에 비해 본 돼지 동맥 혈관편 모델은, 혈관편의 기능 모니터링 (청진, 도플러 초음파)과 안전한 이식편 제거가 가능하여, 이종 이식에서 독특한 감작 연구를 가능하게 한다. 이종 혈관 재이식 모형에서 재이식 시의 혈중

인터류킨 6 의 상승, 보체 매개성 세포 독성의 증가와 이식 혈관편내의 조직 인자의 증가 및 이식 편의 증가된 거부반응을 관찰할 수 있었다.

결론

결론적으로, 본 연구에서는 한국장기이식코호트 자료를 활용하여 급성 거부 반응의 우세 인자를 확인하였고 에플렛 불일치의 임상적 효용을 확인하였다. 이종 재이식 모형을 통하여 감작 거부 반응에서의 항체 매개성 거부 반응의 증대 및 이의 보체 의존 세포독성, 말초 혈액 내 IL-6 의 상승, 이식 편 내의 조직인자 발현과의 관련성을 확인하였다.

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주요어: 장기이식 코호트, 장기이식 레지스트리, 항체매개성 거부반응, 신장이식, 영장류 혈관편 이식

학 번: 2014-30687

의학박사 학위논문

한국 장기이식 코호트 (KOTRY) 에서 신이식의 예후 분석 및 항체매개 거부반응의 영장류 혈관이식 모델 개발

Outcome analysis of renal allograft in Korean Organ Transplantation Registry (KOTRY) and the development of a non-human primate vascular graft model of antibody mediated rejection

2020 년 8 월

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지도교수 안 규 리

이 논문을 의학박사 학위논문으로 제출함

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서울대학교 대학원

의학과 중개의학

정 종 철

정종철의 박사학위논문을 인준함

2020 년 7 월

위 원 장 _____ (인)

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Abstract

Outcome analysis of renal allograft in Korean Organ Transplantation Registry (KOTRY) and the development of a non-human primate vascular graft model of antibody mediated rejection

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Background

Antibody mediated rejection (ABMR) is a significant risk factor for the long-term kidney allograft survival and a hurdle to the highly-sensitized transplantation. Large scale cohort or registry is needed to study clinical transplantation with adequate statistical power. Eplet of human leukocyte antigen is important molecular target to the development of ABMR. ABMR in xenotransplantation are being overcome by the genetic manipulation of donor pig. However, immune sensitization from natural antibody or induced antibody is still important challenge to xenotransplantation. One hurdle to study ABMR in non-human primate xenotransplantation model is the technical complexity, which is being improved by porcine artery patch graft model.

Methods

To study clinical predictors of early post-transplant outcome and clinical usefulness of eplet mismatch, patients included in Korean Organ Transplantation Registry (KOTRY) were used. Kidney transplant recipients who have been transplanted from 2014 to 2018 were enrolled. As variable selection methods, least absolute shrinkage and selection operator (LASSO) and backward stepwise elimination were used.

Dominance analysis was used to rank relative importance of selected variables. Eplet mismatch were imputed by using human leukocyte antigen (HLA) 4 digits transformation based on HLA haplotype distribution. Fractional polynomial was used for the non-linear modeling of eplet mismatches to acute rejection. Also, pathogenesis of ABMR were studied by using xenotransplantation model. As a repeated xenotransplantation model, porcine arterial grafts from GalT knockout pig were transplanted to cynomolgous monkeys under the triple immunosuppressants, anti-CD154 monoclonal antibody and stratification on the anti-thymocyte globulin.

Results

Among 4,839 kidney transplant recipients from KOTRY, overall patient survival rates were 98.4%, 97.8%, and 97.6% at 1, 3, and 5 years, respectively. Death-censored graft survival rates were 98.4%, 97.0%, and 96.9% at 1, 3, and 5 years, respectively. Biopsy-proven acute rejection free survival rates were 90.3%, 87.6%, and 87.3% at 1, 3, and 5 years, respectively. Acute T-cell mediated rejection free survival rates were 92.8%, 91.0%, and 90.6% at 1, 3, and 5 years, respectively. Acute antibody mediated rejection free survival rates were 96.5%, 95.2%, and 95.2% at 1, 3, and 5 years, respectively. In the KOTRY study population, the most dominant predictors to acute rejection within 1 year were donor age, and the mismatch number of HLA. Dominant factors to antibody mediated rejection were desensitization, followed by ATG induction, HLA mismatch numbers. Eplet mismatches were significant independent risk factors to acute rejection even in the low HLA locus mismatch subgroups, which was most prominent in the HLA class II eplet to the association of biopsy-proven T-cell mediated rejection. Compared to the previous porcine patch graft model, this porcine arterial graft model, functional monitoring (auscultation, Doppler) and safe graft removal were possible, which enabled unique sensitization model in xenotransplantation. Elevated serum interleukin 6, enhanced

complement dependent cytotoxicity, elevated tissue factor expression in harvested xenograft, and vigorous rejection histology were observed.

Conclusion

In this study, I found dominant predictors for acute rejection by using KOTRY data, and validated clinical usefulness of eplet mismatches. In repeated xenotransplantation model, increased ABMR were associated with enhanced complement dependent cytotoxicity, elevated level of peripheral IL-6 and prominent tissue factor expression at the xenograft.

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keywords: Organ transplantation registry, antibody mediated rejection, non-human primate study, eplet mismatches

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List of Tables and Figure Legends

1. Introduction

In transplantation field, nationwide or international transplantation registries has provided many valuable data resource and led the development of clinical science of transplantation. [1–3] The Korean Organ Transplantation Registry (KOTRY) group had been operating observational cohort of organ transplantation since 2012. Including myself, In 2014, the authors reported first nationwide retrospective data summary of 4,500 kidney transplantation cases which have received operation between 2009 and 2012. [4] Based on that project, prospective observational cohort of 5 different organ transplantation (kidney, liver, heart, lung and pancreas) started in 2014 with the same name as KOTRY. [5] KOTRY is composed of 5 solid organ transplantation cohorts, including those of kidney, liver, heart, lung, and pancreas transplants. KOTRY is expected to answer the following fundamental questions:

1. What is the primary indication for solid organ transplantation in the Korean population?
2. How severe is the comorbidity burden of solid organ transplantation?
3. What are the immediate post-surgical risks of solid organ transplantation?
4. What is the long-term course of solid organ transplantation?
5. What is the most common cause of death after solid organ transplantation?
6. What is the most common cause of allograft failure?
7. What is the prevalence of induction and maintenance immunosuppression?
8. What is the prevalence of post-transplant comorbidities?
9. What are the genetic factors associated with the deterioration of allograft function?
10. What are the biomarkers that predict the deterioration of allograft function?
11. What are the short- and long-term courses of living donors?

Among these questions, understanding the mechanism of transplanted organ rejection is critical, because it is associated with the early outcome and long-term prognosis of clinical transplantation. To understand the impact of clinical predictors in the real-world kidney transplantation, and to further raise study questions in terms of molecular mechanisms, three-way strategies were adopted: (1) I deployed the exploration study of clinical predictors to early post-transplant outcomes including acute rejections by developing clinical kidney transplantation cohort (KOTRY & Asian society transplantation registry (ASTREG)) and analyzing its early outcomes, (2) I investigated the role of molecular discrepancies of human leukocyte antigen by adopting the concept of eplet mismatches and, (3) I investigated the immunological phenomenon and involved mechanism of antibody-mediated rejection combined with xenogenic coagulation by using novel porcine vascular conduit retransplantation model.

In terms of statistical modelling used in exploratory study, most of clinical epidemiological studies have long used inferential methods which is based on knowledge of expert and predefined hypothesis. On the contrary, data-driven approach does not depend on prior hypothesis, which is usually used to build a prediction or prognostic model. Prognostic models in kidney transplantation is an active area of research, however, it was scarce to compare relative importance or weight of clinical predictors to post-transplant outcomes. [6,7,16,17,8–15] In the present study, I tried to compare relative importance of clinical predictors based on data-driven approach.

Human leukocyte antigen is target antigen in transplantation. Development of molecular and structural biology have led new findings in this molecule, which have been exploded in recent decades. With the advent of immunosuppressant, HLA mismatches are not anymore contraindication in solid organ transplantation. However, it is still significant risk factors for long term graft survival and acute

rejection in spite of modern immunosuppression.[18] Antibody mediated rejection and accompanying donor specific antibody (DSA) is current research topic because it interfere long term graft survival.[19,20] The risk factors for the development de novo DSA are class II HLA mismatching, early T cell-mediated rejection, sensitization status, inadequate immunosuppression, or patient nonadherence. [21–27]

As the antigenicity determining site of HLA has been understood, the concept of molecular mismatch has been developed. Eplets are one of those achievements, which is defined as small configurations of amino acid residues that play dominant roles in HLA epitopes reactive with antibodies. [28] Compared to single amino acid polymorphism as a basic unit of antigen mismatch, an eplet represent the smallest functional unit of an epitope-paratope interface, and are under assumption that it react with the central complementary determining regions of the antibody and locates in the surface of HLA molecules.[29] Clinical outcomes with the molecular mismatches have been reported, however, most of the associations were interpreted as the process of chronic allograft rejection or gradual development of de novo DSA. It is relatively scarce to study the association of early post-transplant outcomes with molecular mismatches. I investigated whether eplet mismatches is associated with early post-transplantation outcome, and gives additional precision value to HLA genotyping by using KOTRY data.

Advancement of genetic manipulation to donor pig kidney and better understanding of immunologic response in xenotransplantation setting are the key drivers for the development of xenotransplantation. Depletion of carbohydrate surface antigen and insertion of human complement regulatory proteins have made long term survival of transplanted graft.[30] Recently, triple knock out and 9 human gene modified pig are prepared, which is expected to extend transplanted organ survival even further.[31] However, proper patient selection is still challenging, and presence of natural antibody or induced antibody from previous exposure to

xenogenic materials might be another challenge to successful xenotransplantation.[32] Only few studies have been done to the sensitized xenotransplantation. [33,34] One of the barrier to sensitization study in xenotransplantation was the complexity of surgical skill to functioning organ transplantation, whereas feasible skin transplantation have limitation to observe humoral immune response. Because solid organ transplantation in xenotransplantation had resulted in vigorous acute rejection, study for antibody mediated rejection and sensitization was difficult. Hence, a pig to non-human primate xenotransplantation model using porcine artery graft was developed during this study as a modified version of artery patch graft, [35] which enables us to harvest after single episode of transplantation and to observe histological changes. ATG was administered to compare whether T cell depletion might affect to the development of induced antibody and maintained transplanted xenograft until 4 weeks to allow antigen exposure during acquired immunity development period.

2. Material and Methods

2.1 Development of Clinical Kidney Transplantation Registry (KOTRY and ASTREG)

First, nationwide solid organ transplantation cohort (Korean Organ Transplantation Registry, KOTRY) were developed to continuously capture the clinical status of kidney transplantation patients in South Korea. The experience of developing transplant registry was expanded to the development of Asian Society Transplantation Registry (ASTREG). For the development of ASTREG, here I briefly describe only the difference compared to KOTRY, because of the similarity of design concept of both registry.

Study Organization

The KOTRY consists of 59 participating centers (30 centers for kidney, 15 for liver, 4 for heart, 5 for lung, 5 for pancreas), a central coordination unit, and a medical research coordinating center (MRCC). The organizational structures include the organ-specific committee, executive committee, and steering committee. A central coordination unit leads the study process, checks enrollment status weekly, and gives feedback to the participating centers. The MRCC is in charge of data validation and statistical consultation. The Korean National Research Institute of Health (KNIH) developed and offered a global web-based electronic data capturing system, named iCReaT. KNIH also participates in the quality assurance of the collected data, regular surveillance of study conductance process, and the management and improvement of the electronic data capturing system. Bio-specimen collection, storage, and quality control are done under contract with

LabGenomics, and part of the deposited biosamples are transferred to KNIH for backup and future collaboration. All of the activities are managed by the KOTRY Foundation (http://www.kotry.org/).

For ASTREG, any kidney transplantation center in Asia can freely use the ASTREG-H platform and contact the ASTREG office after registration as a participating center. Currently, six individual Asian kidney transplantation centers use the ASTREG-H platform, including centers in the Philippines, Mongolia, Myanmar, and South Korea.

Exclusion criteria, Enrollment and Informed Consent

Recipients younger than 19 years are excluded. Except simultaneous pancreas-kidney co-transplantation, those undergoing simultaneous multi-organ transplantation are excluded to ensure the homogeneity of graft-related outcome. However, sequential organ transplantations are not excluded. For liver transplantation, there is no exclusion criteria for age. For the KOTRY, both the donor and recipient are required to register at KOTRY prior to transplantation for living donor organ transplantation. The medical records of eligible individuals are reviewed after receiving their informed consent. Blood samples are taken for DNA and serum/plasma storage before transplantation. In deceased donor organ transplantation, informed consent is taken from the recipient. Under the strengthened data protection laws of Korea, the social security identification number cannot be collected during the KOTRY. However, for outcome matching with the Korean national statistical office data or the centralized health insurance claims data, KOTRY receives an optional informed consent for the use of collected data for study of secondary outcomes. To achieve the best standardized process, the opinions of

each individual institutional review board had been acquired, then a standardized protocol and standardized consent format were submitted. This study was approved by the institutional review boards of all participating centers, and was performed in accordance with the Declaration of Helsinki and the Declaration of Istanbul.

For the ASTREG, data on kidney transplantation can be collected by using the ASTREG-H platform. As an online data collection format, ASTREG-H does not use any specific exclusion criteria for data entry. However, the current data format is oriented toward solitary kidney transplantation, not toward simultaneous kidney pancreas co-transplantation or other solid-organ transplantations. ASTREG provides an online secure and friendly data entry platform that enables each participating center to enter and download their data by using a secure registered ID. The ASTREG-H platform provides automated data verification as a built-in data cleansing system, and the data manager produces data queries to each participating center for data errors in a deidentified manner. ASTREG anonymizes the data to take care of patient confidentiality and privacy issues. The Gachon University Gil Medical Center institutional review boards approved the whole platform system provision (IRB No. GAIRB2019-098), and the waiver of informed consent was approved.

Study Design and Collected Variables

The KOTRY collects solid organ transplantation data to analyze epidemiological trends, graft-related outcomes, and patient mortality. In total, data on 5,014 variables are collected, which are summarized in Tables 1–3. The kidney aspect involves a total of 950 variables, which are comprised of 12 domains of recipient data, 3 domains of donor baseline data, and 4 domains of living donor follow-up data. The liver aspect involves 523 variables in total, which consist of 13

domains of recipient data, 4 domains of donor baseline data, and 3 domains of living donor follow-up. The heart aspect involves 886 variables with 13 domains of recipient data, and 3 domains of donor baseline data. The lung aspect involves 1,495 variables with 22 domains of recipient data, and 3 domains of donor baseline data. The pancreas aspect involves 1,160 variables with 16 domains of recipient data, 4 domains of donor baseline data, and 3 domains of living donor follow-up. Each domain was constructed as a single sheet in electronic case-report format (CRF) on a web-based system (iCReaT). Longitudinal data collection is based on a regular annual interval. For the collection of early comorbidities and adverse outcome, different time points were selected according to each organ's clinical characteristics.

ASTREG-H collects data on pre-transplant clinical and laboratory profiles and post-transplant outcomes. The kidney component collects 227 items across five different domains (Table 4). One domain is for baseline donor characteristics, and the remaining four domains are for recipient data, including recipient baseline characteristics, details about immunosuppression, post-transplant event (irregular interval outcome), and post-transplant annual evaluation. The regular annual evaluation provides a longitudinal panel format, which enables a multilevel longitudinal data analysis. The post-transplant event record is based on the date of outcome occurrence, which enables a time-to-event analysis. Patient death and graft failure are the major outcomes. Graft failure is defined as sustained (>3 months) dependency on dialysis. A pathology report is another important outcome, which is a structured format that follows the BANFF classification scoring system. Few examples of the definitions of other important major post-transplant outcomes are as follows: Cardiovascular disease is defined as cardiovascular death, myocardial infarction, ischemic heart disease with relevant clinical evidence (accompanied by therapeutic intervention or objective findings), and new-onset congestive heart failure requiring hospital admission. Cerebrovascular accident is defined as non-

traumatic hemorrhagic or ischemic brain disease diagnosed using computed tomography or magnetic resonance image. Tuberculosis is defined as a clinically active disease evidenced by typical chest radiographic imaging, microbiological confirmation, or treatment with anti-tuberculosis drugs.

To account for the time-varying nature of post-transplantation comorbidity and to deal with repeated events, post-transplantation comorbidity at every follow-up visit were collected, which allows the analysis of comorbidity duration, and the effects of new-onset comorbidities and their duration on post-transplant outcomes. For example, the duration of transient new-onset diabetes after transplantation (NODAT) or repeated incidence of cardiovascular disease can be collected. Follow-up records will be tracked up to the patients' deaths. However, graft-related variables, including rejection, graft function, and general laboratory profiles, will be tracked until graft loss. To minimize follow-up loss, newsletters regarding registration status and follow-up performance are periodically sent to each participating center and a transfer system is used. If a patient underwent transplantation in center A, and was then followed by center B that also participated in KOTRY, the transfer system allows center B to input that patient's data. To increase the follow-up rate of living donors, the KOTRY emphasizes the importance of follow-up of living donors to each participating center's physicians and surgeons.

Biosamples in KOTRY

For the KOTRY, DNA samples from each donor and recipient are collected prior to organ transplantation. In kidney, heart, lung, and pancreas transplantation, sera are collected from recipients at baseline, prior to transplantation, and again at 1- and 3-years after organ transplantation. Baseline samples are collected in liver transplantation recipients. From 2017, additional plasma samples from the recipients

are collected prior to kidney transplantation, and again at 1- and 3-years post-kidney transplant.

Study Outcomes

The primary outcomes are graft failure and patient death. In kidney transplantation, graft failure is defined as sustained (more than 3 months) dependency on dialysis. In liver, heart, and lung transplantation, graft failure is defined as patient death or re-transplantation. Pancreas graft failure is defined as insulin dependence or death with a full or partially functioning graft.

Pathology data collected included acute or chronic rejection and other diagnoses, such as virus infection and calcineurin inhibitor toxicity. Definitions of the major post-transplantation outcomes are as follows: cardiovascular disease is defined as cardiovascular death, myocardial infarction, ischemic heart disease with relevant clinical evidence (accompanied by therapeutic intervention or objective findings), new-onset congestive heart failure requiring hospital admission and arrhythmia. Stroke includes non-traumatic hemorrhagic or ischemic brain disease confirmed by computed tomography or magnetic resonance image. Tuberculosis is defined as clinically active disease, as evidenced by typical chest radiography imaging, microbiological confirmation, or treatment with anti-tuberculosis drugs. Causes of death are classified into cardiovascular, sudden cardiac death, infection, malignancy, liver disease, accident, suicide, and others.

Living donor outcomes are collected for living liver or kidney transplantation cases. Death, cause of death, and surgical morbidities are collected in both liver and kidney transplantations. Newly developed diseases, including diabetes, hypertension, and urinary stones, are collected in living kidney transplantation donors.

Data validation

Quadruple layers of data validation are available. First, a pre-defined automated data validation system is used at data input, to prevent simple errors. Automated data validation system checks are implemented for essential data elements, to minimize missing variables, and have pre-defined allowed data ranges, to reduce extreme outliers due to simple input error. Additionally, an automated data validation system guide is used to prevent entering of values inconsistent with other variables, by opening or blocking data fields in screens following a logical test of pre-entered data values. Second, manual data validation is performed quarterly by the MRCC by feedback to each participating center. Third, during the outcome adjudication meeting, the distribution of major outcomes is discussed, and outlier values are sent to each investigator. Finally, annual auditing are conducted for all participating centers, to survey their status, including ethical study conductance, adherence to the standardized study protocol, and direct comparison of randomly selected data with the original medical record. These processes are conducted using the Registries for Evaluating Patient Outcomes tool by the Agency for Healthcare Research Quality (AHRQ).

Building statistical analysis files and response to the data request

A statistical analysis file is built thrice a year, following a quarterly data cleansing process. When the participating center requests their own data, the last validated statistical analysis file is sent to the requesting center. A feedback time of 4 hours was aimed at, in parallel with the standard operating protocol of Scientific Registry of Transplant Recipients (SRTR).[3] To request all centers' data, items of the

requested variable are released as a de-identified set (at patient- and center-level) after approval of the organ committee in KOTRY, following review of the study proposal. Each center has access to the main database located in KNIH, and can download their own dataset; however, this is not recommended due to network traffic and incompleteness of data validation. Currently, KOTRY focuses on the ease of data cleansing through an attached online automatic plotting system, and on giving more informative feedback to the participating center, and finally on enforcing information technology-security issues.

Statistical considerations

Descriptive data analysis will be conducted for baseline characteristics. To study outcomes, time–to-event analysis will be primarily used. Life-table methods or Kaplan-Meier curves will be used to represent allograft or patients' survival, and time to major outcomes (cardiovascular disease, cancer, infection, acute rejection, etc.). For the competing nature of outcome events (e.g., patient death vs. cancer occurrence), competing risk models will be adopted for regression modeling.[36] The multilevel characteristics of data were adjusted using a shared-frailty model,[37] in which adjustment should be made for time-dependent confounders or different data hierarchies. To encompass the wide variability of allograft functional decline, the Bayesian smoother will be used.[38] Longitudinal allograft functional changes and associated factors will be analyzed using a mixed linear model. Since the format of follow-up data is a repeated panel structure, the marginal structural model with time-varying confounder adjustment can be applied.[39]

Statistical Power

From 2017, new annual enrollments are estimated as 1,200 for kidney, 700 for liver, 100 for heart, and 30 for lung and pancreas transplantation, respectively. In kidney transplantation, the previous Retro-KOTRY collected the data of 4,987 kidney recipients, and the effort is ongoing to collect the missing information (approximately 1,200 kidney recipient's data) from the end of the previous Retro-KOTRY enrollment and the launch of the prospective KOTRY-kidney. With the assumption of attaining the patient enrollment plan, Table 5 shows the minimum hazard ratios (HRs) detectable at a given prevalence level of risk factors by 2019, using exponential models based on the 20-year patient and graft survival for solid organ transplants from the Organ Procurement and Transplantation Network. The KOTRY-kidney cohort is estimated to detect a relative risk of 1.05 and 1.06 for graft survival and patient survival, respectively, with a 50% prevalent risk factor, at 5% alpha error and 20% beta error in an analysis using a Cox regression model (Table 5). Similarly, the KOTRY-liver, heart, lung, and pancreas cohorts will be able to detect HRs of 1.11, 1.32, 1.87, and 1.82, respectively, for graft survival.

Representativeness

In 2015, the total numbers of organ-transplant centers and KOTRY-participating centers were as follows: for kidney, 30 of 66 centers participated in KOTRY; for liver, 15 of 44; for heart, 4 of 13; for lung, 5 of 7; for pancreas, 5 of 9. As large-volume centers joined KOTRY, the numbers of organ transplantations performed in KOTRY-participating centers were predominantly as follows: for kidney, 1565 of 1891 (82.8%); for liver, 1073 of 1392 (77.1%); for heart, 127 of 145 (87.6%); for lung, 61 of 64 (95.3%); for pancreas, 51 of 59 (86.4%).

2.2 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

Next, I investigated the early outcomes in KOTRY kidney transplant recipients, and dominant predictors for the early outcomes and acute rejection.

Study objective, design, covariables, and statistical approach

For this study, dataset of kidney transplant recipients who received kidney transplantation KT from 2014 to 2018 were used. Total 4,839 KT recipients were analyzed. Mean duration of follow up was 26.0 ± 15.5 months. To derive best prediction model for post-transplantation outcome (patient survival, graft survival, acute rejection, post-transplant eGFR) from baseline (pre-transplant) covariables, diverse variable selection approach were tried depending on the availability of existing methods to the character of variables. For the continuous measures, Leaps and Bound algorithm determined by Akaike's information criteria was used for variable selection. [40,41] For the time to event outcomes and binary outcomes, least absolute shrinkage and selection operator (LASSO) or backward stepwise selection were used. A total 20 covariate candidates for prediction model construction were as follows: recipient age, donor age, recipient sex, donor sex, recipient's history of diabetes, recipients' history of cardiovascular disease, recipient's history of cancer, pre-transplant systolic blood pressure of recipient, pre-transplant body mass index of recipient, donor's diabetes history, donor's hypertension history, waiting times to kidney transplantation, pre-transplant systolic blood pressure of donors, pre-transplant body mass index of donor, deceased donor, total numbers of HLA mismatches, desensitization, ATG as induction agent, smoking history of donors and recipients. When all covariables were entered to the prediction model for the death

censored graft loss, c-statistics was 0.692, which was comparable to the previous prediction studies.[12]

After model has been built, dominance analysis were applied to rank relative importance of each selected variables to target outcome.[42,43] Because dominance analysis can be applied to generalized linear model, application of the methods were conducted only for continuous or binary outcome such as patient/graft survival status at 1 year, acute rejection within 1 year, or post-transplant eGFR at 1 year. Continuous data are presented as mean with standard deviation. Categorical data are presented as count with percent. Cox regression for time to event data was done under the proportional hazard assumption. Statistical analyses were performed using Stata software (version 16; StataCorp LP, College Station, TX) and R (version 3.6.3; R Foundation, Vienna, Austria).

Study Ethics

The study protocol was approved by the Seoul National University Hospital institutional review board (IRB No:H-1902-138-1014). Data analysis was done with de-identified datasets. Patient privacy was preserved in all instances, and the study methods complied with the tenets of the Declaration of Helsinki.

2.3 Significance of eplet mismatch in rejection

Here, I further investigated the molecular representation of human leukocyte antigen mismatch as more precise target of transplantation rejection. I adopted the concept of eplet mistmach.

Study population and eplet estimation

Kidney transplantation donor-recipients pairs of KOTRY were study population. Until now, KOTRY has two separated phase of data collection, the first one was retrospective data collection of 2009 – 2012 kidney transplant patients, and the other one is prospective data collection from 2014. Design and methods and summary data of each phase of KOTRY were described in detail in the previous reports. [4,44] In both dataset, common components were pretransplant evaluation including all HLA genotype (2 digits) results, immunologic risks, induction and maintenance immunosuppressants, biopsy-proven acute rejection, graft function measured as eGFR, graft and patient survival. Details of biopsy reports were available in prospective KOTRY. For this study, dataset of kidney transplant recipients who received KT from 2009 to 2012 (retrospective data) and from 2014 to 2017 (prospective data) were used. Total 7,448 KT donor-recipients pairs were used for eplet estimation.

Previously validated multistep HLA imputation process were conducted to derive 4 digits HLA genotype from 2 digits genotype [45], which is a method based on HLA haplotype frequencies data set for target population. Imputation of HLA-A, -B, -DRB1, and -DQ alleles (4-digit specificity) were done by using Korean HLA haplotype distribution in bone marrow donors to adjust HLA distribution in Korean population. For class I eplet estimation, from total 7,448 patients, 6,834 (91.8%) patients' 4 digits 1st haplotype were successfully called. Among the 6,834 1st haplotype-called patients, 2,857 (41.8%) patients' 4 digits counter-phase haplotype were successfully called. Other 3,977 patients' haplotype were combined by using the mixture of the most frequent allele in each locus. For class II eplet estimation, 6,859 (92.1%) patients' 4 digits 1st haplotype were successfully called. Among the them, 3,012 (43.9%) patients' 4 digits counter-phase haplotype were successfully

called. Other 3,847 patients' haplotype were combined as the same way in class I eplet estimation. If the rare 2 digits genotype were not typed as 4 digits in the distribution reference database, those were considered as failure of imputation, and excluded from data analysis. Finally, 5,871 (78.8%) completely called pairs were used for analysis. The presence of individual eplet and numbers of eplet mismatches for each recipient and donor pair at HLA class I (HLA-A,-B) and class II (HLA-DR,-DQ) loci was imputed by HLAMatchmaker (Version 2.1).

Study Objective and Design

I tested whether eplet mismatches was associated with post-transplant graft outcomes. I performed multivariable analysis and adjustment. I tested whether eplet mismatch gives additional prediction value by the area under ROC curve comparison.[46]

Study outcome, exposure, mediator, and covariables

Post-transplant acute rejection (overall, biopsy-proven total, biopsy-proven cellular, biopsy-proven antibody-mediated) and eGFR were the main outcome. For the secondary outcome, allograft survival, interstitial fibrosis and tubular atrophy at biopsy within post-transplant 1 year were used. As study exposure, number of total eplet mismatches, number of class I or class II eplet mismatches were used, which was calculated described above. In our study, acute rejection was defined as composite outcome of clinical rejection (rejection treatment without kidney biopsy results) and biopsy proven rejection. Pathology reports were based on the reading of pathologist in local center. Data entry format of KOTRY necessitates the entry of individual component of Banff scoring.

Study Ethics, Covariables and Statistical model

The study protocol was approved by the Seoul National University Hospital institutional review board (No:H-1902-138-1014). Deidentified dataset was used, and patient privacy was preserved in all instances. The study was conducted under the Declaration of Helsinki. Missing rates of included covariables in KOTRY datasets were under 0.05%, which enables complete data analysis in the most of our analysis. Continuous data are presented as mean with standard deviation. Categorical data are presented as count with percent. Non-linearity was assumed to the number of eplet mismatches, which were conducted by applying fractional polynomial term to variable of interest. Time to event analysis was conducted by Cox regression under proportional hazard assumption. To compare model's predictability, I constructed multivariable logistic regression models to within 1 years outcome of interest (total rejection within 1 yr, biopsy proven acute rejection within 1 yr, acute T-cell mediated rejection within 1 yr, acute antibody-mediated rejection within 1 yr). As covariables, ten covariables were included in the multivariable logistic regression models: recipient age, recipient sex, donor age, donor sex, deceased donor, ATG induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Statistical significance of eplet mismatches were checked in graphic presentation with 95% confidence interval of predicted coefficients. Performance of overall prediction model was compared by Youden's index of area under ROC curve. All statistical analyses were performed using Stata software (version 16; StataCorp LP, College Station, TX).

2.4 Non-human primate model of antibody mediated rejection

I studied mechanism of antibody mediated rejection in the sensitized setting by using non-human primate xenotransplantation model.

Animals

Cynomolgous monkeys (Macaca fascicularis, M:F = 2:2, 4-6 years old) were used as recipients. Monkeys were obtained from Xenia (Seoul, Korea) and the nonhuman primate center of Korea Institute of Toxicology (Jeongeup, Korea). Genetically modified pigs (n=2, 10 - 20 kgs) which lacks alpha-Gal epitope (GalTKO) were used as donors to provide artery graft. [47] Maintenance care have been offered inside the animal care facility of Seoul National University Hospital. All procedures and medication were approved by the Seoul National University Institutional Animal Care and Use Committee. (IACUC No-15-0218) Experiments were performed under the guidelines in the National Institute of Health guide for the Care and Use of Laboratory Animals.

Surgical procedure of artery graft transplantation and removal

U shaped artery graft patch were produced from GalT-KO porcine aorta. GalT-KO pigs were sacrificed at 36 – 60 weeks after birth. Surgical procedures were as follows: Briefly, femoral artery and vein of recipients were connected by porcine artery graft similar as an arteriovenous shunt. After 1st xenotransplant experiment, all xenograft were removed by surgical exploration of inguinal area. After the periods of immunosuppressant weaning (more than 6 months), 2nd GalT-KO porcine artery graft xenotransplantation were conducted (n=4). All transplantation operation were successful without any significant bleeding complications, localized edema, or

occlusion of graft from immediate thrombus formation. The patency of xenograft were checked by Doppler ultrasound, manual inspection and auscultation. (Figure 1)

Immunosuppression and medical care

The experimental protocol is shown in Figure 2. Immunosuppression was based on the CD40-154 axis blockade (anti-CD154 mAb, 20mg/kg, Genexin, Seongnam, Korea) on Day -1, 0, 3, 7, 10, 14, 21. Cobra venom factor (0.05mg/kg, Quidel, San Diego, CA, USA) were given on Day -1, 0, 1 to suppress post-op immediate coagulation. Oral aspirin (50mg/day), low molecular weight heparin (1mg/kg.day s.c.), cefazolin (10mg/kg.day), and omeprazole (10mg/day) were administered as a maintenance medical care. Clinically applicable triple immunosuppressants (tacrolimus, steroid and mycophenolate) were applied (daily tacrolimus 1mg/kg, methylprednisolone 2mg/kg, and mycophenolate 40mg/kg). To evaluate the impact of anti-thymocyte globulin (ATG) (Genzyme, Cambridge, MA, USA) to the repopulation of memory cell, ATG were given to two recipient animals (ATG group, 5mg/kg/day x 4 days on Day -2, -1, 0, 1) among four animals.

Histology, immunohistochemical stain and immunofluorescence staining

Porcine aortic xenograft were removed at post transplantation day 28 or at the time of necropsy. When animals were living, procedures were conducted under general anesthesia. Xenograft were removed as a whole to preserve the structure of conduit and were fixed 10% formalin and embedded in paraffin blocks for hematoxylin and eosin staining. For immunohistochemical stain, sections (5 micrometer) were labeled with primary antibodies for CD68 (1:200, Invitrogen, Cat no: MA5-13324, CA, USA) and myeloperoxidase (MPO) (1:1000, Abcam, Cat no:

ab9535, Cambridge, UK) and secondary antibody. Monkey spleen was used as positive control. The stained slide were photographed using an Olympus inverted microscope. (Olympus Imaging America, CA, USA) For immunofluorescence staining, deparaffinized sections of xenograft specimen were probed with primary antibodies. (anti-CD-68 and anti-MPO; same as previous, anti-Tissue factor; American Diagnostic, Cat no: 4508 CJ, NY, USA)

Chemical laboratory parameters

SNUH large animal central laboratory provides daily clinical practice laboratory results.

Cytokine analysis

Serum samples from transplanted monkeys were tested for Tumor necrosis factor-alpha, IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1). Luminex-based standard multiplex panel and magnetic beads were used. All assays were done under the provider's manual. (Invitrogen, ProcartaPlex, EXP040-49031-801, CA, USA).

Statistical analysis

Fisher's exact test, Student t-tests were used for the difference comparisons, as appropriately. Significance was defined as $P < 0.05$. GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) were used for the data visualization. Statistical testes were done by using Stata 15 (Statacorp, College Station, TX, USA)

3. Results

3.1 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

Baseline characteristics

In Table 6, baseline characteristics of kidney transplant recipients are described. Mean age of kidney transplant recipients were 49.1 ± 11.5 years old. In deceased donor KT, mean age of recipients was higher (51.7 ± 10.6, $p < 0.001$). Female recipients were 40.6%. More male recipients received deceased donor kidney. Mean body mass index was 23.1 ± 3.6 kg/m^2, mean systolic blood pressure before kidney transplant was 140.1 ± 34.6 mmHg. Proportion of current smoker was 8.6%. As comorbidities, diabetes was in 29.8% and hypertension in 89.7% of recipients. Proportion of cardiovascular disease was 6.1%, which was higher in deceased donor kidney transplant recipients. History of malignancy was present in 6.6%. The most common cause of ESRD was chronic glomerulonephritis (33.5%) followed by diabetic nephropathy (23.5%). Hemodialysis was the most frequently used dialysis modality before transplantation (70.9%). Preemptive kidney transplantation was 24.0% among living donor KT. Mean waiting time for deceased donor KT was 67.1 ± 37.2 months. Retransplantation was in 7.7%. Mean numbers of HLA mismatch was 3.4 ± 1.8. As induction agent, Basiliximab was used in 78.7% of total KT and ATG was used in 31.9% of DDKT. Tacrolimus was the main calcineurin inhibitor (95.7%). Early steroid withdrawal was done in 2.0% of patients.

Donor characteristics

Donor data was described as cases. (Table 7) Mean age of donor cases was 46.9 ± 13.0 years old. Female was more prevalent in living donor, and male was more prevalent in deceased donor. Diabetic donors was 11.9% in deceased donors, and 1.1% in living donors. Donors with hypertension were 24.4% in DDKT and 9.5% in LDKT. Mean BMI of donors was 23.8 and mean pretransplant SBP of donors was 122.4 mmHg. Proportion of smokers was 17.3% in LDKT. Mean cold ischemic time was 289mins in deceased donor. Continuous renal replacement therapy was applied to 6.7% of deceased donors. Extracorporeal membrane oxygenator was applied to 2.7% of deceased donors.

Patient survival and cause of death

Overall patients survival rate were 98.4%, 97.8%, 97.6% at 1,3,5 years respectively. Among living donor kidney transplantation recipients, patient survival were at 1,3,5 years were 99.3%, 99.1%, 98.9%, respectively. Among deceased donor kidney transplantation, patient survival rate were at 1,3,5 years were 97.0%, 95.9%, 95.6% respectively. (Figure 3) The most common cause of death were infection (47.6%) followed by cardiovascular disease (11.9%), the latter occurred exclusively in deceased donor kidney transplantation. (Table 8)

Death-censored graft survival and cause of graft failure

Death-censored graft survival rate were 98.4%, 97.0%, 96.9% at 1,3,5 years respectively. Among living donor kidney transplantation recipients, death-censored graft survival rate were 99.0%, 98.3%, 97.6% at 1,3,5 years respectively. Among deceased donor kidney transplantation, death-censored graft survival rate were 97.4%, 96.1%, 95.9% at 1,3,5 years respectively. Rejection (43.5%) was the most

common cause of graft loss. Primary graft failure was in 11.1% of graft failure. BK virus nephropathy was 3rd common cause (5.6%). (Table 9)

Acute rejection, indication of kidney biopsy and pathology outcomes

Acute rejection free survival rates were 82.4%, 77.0%, and 76.2% at 1, 3, and 5 years, respectively. Among living donor kidney transplant recipients, acute rejection free survival rates were 82.3%, 78.5%, and 76.1% at 1, 3, and 5 years, respectively. Among deceased donor kidney transplants, acute rejection free survival rates were 81.7%, 77.1%, and 76.3% at 1, 3, and 5 years, respectively (Figure 4A). Biopsy-proven acute rejection free survival rates were 90.3%, 87.6%, and 87.3% at 1, 3, and 5 years, respectively. Among living donor kidney transplant recipients, biopsy-proven acute rejection free survival rates were 90.4%, 87.3%, and 87.0% at 1, 3, and 5 years, respectively. Among deceased donor kidney transplant recipients, biopsy-proven acute rejection free survival rates were 90.2%, 88.0%, and 87.7% at 1, 3, and 5 years, respectively (Figure 4B).

Total 2,769 kidney biopsies were performed. Among them, 58.7% were protocol biopsies. (Table 10) The most common indication of kidney biopsy was increased creatinine. (37.5%) Among for-cause biopsies, acute T cell mediated rejection were 26.8%, acute antibody mediated rejection were 14.1%, and borderline rejection were 21.1%. Recurrent glomerulonephritis were 9.2% and BK virus associated nephropathy were 7.7%. If protocol biopsy are included, the proportion of biopsy findings declined, however, the proportion of borderline rejection were not declined. (Table 11) Biopsy-proven acute T-cell mediated rejection free survival rates were 92.8%, 91.0%, and 90.6% at 1, 3, and 5 years, respectively. (Figure 5A). Biopsy proven acute antibody mediated rejection free survival rates were 96.5%, 95.2%, and 95.2% at 1, 3, and 5 years, respectively (Figure 5B).

Predictors to patient survival and dominance analysis

To explore predictors to patient survival, cross-validated LASSO were applied, which resulted all of variables were included at the optimum lambda. I interpret this due to sufficient n to predictors (not p > n condition), where LASSO might not show its strength in variable selection. (Table 12, Table 17, Figure 6) Traditional backward stepwise selection showed reduced predictors from 20 variables to 15 variables. (Table 13) To compare relative importance of predictors, I chose 1 year patient survival as outcome, and applied dominant analysis method. Deceased donor kidney transplantation was the most dominant predictor to 1 year patient death, followed by recipient age, cardiovascular disease history of recipients, duration of dialysis, diabetes of recipients. Interpretation of maintenance immunosuppressant should be cautious because possibility of primary graft failure.

Dominant predictors to graft survival and acute rejection

Dominant predictors for death-censored 1 year graft survival were standard deceased donor kidney transplant, desensitization, donor hypertension, systolic blood pressure of recipients, diabetic recipients. Dominant factor for acute rejection within 1 year were determined by preselected acute rejection predictors (Table 18). Dominant factors to acute rejection were donor age, followed by HLA mismatch numbers, desensitization, female recipients, body mass index of recipients. (Table 19) Dominant factors for antibody mediated rejection within 1 year were determined by preselected predictors (Table 20). Dominant factors to antibody mediated rejection were desensitization, followed by ATG induction, HLA mismatch numbers,

recipient age, and deceased donor. (Table 21) Among re-transplantation recipients, dominant factors for acute rejection within 1 year were deceased donor kidney, donor hypertension, and HLA mismatch numbers. (Table 22) The most dominant predictors to post-transplant 1 year graft eGFR were donor age, followed by acute rejection within 1 yr, BKVAN within 1 yr, female donor, and recipient BMI. (Table 23)

3.2 Significance of eplet mismatch in rejection

Baseline characteristics

Mean age of study population was 46.8 years old. Proportion of deceased donor kidney transplantation was 38%. Female was 42%. Mean HLA mismatch numbers was 3.2 ± 1.7. Mean HLA mismatch numbers in class I was 2.2 ± 1.2, 1.1 ± 0.7 in class II (DR only). The proportion of retransplantation, desensitization, and abo incompatible kidney transplantation were 7%, 18%, and 12%, respectively. Mean cold ischemic time was 1.9 ± 2.3 hrs in deceased donor kidney transplantation. Overall acute rejection including clinical rejection occurred in 16% during follow up period, and biopsy-proven acute rejection occurred in 9% of study population. Compared to 2009-2012 cohorts, newer cohorts showed higher proportion of desensitization, abo incompatibility, overall rejection and biopsy proven acute rejection. Other clinical characteristics are described in Table 24.

Eplet distribution in study population

Mean eplet class I difference was 10.6 ± 6.8, and class II difference was 24.1 ± 17.6. (Table 25) Figure 7 shows the distribution of eplet mismatch. In HLA

zero mismatch subgroup, estimated class I eplet show little deviance from zero eplet mismatch, however, in class II eplet mismatch showed overt increment than serotype mismatch, which could be explained by the inclusion of DQ mismatch in eplet estimation. Except HLA serotype zero mismatch, other estimated eplet mismatch showed Gaussian distribution.

Association of eplet mismatches to acute rejection

Table 26 shows the results of univariate analysis of predictors to acute rejection. Each eplet mismatches were significant predictors to acute rejection. Reduced hazard ratio is due to large eplet mismatch numbers compared to 1 or 2 mismatches in HLA serotype. I tested whether there are any non-linearity in eplet mismatches to the prediction of acute rejection by applying fractional polynomial term in eplet mismatch numbers. In Figure 8, there are downward curvature over 85 eplet mismatches, and it is explained by the downward curvature in non-antibody verified eplet mismatch. However, total eplet mismatches showed statistical significant increased risk to zero eplet mismatches. I investigated the nonlinear association of eplet mismatches to biopsy proven acute rejection only, to test its effect is more precisely explained in those proven outcomes. (Figure 9) Still, eplet mismatches showed increased risks in total eplet, and non-antibody verified eplets. However, eplet mismatches did not show any superior predictability to HLA serotype mismatches when adjusted other multiple covariables, and I could not find any strong non-linear pattern in the association of rejection, and biopsy proven rejection with eplet mismatch numbers.

Association of eplet mismatches to acute rejection in low HLA genotype mismatches

I tried to validate previous finding that eplet mismatches has significant meaning in low HLA-serotype mismatch subpopulation. Figure 10 showed that eplet mismatches show significant risks in low HLA mismatch groups (0 – 2 HLA mismatches), and interestingly, its increased risk is strongly associated in biopsy-proven acute T cell mediated rejection. When class I eplet and class II eplet was analyzed, similar pattern to total eplet mismatches to biopsy-proven acute T cell mediated rejection was shown in class II total eplet mismatches. However, replacement of HLA serotype mismatch to eplet mismatch in multivariable prediction model did not show any statistical improvement in AUC or IDI. (Figure 11)

Studies of individual eplet locus

Finally, I tried to find any significant individual eplet predicting acute rejection. I tested multiple t-tests, however, no significant p-value were achieved. I explored top 10 (least p-value) eplets, and discovered that they were located near the groove of MHC molecule. (Table 27 and 28) (Figure 12)

3.3 Non-human primate model of antibody mediated rejection

Clinical Course of Experiment Group

GalTKO porcine artery xenograft were transplanted into recipient monkeys (n=4). Xenografts were maintained with the described immunosuppressants by post-operative 4 weeks. At day 28, xenografts were removed. Maintenance

immunosuppressant were weaned after xenograft removal. Tacrolimus and mycophenolate were ceased at the day of graft removal, and prednisolone were gradually tapered down until post-transplant 7 weeks. The 2nd transplantation were conducted at least after 5 months from the removal of 1st xenograft (150 days, and 300 days after first transplantation). Group allocation of ATG vs control were the same as first xenotransplantation. Post-operative immediate assessment of xenograft showed that all graft were patent. Three of 4 recipients survived healthy until the 2nd removal of xenograft. One recipient in non-ATG group (R23-10) died at the day 22 of 2nd transplantation. The patency of graft have been maintained until the removal of xenograft or the day before of mortality.

Coagulation profile

Thrombocytopenia was not prominent in 1st transplantation, and there was no significant difference between ATG treatment vs control group. However, in the 2nd transplantation, the non-ATG group recipient R23-10 experienced with lethal thrombocytopenia. (Figure 13A ~ 13D) In R23-10, development of thrombocytopenia was accompanied by systemic inflammation represented as increased CRP level. Similar to previous report [48], systemic inflammation preceded thrombocytopenia. Deterioration of clinical course in R23-10 was quite abrupt, leading to death on the course of pondering euthanasia and giving antibiotics. Blood culture tests were conducted post-mortem, which did not show any growth of microorganism. Except deceased recipient, both 1st and 2nd transplantation showed similar pattern of increment of systemic inflammation along the experimental course and there was no significant difference between ATG treatment vs control group.

To test whether those intra-graft coagulation reaction is represented by systemic markers, Fibrinogen and Anti-thrombin III were checked. (Figure 13E ~ 13J) Post-

operative early fibrinogen consumption was found in the 2nd transplant of recipient of ATG non-treated group. However, it recovered within 1 week and there was not any significant bleeding or ischemic complications. Systemic measurement of fibrinogen did not reveal any significant difference in deceased recipient. Anti-thrombin III dropped to almost 60% in deceased recipient R23-10, which represents the severity of intravascular coagulation responses. However, change of anti-thrombin III level did not precede mortality event, and the similar level change was also shown in another surviving recipient.

Histopathology and immunohistochemical staining

Histopathology of 1st and 2nd xenograft were compared. (Figure 14) In low power light microscope, the 2nd transplanted graft showed more distorted gross morphology of vascular graft. Also in the 2nd transplanted graft, more inflammatory cells infiltrate in tunica adventitia, which is filled with many blood plug. Loss of endothelial cell in tunica intima and loss of nuclei in tunica media were also observed in 1st xenograft, however, those findings were much more prominent in 2nd xenograft. The administration of ATG did not affect the histology in 1st xenograft nor 2nd xenograft. In the 2nd xenograft, endothelial lining was completely lost regardless of ATG treatment. Large intraluminal thrombus indicates the severe inflammatory reaction in those graft.

Immunohistochemical staining reveals prominent infiltration of CD68+ cells and MPO+ cells. The feature was comparable both in ATG (-) group and ATG (+) group. Anti-tissue factor antibody were stained strongly in first and second transplant. Tissue factor stain was prominent inside the xenograft along the tunica media to tunica adventitia. Luminal area showed relatively weak expression of tissue factor. Tissue factor expression were prominent in the 2nd transplantation group,

which indicated much strong coagulation response in the 2nd transplantation group. (Figure 15)

Cytokine & lymphocyte population

As expected, lymphocyte depletion was achieved by using ATG administration. Total lymphocyte count decreased under the $1x10^3$ cells/μL and was maintained until the weaning of immunosuppression. Unlike the depleted lymphocytes, monocytes and neutrophils showed no significant difference between ATG administration or not. (Figure 16) Although the circulating monocyte was not different between ATG usage. Infiltrating CD-68+ cells were more prominent in ATG (-) groups. When peripheral blood circulating cytokine level was analyzed, MCP-1 level was significantly high at the 1 week of xenotransplantation. At the second transplantation, IL-6 level were prominently higher in the ATG (-) groups than ATG (+) group. Both MCP-1 and IL-6 level were generally higher than one in 1^{st} xenotransplantation, and the phenomenon that showed reduced level of MCP-1 in the ATG (+) group at the 1^{st} xenotransplantation was attenuated at the 2^{nd} xenotransplantation experiment. (Figure 17)

Total IgG and IgM level were measured. During the period when xenograft exposed to the recipient, there was no significant trend of total antibody level elevation or depletion. However, when we check the donor specific complement dependent cytotoxicity, much strong cytotoxicity reaction were observed at the serum from 2^{nd} transplantation. Interestingly, after the removal of second transplant xenograft, one animal from ATG receiving group showed decrement of total IgG level, which coincides the reduced cytotoxic reaction in the donor specific cytotoxicity assay. (Figure 18)

4. Discussion

4.1 Dominant predictors of post-transplantation early outcomes and rejection in KOTRY

In the present study, I reported the baseline characteristics and early outcome of Korean Organ Transplantation Registry (KOTRY). Baseline predictors to early outcomes were explored, and dominant factors to patient and graft outcomes were reported. Dominant factors to patient survival were found as predictors associated with recipient's age or recipient's comorbidities. To graft survival, dominant factors were proper immunosuppression, and donor kidney function. It was interesting to see that donor age was found as the most dominant factor to acute rejection. Donor age and donor sex were dominant factors to the graft function at 1 year.

When the KOTRY launched, annual transplantation numbers were 1,400. At the design stage of KOTRY, annual enrollment of 1,200 cases were aimed to cover more than 80% of total kidney transplantation in South Korea. However, recent rapid increment of kidney transplantation numbers have made KOTRY covers about 50-60% of total kidney transplantation in South Korea. Still, KOTRY projects is the largest multi-center cohorts in this country. In KOTRY, clinical details which claim data cannot capture are important resources to future research. Another strength of KOTRY is it's role as a biobank. Prospective sample collection will be invaluable research resources.

The most common cause of ESRD in South Korea is diabetic nephropathy [49], which is reflected as the high proportion of diabetes in KOTRY. High proportion of glomerulonephritis as cause of ESRD could represent selection criteria of comorbidities for kidney transplantation. Another important feature of Korean kidney transplantation is high proportion of living donor kidney transplantation.

Among living donor kidney transplantation, 24% were preemptive kidney transplantation. Long waiting is another feature of Korean kidney transplantation, of which reduction is important future task. Aside from standard triple maintenance immunosuppressants, ATG induction was observed variation in immunosuppressant. It was of note to see the proportion of steroid withdrawal was 2%. Cold ischemic time is short in Korea due to its concentrated population structure. The most common cause of death was infection, followed by cardiovascular disease. These cause of death is compatible with the predictors selected in data-driven approach, because the recipient age and history of cardiovascular disease were selected as dominant predictors to 1 year mortality.

Recent investigations of donor safety have concerned higher lifetime ESRD risk in young donors.[50] In terms of graft survival of recipient side, it is interesting to see selected predictors were donor characteristics such as donor age, donor hypertension, and donor diabetes. However, extension of this finding to long term risk predictors needs caution, because non-modifiable donor factor could be exaggerated in early transplantation outcomes. When we think about donor safety, marginal kidney function would also affect donor's long term outcome, therefore this data is an evidence to the importance of proper donor selection.

It was interesting to see that donor age was the most dominant factor to acute rejection. There were several publication to see the significance of donor age as the risk factors of acute rejection, however, to the best of our knowledge, this is the first study to find that donor age is the most dominant factor to the acute rejection in a quantitative comparison. HLA incompatibility was the 2nd dominant predictors to acute rejection. This finding could be an epidemiological evidence that support the importance of passenger leukocyte and its memory, or endothelial cell damage and PAMP expression. Desensitization was selected as important predictors to acute rejection, which implies although mitigation of immunological risk was performed

by desensitization, residual risk still persist. Future studies are anticipated to investigate the details about desensitization.

Dominant predictors to 1 year post-transplant recipient's eGFR were donor age, recipient BMI, HLA mismatches. Because donor age and HLA mismatches were both dominant predictors to acute rejection, I could prove that both predictors were mediating acute rejection to post-transplant graft eGFR. The importance of donor kidney-recipient weight gap was well known factor to post-transplant eGFR [51,52]. In this study, its importance to predict post-transplant eGFR was high.

The limitation of study are as follows: First, this project have enrolled about 50% of total kidney transplantation in South Korea. Informed consent is requirement to observational cohort, therefore information bias might exist. For example, recipients with poor compliance could refuse study enrollment, and urgent transplantation performed during weekends or late night might not be enrolled in this project. However, when early outcome was compared to previous reports which covered over 92% of total kidney transplantation, there was no statistical difference in early outcomes. Second, dominance of predictors was based on variable selection in traditional stepwise regression, which is not completely independent on the randomness of entering variables. I tried to overcome this limitation by applying regularized regression methods (LASSO), which was unsuccessful due to large numbers of cases compared to selected predictors. However, I think this quantitative comparison of relative importance of variables is noble to transplantation field. Provided model can produce better AUC than EPTS, which assures that the finding is significant.

In summary, I described clinical characteristics of patient enrolled in KOTRY during recent 5 years, and presented dominant predictors to early post-transplantation outcomes by comparing relative contribution to outcome prediction. The dominant predictors to recipient mortality within 1 years were deceased donor,

steroid usage, recipient age, and recipient cardiovascular disease history. The dominant predictors to death censored graft loss within 1 year were immunosuppressant usage, deceased donor. The dominant predictors to acute rejection within 1 year were donor age, and HLA mismatches. Finally, donor age, donor sex, recipient BMI were the dominant predictors to post-transplant 1 year recipient's eGFR.

4.2 Significance of eplet mismatch in rejection

In this study, I investigated the association of eplet mismatch with acute rejection in two sets of Korean kidney transplantation cohorts. Eplet mismatch was not a superior predictor to HLA serotype when it was added to multivariable clinical variables. However, eplet mismatches was significant risk factors in low MHC mismatch group, which is the external validation for previous study results. Interestingly, in the present study, class II eplet mismatches were strongly associated with acute T cell mediated rejection.

Several approaches have been used to investigate the association of molecular mismatch to the clinical phenotype in organ transplantation. Differences in single amino acid and its position information were associated with delayed graft function and allograft survival. [53,54] Electrostatic mismatch concept based on surface electrostatic potential differences between HLA molecules revealed that this amino acid mismatch approach predicts de novo alloimmunization against HLA-A,B,DRB, and DQB. [55–57] Eplet mismatches proposed by Rene Duquesnoy was the most popular method to be used. Eplet mismatches have been reported to be associated with development of de novo DSA, transplant glomerulopathy, antibody mediated rejection, graft survival, and acute T-cell mediated rejection including borderline phenotype. [58–63] Finally, consideration of indirect presentation of class I MHC

peptide onto class II MHC in addition to eplet mismatches have been suggested, and also was associated with the development of dnDSA.[64,65] Each method has own unique interpretation and limitations, however, in terms of predictability to the development of dnDSA, it was reported that there was no significant differences between methods.[66]

Previous studies reported good association of eplet mismatches with the development of de novo DSA. Because of non-invasive nature, monitoring of de novo DSA has strengths with the completeness of measurements and serial measurements. Previous studies determined the cutoff points to categorize alloimmune risks by using ROC curve to the development of dnDSA.[58,61] In this study, I tried to find the cutoff points to categorize alloimmune risks also, whereas the target outcome were acute rejection, biopsy proven acute rejection, acute T cell mediated rejection, or acute antibody mediated rejection in the present study. Therefore, the cutoff points of eplet mismatch numbers were higher than the numbers derived from previous studies which used dnDSA as target outcome.

Compared to the development of dnDSA, association of biopsy proven cellular rejection was scarce, which could be explained by invasive nature of measurement, administration of induction agents, T cell as main target of modern maintenance immunosuppression. Therefore, association with acute rejection was reported in large scale registry study, [67] of which the present study function as external validation although there is differences in the proportion of deceased donor kidney transplantation, ethnicity, and ABO incompatibility. Acute antibody mediated rejection was not associated with eplet mismatches in this study. I interpret this phenomenon derives from the relatively short duration of current study, and gradual progression of transplant glomerulopathy might not be properly captured at 1st biopsy. In future study, a proper analysis for repeated biopsy samples are warranted. Strong association of class II eplet mismatch with T cell mediated rejection in low HLA

mismatch pairs is a new finding in this study. Eplet mismatches was proposed to represent the interaction between MHC molecule and antibody, therefore it focused on the surface amino acid residue, and structural information as intact molecule. However, it is well known phenomenon that class I or II MHC peptide can be digested inside recipient APC, then can be presented to immune responder cell. (indirect presentation) Donor MHC fragmented peptide presented on recipient MHC can induce an activation of helper T cells, which can offer helper signal to effector T cells or B cell activation. Although the association was not clearly shown as in the present study, one of the early studies of eplet mismatches also reported the preceding T-cell mediated rejection was associated with the development of dnDSA according to eplet mismatches, and cellular rejection including borderline phenotype was reported to be associated with DR/DQ eplet mismatches.[21,68]

The limitation of study as follows: First, eplet determination is based on imputation by HLA haplotype distribution. Second, measurement of de novo DSA were scarce and excluded from analysis. Third, follow up duration was too short to delineate chronic manifestation such as transplant glomerulopathy, the development of dnDSA, or allograft survival. Fourth, pathology reporting was dependent on pathologist from individual center.

In summary, eplet mismatches in class II MHC was found as significant risk factors to biopsy proven acute T cell mediated rejection in low degree HLA mismatches (1 or 2 mismatches).

4.3. Non-human primate model of antibody mediated rejection

In this study, GalT-KO porcine vascular conduit xenotransplantation to cynomolgous monkeys were conducted. Conventional triple immunosuppressant with anti-CD-154 monoclonal antibody and cobra venom factors were applied as immunosuppressants. Additionally, experiment groups were divided by the presence

of ATG usage. Although the ATG successfully depleted lymphocyte, there was no significant difference of cellular infiltration or tissue factor expression inside the graft. However, circulating IL-6 level and platelet consumption in the second transplantation was elevated in the ATG non-use group, suggesting partial role of T cell depletion for attenuating systemic inflammation in the second transplantation.

Vascular conduit was used as the model of xenotransplantation in the present study. Previous studies used pig artery patch model, which has strengths of technical easiness and ability to test humoral immunological response. Vascular conduit model has similar strength of technical feasibility and exposure to humoral immune system. In addition, this conduit model can offer the chance of functional monitoring (auscultation, doppler) and safe graft removal, which enables unique sensitization model in xenotransplantation. In this experiment, all monkeys were alive during 4 weeks of first vascular xenograft transplantation, and the rejected xenograft were successfully removed. After those 4 weeks exposure to porcine vascular conduit could elicit very strong sensitization which was confirmed by strong complement dependent cytotoxicity assay and vigorous rejection confirmed by histology in 2nd transplanted xenograft.

In this experimental model, whether T-cell depletion by ATG could affect the development of sensitization in the 2nd xenotransplantation were tried to be delineated. GalTKO pig and old world monkey which express Neu5Gc antigen in their cell surface were used. In this system, the two major proposed carbohydrate xenoantigen (alpha 1,3-gal, Neu5Gc) were compatible. Therefore, overt hyperacute rejection due to profound preformed natural antibodies could be avoided and the importance of non-Gal antibodies were tested. After the removal of first rejected xenograft, 2nd transplantation were conducted after more than 6 months, which led sufficient time to develop induced memory and recovery from the effect of immunosuppressants. Although more vigorous rejection and enhanced complement

dependent cytotoxicity of sensitized serum in 2nd transplantation were observed, any biochemical difference across T cell depletion were found. However, one monkey in ATG-non treated group had expired during 2nd transplantation course.

T-cell help to antibody development is thought to be mediated through follicular helper T cell. In secondary lymphoid organs, primed follicular helper T cell can engage with B cells in the T-B border, and can prime B cells to differentiate into either plasma cells or germinal center B cells which subsequently produce high affinity antibodies.[6970] Enhanced complement dependent cytotoxicity of recipient serum in the 2nd transplantation clearly shows the affinity maturation. The lymphocyte depletion in the present study was successful by ATG, however, the susceptibility of follicular helper T cell to ATG have been reported as mixed results [71,72], which is one probable explanation of similar rejection phenomenon across ATG usage. Analysis of circulating follicular helper T cell is undergoing. Another explanation for non-difference between ATG vs non-ATG group is the resistance of memory T cells to ATG. Memory T cells proliferates quickly when encountered target antigen, expresses qualitatively enhanced antigen responsiveness, does not need costimulation signal to be activated, and are not restricted to lymphoid organs. [73] T cell depletion can make empty space where homeostatic proliferation of T cells could happen, which is advantageous condition for memory T cell to proliferate in a more fast way.[74] Xenoantigen from pig might be thought as new antigen to recipient monkeys, however large animals who was grown in non-SPF conditions have presensitized memory T cells compared to SPF mice due to heterologous immunity. The presence of sensitized T cells at pre-transplant stages were proven to be associated with transplanted graft rejection.[75,76] Following results of immunophenotyping for the circulating peripheral blood cells are needed. Another explanation of inefficacy of ATG is the presence of anti-CD154 monoclonal antibody which is a potent costimulatory blocker. Because CD40 ligand is offered by follicular

helper T cell, superimposed ATG depletion might have not added further effects on the already blocked costimulation signal.

In recent xenotransplantation studies, long-term survival is achieved by multi-faceted approach, which includes intervention of innate inflammation response (IL-6R antibody, Anti-TNF alpha receptor blockade) [77,78], regulation of complement propagation (human thrombomodulin, human EPCR, hCD55, membrane cofactor protein (CD46))[79,80], and depletion of B cells. (Rituximab) In this study, rituximab was not used intentionally to study antibody formation after xenoantigen sensitization. Minimal blockade of innate immunity enabled to study innate immunity and coagulation phenomenon. Consumptive coagulopathy is a manifestation of severe inflammation. Immune-thrombosis is a recent active research area. In this study, there was no definite measured differences of coagulopathy between ATG administration or re-transplantation. However, more vigorous rejection in 2nd transplantation, and a platelet consumption with mortality during 2nd transplantation suggest more enhanced immune-thrombosis might have occurred in 2nd transplantation, which is evidence by elevated tissue factor expression in 2nd xenograft.

Elevated IL-6 was observed in 2nd transplantation. IL-6 is known to be excreted from various cell sources including neutrophil, macrophage, or activated endothelial cells.[81] Accompanying this phenomenon, at the histology level macrophage infiltration or neutrophil infiltration to xenograft were more prominent in the 2nd transplantation, and the extent of damage in vascular xenograft were also more prominent in the 2nd transplantation. Because the histological phenotype of 2nd xenograft resembles chronic rejection, earlier graft recovery and histological assessment might have revealed more comparable active rejection phenomenon.

In terms of second transplantation, one might think it is too far future in xenotransplantation. However, in the scope of sensitization, it is very close topic to

contemporaneous situation. With the advancement of genetic manipulation of donor pig, xenotransplantation are at the gate of clinical application, where careful patient selection is critical. The result of present study might be interpreted in that context. Xenogenic material exposure (i.e. cardiac tissue valves) could affect induced antibody levels. Heterologous immunity might explain the various degree of natural antibodies, however, functional difference and consequential immunologic reponses are not well defined in xenotransplantation. In recent study, significant difference of endothelial cell activation between natural antibody and induced antibody was reported.[82] The present study shows more vigorous rejection phenomenon in sensitized setting, however, the results of present study examines overall immunologic phenomenon, not restricted in endothelial cell activation. To evaluate endothelial cell response to sensitized serum is another important topic[83], and I expect future investigation would come out.

There are several limitation of this study as follows: Though the non-human primate was used as experimental object, small numbers are limitation. Cobra venom factor and anti CD-154 monoclonal antibody is not clinically applicable drugs. Vascular conduit graft is a feasible model to study histology and sensitization, however, its applicability is limited because it is a model system rather than real target organ.

In summary, a repeated GalT knockout porcine artery transplantation model to non-human primate were developed. More severe rejection phenotype in 2nd transplantation was accompanied by circulating elevated IL-6 and tissue factors expression in the affected graft under the CD154-40 costimulation blockade.

5. Conclusion

To understand the impact of clinical predictors in the real-world kidney transplantation to study the underlying mechanism of graft failure and rejection, kidney transplantation cohort (KOTRY and ASTREG) were developed and were used for analysis. Study of KOTRY revealed that donor age and HLA mismatch were the dominant predictors for acute rejection. Detailed analysis of HLA was further done by adopting eplet mismatch concept, which revealed that class II eplet mismatches were a significant risk factor to the development of acute T-cell mediated rejection. Repeated vascular artery xenotransplantation of GalT-knockout pig to cynomolgous monkeys revealed that at the second transplantation, vigorous rejection was accompanied by the elevated IL-6 and tissue factor expression in the CD154-40 cosimulation blockade. These findings collectively suggest the importance of donor antigenicity in relation to recipient's sensitization status, the necessity to control overall inflammation not only sole B or sole T cell, and the importance of interplay between distinct immune pathways including interplay of innate and adaptive immunity or interplay between B-cell and T-cell, and interplay between preformed antibody to the coagulation pathway. These findings generate the hypothesis whether target the linker cell or linker system between different immune pathways is effective to the control of immunological rejection of kidney transplantation, which could be a new therapeutic option in controlling multi-faceted rejection in high-risk transplantation settings.

Table 1. KOTRY data collection formats for organ recipients: common variables in all organ transplantation

Categories	Variables	Collection timing		
		B	<1y	A
Demographics	Age, gender, ethnicity, date of transplantation, cause of organ failure, number of transplantation	O		
Comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including diabetes, hypertension, cardiovascular disease, malignancies and selected medication	O		
Laboratory assessment	CBC, routine chemistry, uric acid, lipid panel, urinalysis	O	O	O
Immunologic assessment	ABO/HLA typing, crossmatch, PRA, DSA	O		
Viral markers	HBsAg, anti-HBsAb, anti-HCV Ab, anti-CMV Ab, anti-EBV Ab, anti-HIV Ab	O		

Immunosuppressants	Induction and maintenance immunosuppressants, concentration of immunosuppressants (calcineurin inhibitors and mTOR inhibitors)	O	O	O
Immediate complications	Surgical complications		O	
Discharge data	Date of discharge, functioning parameter of transplanted organ		O	
Post-transplant outcomes	Allograft rejection, graft failure, infection, patient death		O	O
Post-transplant comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including new-onset diabetes, hypertension, cardiovascular disease, tuberculosis, fracture and malignancies		O	O
Biosamples	DNA	O		
	Serum and plasma	O		O*

Abbreviations: <1yr, post-transplant visits within 1 year; A, annual visit; B, baseline visit; CBC, complete blood count; CMV, cytomegalovirus; DSA, donor specific antibody; EBV, Epstein-Barr virus; HBsAg, hepatitis B virus surface antigen; anti-HBsAb, anti-hepatitis B virus surface antibody; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin; PRA, panel reactive antibody

Post-transplant visits within 1 year are differently set among each organ. In kidney transplantation, visits are set at 6 month, in liver and heart transplantation, at 1 and 6 month, in pancreas transplantation, at 3 and 6 month, and in lung transplantation, at 3, 6, and 9 month. Baseline recipients' DNAs are collected in all organ transplantation. Post-transplant sera are collected at 1- and 3- years after transplantation in kidney, heart, lung and pancreas transplantation. In kidney transplantation, post-transplant plasma is additionally collected at 1- and 3- years after transplantation.

Table 2. KOTRY data collection formats for organ donors: common variables in all organ transplantation

Categories	Variables	Collection timing	
		B	A
Demographics	Age, gender, relationship to recipients, ethnicity	O	
Comorbidities	Height, weight, blood pressure, heart rate, smoking history, medical history including diabetes, hypertension, cardiovascular disease, and malignancies	O	
Deceased donor profile	Deceased donor profile (cause of brain death, inotropics management, vital-supporting devices, cold ischemic time)	O	
Laboratory assessment	CBC, routine chemistry, uric acid, lipid panel, ABO typing, urinalysis	O	O
Immunologic assessment	ABO/HLA typing	O	
Viral markers	HBsAg, anti-HBsAb, anti-HCV Ab, anti-CMV Ab, and anti-EBV Ab	O	
Living donor outcome	Post-operative surgical comorbidities, death, ESRD		O
Biosamples	DNA	O	

Abbreviations: A, annual visit; B, baseline visit; CBC, complete blood count; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ESRD, end stage renal disease; HBsAg, hepatitis B virus surface antigen; anti-HBsAb, anti-hepatitis B virus surface antibody; HCV, hepatitis C virus; HDL, high-density lipoprotein ; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin inhibitor

Baseline donors' DNAs are collected in all organ transplantation.

Table 3. Organ-specific information of Korean Organ Transplantation Registry

Organ	At baseline	At follow-ups
Kidney		Allograft biopsy based on Banff reports, living donor outcome (ESRD, urinary stone, hypertension)
Liver	Child-Pugh score, MELD/PELD score, donor-recipient liver volumetry, treatment history of hepatocellular carcinoma, surgical type of liver transplantation	Post-transplant rehabilitation status, recurrence of HBV or HCV, living donor outcome (hepatic morbidity)
Heart	Usage of cardiac assisting device and ventilator, intraoperative cardiopulmonary bypass usage	Serum cardiac markers (NT-proBNP, troponin I and T) at discharge, echocardiography
Lung	Latent tuberculosis infection (TST, IGRA), bone mineral density, lung size measure (donor & recipient), arterial blood gas analysis, donor bronchoscopic exam	Primary graft dysfunction, 6 minutes walking test, pulmonary function test (spirometry), post-transplant functioning status (tracheostomy, home O2, BiPAP)

Pancreas	C-peptide, anti-GAD antibody, HbA1c, surgical technique (drainage type, portal vein extension, arterial Y graft, artery and vein anastomosis type)	Insulin, C-peptide, HbA1c

Abbreviations: BiPAP, bi-level positive airway pressure; BNP, blood natriuretic peptide; ESRD, end stage renal disease; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; IGRA, interferon gamma releasing assay; MELD, model for end-stage liver disease; PELD, pediatric end -stage liver disease; TST, tuberculin skin test

Table 4. Representative items included in ASTREG-H

Domains	Variables
Baseline recipient characteristics	Age, sex, ethnicity, number of kidney transplant, smoking history, cause of end stage renal disease, previous history of renal replacement therapy, date of end stage renal disease diagnosis, comorbidities (diabetes, hypertension, cardiovascular disease, cerebrovascular attack, peripheral arterial disease, malignancy), height, weight, blood pressure, serostatus (CMV, HBsAg, HBsAb, HCV, EBV, HIV), ABO blood type
Baseline donor characteristics	Age, sex, ethnicity, donor relationship with recipients, deceased donor, comorbidities (diabetes, hypertension, malignancy), serostatus (CMV, HBsAg, HBsAb, HCV, EBV, HIV), ABO blood type, measured glomerular filtration rate in living donors, cause of brain death in deceased donor, serum creatinine before donation, complications after kidney donation
Immunologic parameters	Human leukocyte antigen mismatch (A,B,DR), crossmatch, panel reactive antibody profiles, baseline donor specific antibodies, baseline ABO titer, desensitization

	regimen, induction agent, maintenance immunosuppressants, trough level of calcineurin inhibitors
Post-transplant event of recipients (irregular outcome)	Delayed graft function, surgical complications, acute rejection, report of every kidney biopsy, vascular disease, infection, malignancy
Post-transplant annual surveillance of recipients (regular annual evaluation)	Height, weight, blood pressure, serum creatinine, parathyroid hormone, cholesterol, development of donor specific antibodies, plasma BK virus titer

Abbreviations: ASTREG, Asian Society of Transplantation Registry; CMV, cytomegalovirus; HBsAg, hepatitis B virus surface antigen; HBsAb, Antibody to the hepatitis B virus surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; EBV, Epstein-Barr virus

Table 5. Minimum detectable increase in relative risk of graft survival, patient survival and acute rejection from Korean Organ Transplantation Registry (KOTRY)

Organ (Expected enrollment number by 2019)	Survival Outcomes	Estimated Number of outcomes by 2019 based on observed events*	Detectable statistically significant minimum of hazard ratios		
			Risk Factor with 10% Prevalence	Risk Factor with 20% Prevalence	Risk factor with 50% Prevalence
Kidney Transplant (12,000)	Graft	1,379	1.09	1.07	1.06
	Patient	736	1.09	1.07	1.05
Liver Transplant (3,900)	Graft	839	1.18	1.13	1.11
	Patient	636	1.17	1.13	1.10
Heart Transplant (570)	Graft	130	1.51	1.38	1.32
	Patient	119	1.50	1.38	1.31

Lung Transplant (150)	Graft	48	2.34	1.98	1.87
	Patient	51	2.37	2.00	1.89
Pancreas Transplant (150)	Graft	41	2.25	1.92	1.82
	Patient	25	2.09	1.81	1.72

Table 6. Baseline clinical characteristics of the kidney transplant recipients of Korean Organ Transplantation Registry (2014 – 2018)

Variables	Total (n=4,839)	Living (n=3,039)	Deceased (n=1,800)	P
Age, yrs	49.1 ± 11.5	47.6 ± 11.7	51.7 ± 10.6	<0.001
Female sex	1,965 (40.6)	1,265 (41.6)	700 (38.9)	0.061
Body mass index, kg/m^2	23.1 ± 3.6	23.2 ± 3.7	23.0 ± 3.3	0.187
SBP, mmHg	140.1 ± 34.6	137.4 ± 39.8	144.7 ± 22.3	<0.001
DBP, mmHg	84.6 ± 32.1	84.7 ± 39.2	84.4 ± 13.3	0.002
Smoking				<0.001
Never	3,670 (75.8)	2,287 (75.3)	1,383 (76.8)	
Current	414 (8.6)	235 (7.7)	179 (9.9)	
Former	702 (14.5)	490 (16.1)	212 (11.8)	
Unknown	53 (1.1)	27 (0.9)	26 (1.4)	
Comorbidities				
Diabetes	1,442 (29.8)	913 (30.0)	529 (29.4)	0.631
Hypertension	4,340 (89.7)	2,727 (89.7)	1,613 (89.6)	0.976
Cardiovascular disease	294 (6.1)	158 (5.2)	136 (7.6)	<0.001
Malignancies	317 (6.6)	183 (6.0)	134 (7.4)	0.115

Cause of end stage renal disease				<0.001
Diabetic nephropathy	1,135 (23.5)	708 (23.3)	427 (23.7)	
Hypertension	763 (15.8)	413 (13.6)	350 (19.4)	
Glomerulonephritis	1,610 (33.3)	1,067 (35.1)	543 (30.2)	
ADPKD	231 (4.8)	148 (4.9)	83 (4.6)	
Other	150 (3.1)	95 (3.1)	55 (3.1)	
Unknown	950 (19.6)	608 (20)	342 (19)	
Dialysis before transplantation				<0.001
Hemodialysis	3,429 (70.9)	2,009 (66.1)	1,420 (78.9)	
Peritoneal dialysis	619 (12.8)	241 (7.9)	378 (21)	
Kidney transplant	59 (1.2)	59 (1.9)		
Preemptive	732 (15.1)	730 (24.0)	2 (0.1)	
Duration of waitlist, mos	55.6 ± 41.6	8.1 ± 18.2	67.1 ± 37.2	<0.001
2nd Kidney transplantation	375 (7.7)	216 (7.1)	159 (8.8)	0.137
Desensitization	1,106 (22.9)	1,064 (35.0)	42 (2.3)	<0.001
HLA mismatch numbers	3.4 ± 1.8	3.3 ± 1.7	3.4 ± 1.9	0.021
Induction agent				<0.001

Anti-thymocyte globulin	1,005 (20.9)	434 (14.4)	571 (31.9)	
Basiliximab	3,780 (78.7)	2,577 (85.5)	1,203 (67.2)	
No induction	21 (0.4)	4 (0.1)	17 (0.9)	
Calcineurin inhibitor				<0.001
Tacrolimus	4,631 (95.7)	2,872 (94.5)	1,759 (97.7)	
Cyclosporin A	153 (3.2)	132 (4.3)	21 (1.2)	
No Calcineurin inhibitors	55 (1.1)	35 (1.2)	20 (1.1)	
mTOR inhibitor				0.283
Sirolimus or everolimus	53 (1.1)	37 (1.2)	16 (0.9)	
Steroid				0.194
Yes	4,739 (97.9)	2,983 (98.2)	1,756 (97.6)	
No	99 (2.0)	56 (1.8)	43 (2.3)	

Abbreviations) ADPKD, autosomal dominant polycystic kidney disease; DBP, diastolic blood pressure; SBP, systolic blood pressure

Table 7. Baseline clinical characteristics of the kidney transplant donors of Korean Organ Transplantation Registry (2014 – 2018)

Variables	Total (n=4,838)	Living (n=3,039)	Deceased (n=1,799)	P
Age, yrs	46.9 ± 13	46.1 ± 11.8	48.4 ± 14.8	<0.001
Female sex	2,245 (46.4)	1,709 (56.2)	536 (29.8)	<0.001
Comorbidities				
Diabetes	248 (5.1)	34 (1.1)	214 (11.9)	<0.001
Hypertension	728 (15.0)	288 (9.5)	440 (24.4)	<0.001
Body mass index, kg/m^2	23.8 ± 3.4	24.2 ± 3.2	23.2 ± 3.7	<0.001
SBP, mmHg	122.4 ± 17.2	122.2 ± 13.9	122.7 ± 21.8	0.418
DBP, mmHg	75.3 ± 12.7	76.3 ± 10.0	73.6 ± 16.2	<0.001
Smoking				<0.001
Never	3,105 (64.2)	2,229 (73.4)	876 (48.7)	
Current	1,203 (24.9)	525 (17.3)	678 (37.7)	
Former	313 (6.5)	243 (8)	70 (3.9)	
Unknown	218 (4.5)	42 (1.4)	176 (9.8)	
Cold ischemic time, mins	140.2 ± 138.0	61.9 ± 41.8	289.0 ± 134.6	<0.001

CRRT	110 (6.7)	0	110 (6.7)
ECMO	45 (2.7)	0	45 (2.7)

Abbreviations) CRRT, continuous renal replacement therapy; DBP, diastolic blood pressure; ECMO, extracorporeal membrane oxygenation; SBP, systolic blood pressure

Table 8. Causes of Death of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=84)	Living (n=23)	Deceased (n=61)
Cardiovascular	10 (11.9%)	0 (0%)	10 (16.4%)
Infection	40 (47.6%)	11 (47.8%)	29 (47.5%)
Malignancy	4 (4.8%)	0 (0%)	4 (6.6%)
Sudden cardiac death	3 (3.6%)	3 (13.0%)	0 (0%)
Liver disease	1 (1.2%)	0 (0%)	1 (1.6%)
Others	16 (19.0%)	5 (21.7%)	11 (18.0%)
Unknown	10 (11.9%)	4 (17.4%)	6 (9.8%)

Table 9. Causes of graft loss of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=108)	Living (n=50)	Deceased (n=58)
Rejection	47 (43.5%)	24 (48%)	23 (39.7%)
BK virus nephropathy	6 (5.6%)	3 (6%)	3 (5.2%)
Glomerulonephritis	4 (3.7%)	0 (0%)	4 (6.9%)
Non-compliance	4 (3.7%)	3 (6%)	1 (1.7%)
Early surgical complication	3 (2.8%)	2 (4%)	1 (1.7%)
Primary graft failure	12 (11.1%)	5 (10%)	7 (12.1%)
Others	16 (14.8 %)	6 (12%)	10 (17.2%)
Unknown	16 (14.8%)	7 (14%)	9 (15.5%)

Table 10. Causes of Biopsies of kidney transplantation recipients in Korean Organ Transplantation Registry

Variables	Total (n=2,769)	Living (n=1,617)	Deceased (n=1,152)
Increased creatinine	1,039 (37.5%)	579 (35.8%)	460 (39.9%)
Increased proteinuria	51 (1.8%)	21 (1.3%)	30 (2.6%)
Protocol biopsy	1,625 (58.7%)	987 (61.0%)	638 (55.4%)
Others	54 (2.0%)	30 (1.9%)	24 (2.1%)

Table 11. Result of kidney allograft biopsy (all kidney biopsy)

Variables	Including protocol biopsies			Only indication biopsies		
	Total (n=2,769)	Living (n=1,617)	Deceased (n=1,152)	Total (n=1,144)	Living (n=630)	Deceased (n=514)
Borderline change	552 (19.9%)	332 (20.5%)	220 (19.1%)	241 (21.1%)	128 (20.3%)	113 (22.0%)
Acute T-cell mediated rejection	437 (15.8%)	268 (16.6%)	169 (14.7%)	306 (26.8%)	192 (30.5%)	114 (22.2%)
Acute antibody mediated rejection	203 (7.3%)	124 (7.7%)	79 (6.9%)	161 (14.1%)	94 (14.9%)	67 (13.0%)

Chronic active T cell mediated rejection	28 (1.0%)	14 (0.9%)	14 (1.2%)	27 (2.4%)	13 (2.1%)	14 (2.7%)
Chronic active antibody mediated rejection	29 (1.1%)	20 (1.2%)	9 (0.8%)	25 (2.2%)	17 (2.7%)	8 (1.6%)
Interstitial fibrosis and tubular atrophy	358 (12.9%)	177 (11.0%)	181 (15.7%)	188 (16.4%)	93 (14.8%)	95 (18.5%)
BK nephropathy	99 (3.6%)	54 (3.3%)	45 (3.9%)	88 (7.7%)	47 (7.5%)	41 (8.0%)
Glomerulonephritis	146 (5.3%)	75 (4.6%)	71 (6.2%)	105 (9.2%)	53 (8.4%)	52 (10.1%)
Calcineurin inhibitor toxicity	164 (5.9%)	79 (4.9%)	85 (7.4%)	90 (7.9%)	47 (7.5%)	43 (8.4%)

Others	587 (21.2%)	310 (19.2%)	277 (24.1%)	326 (28.5%)	193 (30.6%)	135 (5.9%)

- Multiple selections are allowed

Table 12. Comparison of predictors to death of patient estimated by least absolute shrinkage and selection operator (LASSO) and multivariable Cox regression

Variables	Selective Inference by LASSO variable selection			Multivariable Cox regression		
	Coefficient	P-value	Post-selection interval	Coefficient	P-value	95% C.I.
Age (Recipients)	0.434	0.070	0.707 – 0.724	0.434	0.010	0.103 – 0.764
Age (Donors)	-0.035	0.705	1.407 – 0.273	-0.035	0.786	-0.287 – 0.217
Sex (Recipients)	-0.358	0.247	0.522 - -0.070	-0.358	0.248	-0.966 – 0.249
Sex (Donors)	-0.571	0.069	0.074 – 0.668	-0.571	0.069	-1.185 – 0.043
DM history (Recipients)	0.729	0.010	1.197 – 1.003	0.729	0.010	0.175 – 1.283
CVD history (Recipients)	0.888	0.001	1.344 – 1.278	0.888	0.001	0.349 – 1.427

Cancer history (Recipients)	0.816	0.016	1.437 – 1.341	0.816	0.016	0.151 – 1.480
SBP (Recipients)	-0.054	0.660	0.793 – 0.914	-0.054	0.660	-0.295 – 0.187
BMI (Recipients)	-0.050	0.727	1.126 – 7.583	-0.05	0.727	-0.327 – 0.228
DM history (Donors)	0.557	0.209	1.146 – 1.241	0.557	0.126	-0.156 – 1.269
HTN history (Donors)	-0.067	0.801	4.810 – 0.840	-0.067	0.838	-0.707 – 0.574
Dialysis duration	0.235	0.053	0.447 – 1.007	0.235	0.053	-0.003 – 0.472
SBP (Donors)	0.051	0.632	0.204 – 0.652	0.051	0.631	-0.158 – 0.261
BMI (Donors)	-0.166	0.198	0.170 - -0.011	-0.166	0.198	-0.419 – 0.087
Deceased donor	1.311	0.018	2.177 – 1.965	1.311	0.001	0.504 – 2.117
HLA mismatch numbers	0.313	0.067	0.538 – 0.577	0.313	0.023	0.043 – 0.583
Desensitization	1.047	0.006	1.739 – 1.792	1.047	0.006	0.299 – 1.794

ATG induction	0.086	0.761	0.435 – 0.488	0.086	0.760	-0.467 – 0.640
Ever smoker (recipients)	0.225	0.447	0.700 - -0.180	0.225	0.446	-0.353 – 0.802
Ever smoker (donors)	-0.292	0.300	0.582 – 0.595	-0.292	0.299	-0.842 – 0.258

Abbreviations) ATG, anti-thymocyte globulin; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HLA, human leukocyte antigen; SBP, systolic blood pressure;

Table 13. Selected predictors to patient death by stepwise backward selection

Variables	Coefficient	95% C.I.	P
Recipient age, yrs	0.037	0.011 - 0.063	0.005
Donor BMI, kg/m^2	-0.059	-0.129 - 0.011	0.098
Female recipient	-0.530	-1.066 - 0.005	0.052
Female donor	-0.339	-0.853 - 0.175	0.196
Diabetes (recipient)	0.673	0.164 - 1.181	0.009
Cardiovascular disease (recipient)	0.831	0.322 - 1.340	0.001
Cancer (recipient)	0.672	0.023 - 1.321	0.042
Desensitization	0.903	0.199 - 1.608	0.012
HLA mismatch numbers	0.176	0.034 - 0.317	0.015
RRT duration, months	0.005	0.001 - 0.008	0.006
Deceased donor	1.179	0.476 - 1.882	0.001

Abbreviations) BMI, body mass index; HLA, human leukocyte antigen; RRT, renal replacement therapy

Table 14. Selected predictors to 1 year patient death and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	1.202	0.420 - 1.984	0.003	0.578	1
Age (recipients), yrs	0.043	0.013 - 0.073	0.004	0.498	2
Cardiovascular disease (recipients)	0.876	0.296 - 1.455	0.003	0.269	3
Duration of renal replacement therapy, months	0.006	0.002 - 0.009	0.005	0.341	4
Diabetes (recipients)	0.713	0.139 - 1.286	0.015	0.323	5
Diabetes (donors)	0.545	-0.203 - 1.294	0.153	0.121	6
Body mass index (donors), kg/m^2	-0.079	-0.158 - 0.001	0.052	-0.264	7

Female recipients	-0.543	-1.145 - 0.059	0.077	-0.268	8
HLA mismatch numbers	0.120	-0.037 - 0.276	0.134	0.210	9
Desensitization	0.934	0.131 - 1.737	0.023	0.394	10
Systolic blood pressure (donors), mmHg	-0.009	-0.022 - 0.005	0.198	-0.150	11

Abbreviations) HLA, human leukocyte antigen

Table 15. Selected predictors to death-censored graft loss by stepwise backward selection

Variables	Coefficient	95% C.I.	P
BMI (recipient), kg/m^2	0.067	0.013 - 0.121	0.015
Age (donor), yrs	0.015	-0.002 - 0.032	0.081
HLA mismatch numbers	0.092	-0.028 - 0.212	0.132
Female donor	0.299	-0.126 - 0.724	0.168
Donor diabetes	-1.004	-2.181 - 0.172	0.094
Desensitization	0.691	0.163 - 1.220	0.010
Donor systolic blood pressure, mmHg	-0.014	-0.025 - -0.002	0.020
Deceased donor	0.915	0.412 - 1.418	<0.001

Abbreviations) BMI, body mass index; HLA, human leukocyte antigen

Table 16. Selected predictors to 1 year death-censored graft loss and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	1.442	0.781 - 2.103	<0.001	0.694	1
Desensitization	1.129	0.441 - 1.817	0.001	0.476	2
Donor hypertension	0.691	0.091 - 1.291	0.024	0.249	3
Systolic blood pressure (recipients), mmHg	-0.013	-0.026 - -0.001	0.039	-0.272	4
Diabetes (recipients)	0.441	-0.088 - 0.971	0.102	0.200	5
Diabetes (donors)	-1.363	-2.825 - 0.099	0.068	-0.304	6
Body mass index (recipients), kg/m^2	0.070	0.001 - 0.139	0.048	0.247	7
Systolic blood pressure (donors), mmHg	-0.013	-0.027 - 0.001	0.067	-0.228	8

Cancer (recipients)	0.535	-0.272 - 1.341	0.194	0.132	9

Table 17. Comparison of predictors to acute rejection estimated by least absolute shrinkage and selection operator (LASSO) and multivariable Cox regression

Variables	Selective Inference by LASSO variable selection			Multivariable Cox regression		
	Coefficient	P-value	Post-selection interval	Coefficient	P-value	95% C.I.
Age (Recipients)	-0.101	0.008	-0.164 - -0.035	-0.101	0.008	-0.176 - -0.027
Age (Donors)	0.214	<0.001	0.147 – 0.300	0.214	<0.001	0.136 – 0.293
Sex (Recipients)	-0.310	<0.001	-0.446 - -0.173	-0.310	<0.001	-0.471 - -0.149
Sex (Donors)	0.103	0.116	-0.091 – 0.919	0.103	0.230	-0.065 – 0.270
DM history (Recipients)	-0.086	0.316	-0.224 – 0.188	-0.086	0.314	-0.254 – 0.081
CVD history (Recipients)	-0.150	0.232	-0.353 – 0.195	-0.150	0.231	-0.395 – 0.095

Cancer history (Recipients)	0.261	0.052	-0.007 – 0.482	0.261	0.052	-0.002 – 0.523
SBP (Recipients)	-0.044	0.227	-0.104 – 0.056	-0.044	0.226	-0.116 – 0.027
BMI (Recipients)	0.080	0.161	-0.056 – 0.139	0.080	0.028	0.009 – 0.152
DM history (Donors)	-0.339	0.056	-0.629 – 0.016	-0.339	0.055	-0.685 – 0.008
HTN history (Donors)	0.041	0.691	-0.811 – 0.181	0.041	0.689	-0.160 – 0.242
Dialysis duration	-0.067	0.129	-0.144 – 0.035	-0.067	0.128	-0.154 – 0.019
SBP (Donors)	-0.010	0.759	-0.072 – 0.368	-0.010	0.778	-0.080 – 0.060
BMI (Donors)	-0.005	0.901	-0.029 – 0.888	-0.005	0.900	-0.077 – 0.068
Deceased donor	0.249	0.053	-0.007 – 0.632	0.249	0.017	0.044 – 0.454
HLA mismatch numbers	0.132	<0.001	0.070 – 0.193	0.132	<0.001	0.060 – 0.205

Desensitization	0.359	<0.001	0.213 – 0.505	0.359	<0.001	0.187 – 0.532
ATG induction	0.082	0.353	-0.221 – 0.226	0.082	0.356	-0.092 – 0.256
Ever smoker (recipients)	-0.158	0.081	-0.321 – 0.032	-0.158	0.079	-0.335 – 0.019
Ever smoker (donors)	-0.013	0.883	-0.085 – 1.791	-0.013	0.883	-0.184 – 0.159

Abbreviations) ATG, anti-thymocyte globulin; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HLA, human leukocyte antigen; SBP, systolic blood pressure;

Table 18. Selected predictors to acute rejection by stepwise backward selection

Variables	Hazard Ratio	95% C.I.	P
Age (Recipients)	0.990	0.984 – 0.996	0.001
Age (Donors)	1.016	1.011 – 1.022	<0.001
Sex (Recipients)	0.762	0.661 – 0.877	<0.001
Desensitization	1.493	1.269 – 1.756	<0.001
Deceased donor	1.212	1.033 – 1.423	0.019
Mycophenolate mofetil	0.639	0.540 – 0.756	<0.001
HLA mismatch numbers	1.084	1.042 – 1.128	<0.001
SBP (Recipients)	0.997	0.994 – 1.001	0.129
Body mass index (Recipients)	1.017	0.998 – 1.037	0.073
DM history (Donors)	0.732	0.527 – 1.018	0.064
Steroid	1.451	0.854 – 2.466	0.169

Abbreviations) HLA, human leukocyte antigen; SBP, systolic blood pressure

Table 19. Selected predictors to acute rejection with post-transplant 1 year and dominance

Variables	Coeffieicnt	95% C.I.	P	Standardized Beta	Rank
Donor age, yrs	0.019	0.012 - 0.025	<0.001	0.235	1
HLA mismatch numbers	0.092	0.043 - 0.141	<0.001	0.163	2
Desensitization	0.443	0.239 - 0.647	<0.001	0.188	3
Female recipients	-0.368	-0.556 - -0.181	<0.001	-0.181	4
Body mass index (recipients), kg/m^2	0.023	-0.001 - 0.046	0.063	0.080	5
Diabetes mellitus (donors)	-0.523	-0.939 - -0.106	0.014	-0.116	6
Recipient age, yrs	-0.009	-0.016 - -0.001	0.021	-0.100	7
Systolic blood pressure (recipients), mmHg	-0.003	-0.007 - 0.001	0.125	-0.067	8
Deceased donor	0.196	-0.003 - 0.394	0.053	0.094	9

Ever smoker (recipients)	-0.162	-0.370 - 0.046	0.127	-0.070	10

Abbreviations) HLA, human leukocyte antigen

Table 20. Selected predictors to antibody mediated rejection by stepwise backward selection

Variables	Coefficients	95% C.I.	P
Age (Recipients)	-0.020	-0.033 - -0.007	0.003
Age (Donors)	0.011	-0.003 – 0.024	0.114
ATG induction	0.385	0.048 – 0.723	0.025
Female donor	0.271	-0.092 – 0.634	0.144
SBP (Recipients)	-0.007	-0.016 – 0.002	0.131
Ever smoker (donors)	0.404	0.037 – 0.771	0.031
Desensitization	0.441	0.035 – 0.848	0.033
Deceased donor	1.143	0.784 – 1.502	<0.001
Donor hypertension	-0.404	-0.897 – 0.090	0.109
HLA mismatch numbers	0.144	0.052 – 0.237	0.002

Abbreviations) ATG, anti-thymocyte globulin; HLA, human leukocyte antigen; SBP, systolic blood pressu

Table 21. Selected predictors to antibody mediated rejection with post-transplant 1 year and dominance

Variables	Coeffieicnt	95% C.I.	P	Standardized Beta	Rank
Desensitization	1.284	0.868 – 1.699	<0.001	0.542	1
ATG induction	0.547	0.167 – 0.926	0.005	0.217	2
HLA mismatch numbers	0.118	0.013 – 0.224	0.027	0.208	3
Age, yrs	-0.019	-0.034 - -0.004	0.015	-0.218	4
Deceased donor	0.394	-0.070 – 0.859	0.096	0.190	5
Donor hypertension	-0.451	-1.015 – 0.112	0.116	-0.162	6
Ever smoker (donors)	0.273	-0.084 – 0.630	0.134	0.131	7
Donor age, yrs	0.010	-0.005 – 0.025	0.182	0.128	8

Abbreviations) ATG, anti-thymocyte globulin; HLA, human leukocyte antigen

Table 22. Selected predictors to acute rejection with post-transplant 1 year and dominance in re-transplantation patients

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Deceased donor	0.923	0.143 – 1.704	0.020	0.456	1
Donor hypertension	0.569	-0.186 – 1.323	0.140	0.214	2
HLA mismatch numbers	0.147	-0.034 – 0.328	0.112	0.264	3
ATG induction	0.484	-0.169 – 1.137	0.146	0.236	4
Desensitization	0.741	-0.034 – 1.517	0.061	0.348	5
DM history (Recipients)	-0.788	-1.792 – 0.216	0.124	-0.302	6
Mycophenolate mofetil	-0.583	-1.345 – 0.179	0.134	-0.225	7

Abbreviations) ATG, anti-thymocyte globulin; DM, diabetes mellitus; HLA, human leukocyte antigen

Table 23. Selected predictors to post-transplant 1 year estimated glomerular filtration rate and dominance

Variables	Coefficient	95% C.I.	P	Standardized Beta	Rank
Donor age, yrs	-0.590	-0.639 - -0.540	<0.001	-7.515	1
Acute rejection within 1 yr	-10.122	-11.701 - -8.543	<0.001	-3.890	2
BKVAN within 1 yr	-23.426	-28.413 - -18.438	<0.001	-2.834	3
Female donor	-2.305	-3.761 - -0.850	0.002	-1.151	4
Body mass index (recipients), kg/m^2	-0.471	-0.649 - -0.292	<0.001	-1.664	5
Diabetes mellitus (donors)	-2.777	-5.633 - 0.080	0.057	-0.615	6
Ever smoking (donors)	1.808	0.342 - 3.273	0.016	0.866	7
Body mass index (donors), kg/m^2	0.396	0.206 - 0.585	<0.001	1.303	8
Deceased donor	-2.470	-4.171 - -0.768	0.004	-1.189	9

Systolic blood pressure (recipients), mmHg	0.063	0.032 - 0.093	<0.001	1.275	10
Systolic blood pressure (donors), mmHg	-0.043	-0.079 - -0.008	0.017	-0.746	11
Age (recipients), yrs	-0.042	-0.096 - 0.012	0.125	-0.487	12
Female recipients	0.762	-0.513 - 2.037	0.241	0.375	13
Duration of renal replacement therapy, months	0.011	-0.001 - 0.023	0.068	0.669	14
Desensitization	-1.474	-3.031 - 0.083	0.064	-0.616	15

Abbreviations) BKVAN, BK virus associated nephropathy

Table 24. Baseline clinical characteristics of the study population

Variables	Total (n=5,871)	Cohort 1 (n=2,806)	Cohort 2 (n=3,065)	P
Age, yrs	46.8 ± 11.8	48.7 ± 11.6	45.0 ± 11.7	<0.001
Female sex	2,440 (42)	1,148 (41)	1,292 (42)	0.335
Deceased donor	2,236 (38)	1,098 (39)	1,138 (37)	0.122
HLA mismatch (total)	3.2 ± 1.7	3.2 ± 1.8	3.2 ± 1.7	0.382
Class I mismatch	2.2 ± 1.2	2.2 ± 1.2	2.1 ± 1.2	0.400
Class II mismatch	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	0.496
Eplet mismatch, class I	10.6 ± 6.8	10.5 ± 6.7	10.6 ± 6.9	0.801
Eplet mismatch, class II	24.1 ± 17.6	23.7 ± 17.2	24.3 ± 17.6	0.197
Retransplantation	432 (7)	212 (8)	220 (7)	0.580
Desensitization	1,063 (18)	626 (22)	437 (14)	<0.001
ABO incompatible KT	693 (12)	425 (15)	268 (9)	<0.001
Cold ischemic time (hrs)	710 (12)	548 (20)	162 (5)	<0.001
Total acute rejection	1.9 ± 2.3	1.9 ± 2.2	1.9 ± 2.3	0.838
Biopsy-proven acute rejection	520 (9)	335 (12)	185 (6)	<0.001

Cause of end stage renal disease				<0.001
Diabetic nephropathy	1,176 (20)	622 (22)	554 (18)	
Hypertension	909 (15)	461 (16)	448 (15)	
Glomerulonephritis	1,988 (34)	950 (34)	1,038 (34)	
Others	260 (4)	233 (8)	27 (1)	
Unknown	1,538 (26)	540 (19)	998 (33)	
Diabetes (recipient)	1,496 (25)	787 (28)	709 (23)	<0.001
Cardiovascular disease (recipients)	562 (10)	296 (11)	266 (9)	0.016
Donor age, yrs	44.8 ± 12.9	46.6 ± 12.8	43.1 ± 12.8	<0.001
Donor sex	2,611 (44)	1,278 (46)	1,333 (44)	0.121
Donor diabetes	220 (4)	146 (5)	74 (3)	<0.001
Donor hypertension	710 (13)	419 (15)	291 (10)	<0.001

Abbreviations) ADPKD, autosomal dominant polycystic kidney disease; DBP, diastolic blood pressure; SBP, systolic blood pressure

Table 25. Estimated eplet of the study population

Variables	Total (n=5,871)	Cohort 1 (n=2,806)	Cohort 2 (n=3,065)	P
Eplet mismatch class I	10.6 ± 6.8	10.5 ± 6.7	10.6 ± 6.9	0.801
Antibody verified eplet	5.9 ± 4.0	5.9 ± 4.0	6.0 ± 4.1	0.656
Other eplet	4.6 ± 3.2	4.6 ± 3.2	4.6 ± 3.2	0.980
Eplet mismatch class II	24.1 ± 17.6	23.7 ± 17.2	24.3 ± 17.6	0.197
DR	10.6 ± 8.6	10.6 ± 8.5	10.6 ± 8.7	0.986
Antibody verified DR	4.3 ± 3.9	4.3 ± 3.8	4.2 ± 3.9	0.202
Other DR	6.3 ± 5.3	6.3 ± 5.4	6.4 ± 5.3	0.341
DQ	13.5 ± 10.6	13.2 ± 10.5	13.7 ± 10.7	0.036
Antibody verified DQ	5.3 ± 4.9	5.0 ± 4.8	5.5 ± 5.0	<0.001
Other DQ	8.2 ± 6.4	8.1 ± 6.4	8.2 ± 6.5	0.701

Table 26. Association of HLA eplet mismatches with acute rejection

Variables	Unadjusted Hazard Ratio	95% C.I.	P	Adjusted hazard ratio	95% C.I.	P
HLA mismatches						
HLA-A	1.182	1.050 – 1.331	0.006	0.965	0.806 – 1.155	0.695
HLA-B	1.389	1.222 – 1.578	<0.001	1.169	0.973 – 1.406	0.096
HLA-DR	1.382	1.222 – 1.563	<0.001	1.240	1.020 – 1.509	0.031
Eplet mismatches						
Class I	1.024	1.011 – 1.037	<0.001	1.007	0.985 – 1.029	0.523
Class II	1.001	1.004 – 1.014	<0.001	N/A	N/A	N/A
DR	1.018	1.009 – 1.028	<0.001	1.003	0.989 – 1.017	0.685
DQ	1.012	1.004 – 1.020	0.003	0.997	0.986 – 1.008	0.546

Recipient age (10yrs)	0.956	0.889 – 1.029	0.231	0.895	0.829 – 0.965	0.004
Recipient female sex	0.853	0.714 – 1.018	0.079	0.856	0.715 – 1.024	0.088
Donor age (10yrs)	1.241	1.156 – 1.332	<0.001	1.229	1.142 – 1.323	<0.001
Deceased donor	1.124	0.943 – 1.339	0.192	1.048	0.875 – 1.256	0.611

Table 27. Identification of individual eplet to biopsy proven acute rejection

Eplets	Non-rejection (n=2,471)	Rejection (n=335)	Total (n=2,806)	P
Class I				
113-76ED	141 (5.7)	30 (9.0)	171 (6.1)	0.242
120-143S	221 (8.9)	42 (12.5)	263 (9.4)	0.297
121-147L	221 (8.9)	42 (12.5)	263 (9.4)	0.297
19-180E	323 (13.1)	57 (17.0)	380 (13.5)	0.341
109-71KA	95 (3.8)	20 (6.0)	115 (4.1)	0.351
4-65QIA	350 (14.2)	60 (17.9)	410 (14.6)	0.385
107-66KA	318 (12.9)	54 (16.1)	372 (13.3)	0.428
5-69AA	411 (16.6)	68 (20.3)	479 (17.1)	0.430
108-66IS	378 (15.3)	63 (18.8)	441 (15.7)	0.432

6-69TNT	116 (4.7)	22 (6.6)	138 (4.9)	0.453
Class II				
DR-2-11-STS	381 (15.4)	75 (22.4)	456 (16.3)	0.124
DQB-6-37YA	187 (7.6)	41 (12.2)	228 (8.1)	0.145
DR-8-77N	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-10-98Q	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-1-13SE	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-17-71K	401 (16.2)	77 (23.0)	478 (17.0)	0.146
DR-7-73A	0 (0)	1 (0.3)	1 (0.03)	0.160
DR-8-77T	0 (0)	1 (0.3)	1 (0.03)	0.160
DQB-14-86G	226 (9.1)	47 (14.0)	273 (9.7)	0.164
DR-9-	226 (9.1)	47 (14.0)	273 (9.7)	0.164

Table 28. Characteristics of suggested individual eplet

Eplets	Antibody verified	Ellipro score	Luminex Allele of Epitope
Class I			
76ED	No	High	B*27:03, B*27:05, B*37:01, B*47:01
143S	Yes	High	B*40:01, B*48:01, B*81:01, C*17:01
147L	No	High	B*40:01, B*48:01, B*81:01, C*07:01, C*07:02, C*07:04, C*17:01
180E	Yes	High	B*07:02, B*07:03, B*08:01, B*40:01, B*41:01, B*41:02, B*42:01, B*48:01, B*81:01
71KA	No	Low	B*27:03, B*27:05, B*27:08, B*73:01
65QIA	Yes	Intermediate	B*07:02, B*27:03, B*27:05, B*27:08, B*42:01, B*54:01, B*55:01, B*56:01, B*67:01, B*73:01, B*81:01, B*82:01, B*82:02

66KA	No	Intermediate	A*02:01, A*02:02, A*02:03, A*02:05, A*02:06, A*23:01, A*23:02, A*24:02, A*24:03, A*34:01
69AA	Yes	Intermediate	B*07:02, B*15:16, B*27:03, B*27:05, B*27:08, B*42:01, B*54:01, B*55:01, B*56:01, B*57:01, B*57:03, B*58:01, B*67:01, B*73:01, B*81:01, B*82:01, B*82:02
66IS	No	Low	B*13:01, B*13:02, B*15:01, B*15:02, B*15:03, B*15:12, B*15:13, B*18:01, B*37:01, B*40:01, B*40:02, B*40:05, B*40:06, B*41:01, B*41:02, B*44:02, B*44:03, B*45:01, B*47:01, B*48:01, B*49:01, B*50:01, B*52:01
69TNT	Yes	Intermediate	B*07:03, B*08:01, B*13:01, B*13:02, B*14:01, B*14:02, B*14:05, B*14:06, B*15:01, B*15:02, B*15:03, B*15:10, B*15:11, B*15:12, B*15:13, B*15:18, B*18:01, B*35:01, B*35:08, B*37:01, B*38:01, B*39:01, B*39:05, B*40:01, B*40:02, B*40:05, B*40:06, B*41:01, B*41:02, B*44:02, B*44:03, B*45:01, B*47:01, B*48:01, B*49:01, B*50:01, B*51:01, B*51:02, B*52:01, B*53:01, B*59:01, B*78:01

Class II

DR-2-11-STS	Yes	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02, DRB1*14:03, DRB1*14:54
DQB-6-37YA	No	DQB1*03:01, DQB1*03:02, DQB1*03:03, DQB1*03:19, DQB1*04:01, DQB1*04:02, DQB1*06:02, DQB1*06:03, DQB1*06:04, DQB1*06:09
DR-8-77N	Yes	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-10-98Q	Yes	DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-1-13SE	No	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02,

		DRB1*14:03, DRB1*14:54, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-17-71K	No	DRB1*03:01, DRB1*03:02, DRB1*03:03, DRB1*04:01, DRB1*13:03, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB3*03:01
DR-7-73A	Yes	DRB1*01:01, DRB1*01:02, DRB1*01:03, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05, DRB1*08:01, DRB1*08:02, DRB1*09:01, DRB1*09:02, DRB1*10:01, DRB1*11:01, DRB1*11:03, DRB1*11:04, DRB1*12:01, DRB1*12:02, DRB1*13:01, DRB1*13:02, DRB1*13:03, DRB1*13:05, DRB1*14:01, DRB1*14:02, DRB1*14:03, DRB1*14:04, DRB1*14:54, DRB1*15:01, DRB1*15:02, DRB1*15:03, DRB1*16:01, DRB1*16:02, DRB4*01:01, DRB4*01:03, DRB5*01:01, DRB5*02:02
DR-8-77T	Yes	DRB1*01:01, DRB1*01:02, DRB1*01:03, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05,

		DRB1*07:01,	DRB1*08:01,	DRB1*08:02,	DRB1*09:01,
		DRB1*09:02,	DRB1*10:01,	DRB1*11:01,	DRB1*11:03,
		DRB1*11:04,	DRB1*12:01,	DRB1*12:02,	DRB1*13:01,
		DRB1*13:02,	DRB1*13:03,	DRB1*13:05,	DRB1*14:01,
		DRB1*14:02,	DRB1*14:03,	DRB1*14:04,	DRB1*14:54,
		DRB1*15:01,	DRB1*15:02,	DRB1*15:03,	DRB1*16:01,
		DRB1*16:02,	DRB4*01:01,	DRB4*01:03,	DRB5*01:01,
		DRB5*02:02			
DQB-14-86G	No	DQB1*06:04, DQB1*06:09			
DQB130Q-	No	DQB1*06:04, DQB1*06:09			

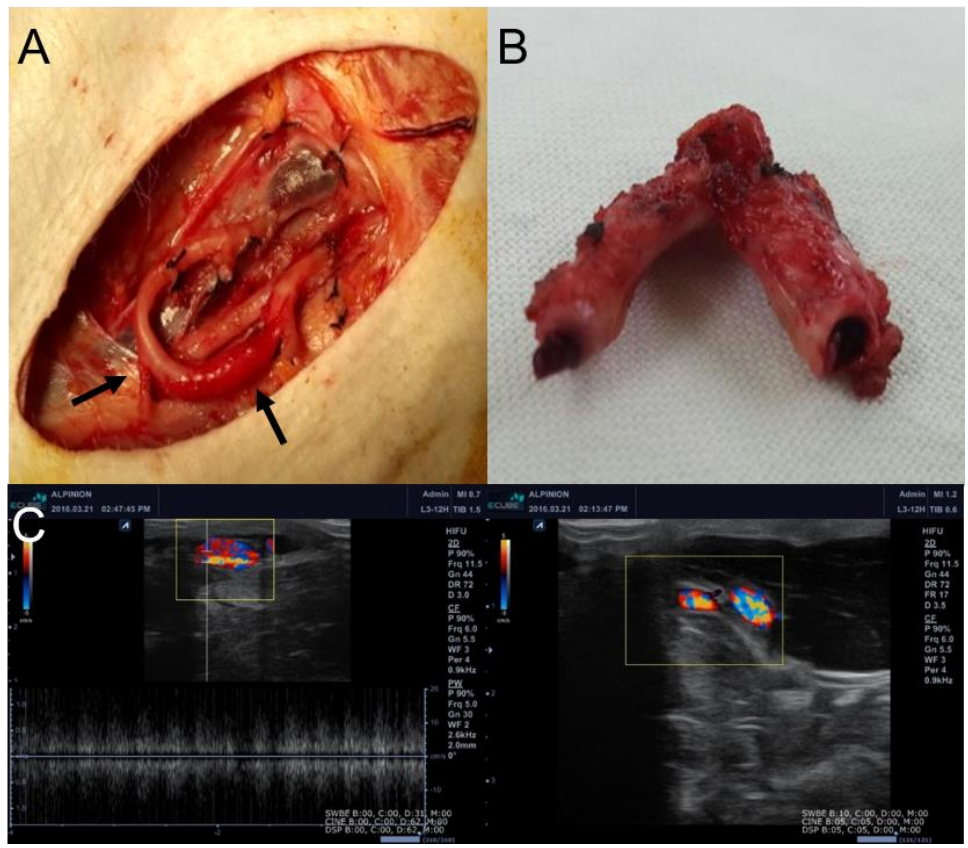


Figure 1. Alpha-galactosyltransferase knock out (GTKO) porcine vascular transplantation to Cynomolgus monkey

(A) Porcine artery graft anastomosed to femoral artery and femoral vein of cynomolgous monkey (B) Excised porcine artery graft after 4 weeks of transplantation periods. Note intraluminal thrombus. (C) Doppler ultrasonographical assessment of transplanted graft.

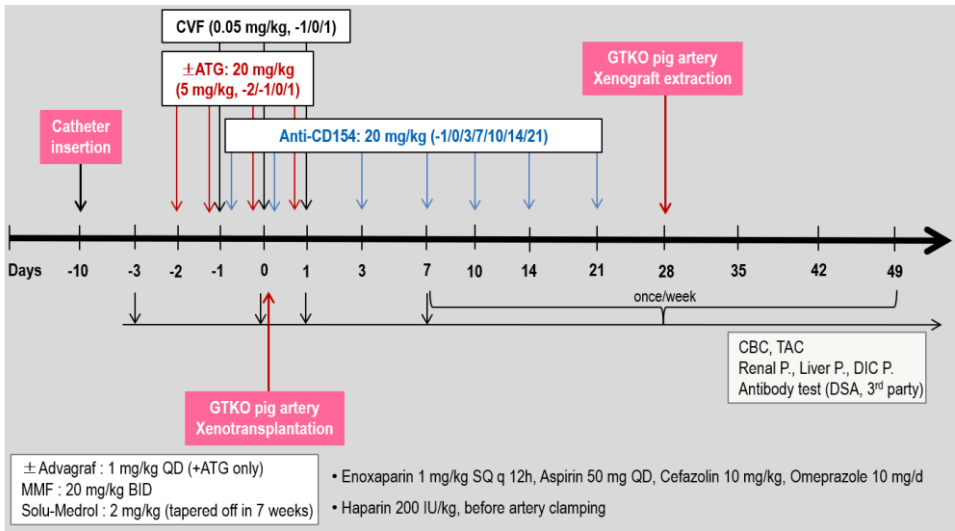
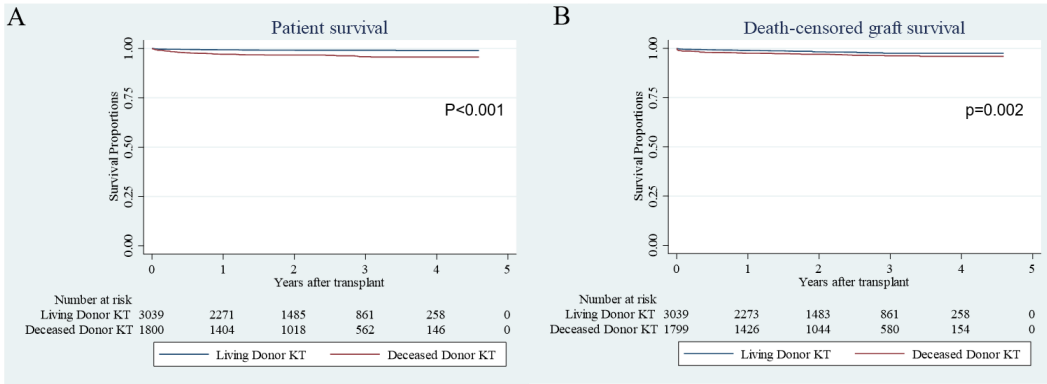


Figure 2. Immunosuppressive regimens of the GTKO pig artery transplantation in Cynomolgus monkey

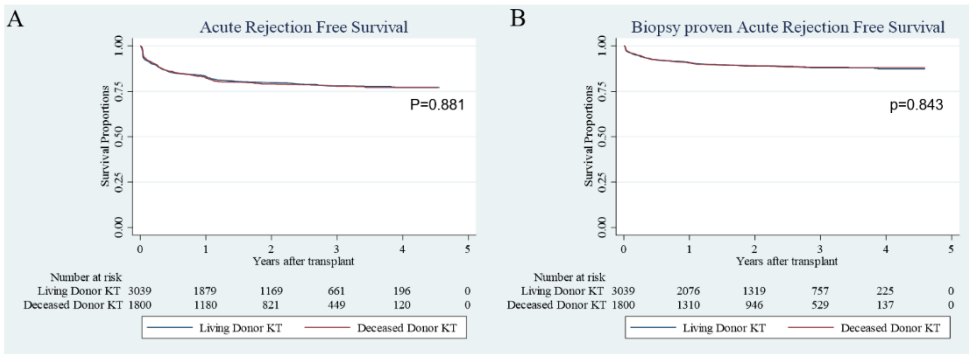


	1yr	2yr	3yr	4yr	5yr
Total	98.4 (98.0 – 98.8)	98.2 (97.8 – 98.6)	97.8 (97.3 – 98.3)	97.6 (97.0 – 98.1)	97.6 (97.0 -98.1)
LDKT	99.3 (98.5 – 99.2)	99.2 (97.6 – 98.7)	99.1 (96.8 – 98.2)	98.9 (96.8 – 98.2)	98.9 (96.8 – 98.2)
DDKT	97.0 (96.0 – 97.8)	96.6 (95.6 – 97.4)	95.9 (94.7 – 96.8)	95.6 (94.2 – 96.6)	95.6 (94.2 – 96.6)

	1yr	2yr	3yr	4yr	5yr
Total	98.4 (98.0 – 98.7)	97.8 (97.3 – 98.2)	97.0 (96.4 – 97.6)	96.9 (96.2 – 97.5)	96.9 (96.2 -97.5)
LDKT	99.0 (98.5 – 99.2)	98.3 (97.6 – 98.7)	97.6 (96.8 – 98.2)	97.6 (96.8 – 98.2)	97.6 (96.8 – 98.2)
DDKT	97.4 (96.5 – 98.1)	97.0 (96.0 – 97.7)	96.1 (94.9 – 97.0)	95.9 (94.5 – 96.9)	95.9 (94.5 – 96.9)

Figure 3. Patient and death-censored graft survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of patient survival (B) Kaplan-Meier curve of death-censored graft survival

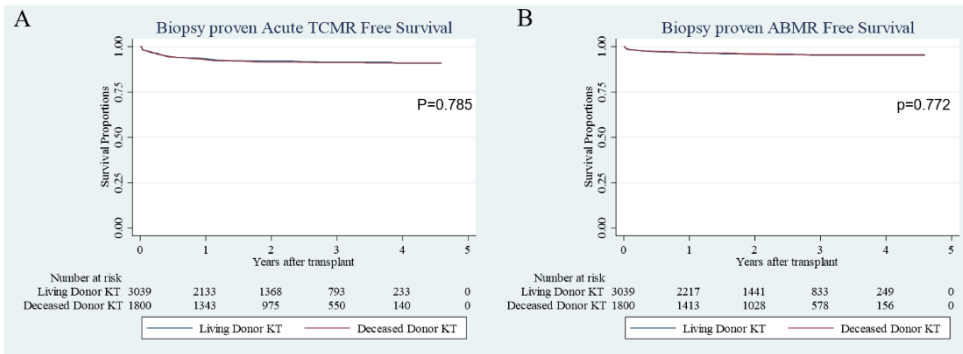


	1yr	2yr	3yr	4yr	5yr
Total	82.4 (98.0 – 98.8)	78.4 (97.8 – 98.6)	77.0 (97.3 – 98.3)	76.2 (97.0 – 98.1)	76.2 (97.0 -98.1)
LDKT	82.3 (80.8 – 83.7)	78.5 (76.9 – 80.1)	76.8 (75.0 – 78.5)	76.1 (74.2 – 77.9)	76.1 (74.2 – 77.9)
DDKT	81.7 (79.7 – 83.4)	78.2 (76.1 – 80.1)	77.1 (74.9 – 79.2)	76.3 (73.9 – 78.5)	76.3 (73.9 – 78.5)

	1yr	2yr	3yr	4yr	5yr
Total	90.3 (89.4 – 91.2)	88.5 (87.5 – 89.5)	87.6 (86.5 – 88.6)	87.3 (86.1 – 88.3)	87.3 (86.1 -88.3)
LDKT	90.4 (89.2 – 91.5)	88.5 (87.2 – 89.7)	87.3 (85.9 – 88.6)	87.0 (85.4 – 88.4)	87.0 (85.4 – 88.4)
DDKT	90.2 (88.7 – 91.6)	88.6 (86.9 – 90.0)	88.0 (86.2 – 89.5)	87.7 (85.8 – 89.3)	87.7 (85.8 – 89.3)

Figure 4. Acute rejection free- and biopsy-proven acute rejection free- survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of acute rejection-free survival (B) Kaplan-Meier curve of biopsy-proven acute rejection-free survival



	1yr	2yr	3yr	4yr	5yr
Total	92.8 (92.0 – 93.5)	91.4 (90.5 – 92.2)	91.0 (90.0 – 91.8)	90.6 (89.6 – 91.6)	90.6 (89.6 - 91.6)
LDKT	93.0 (91.9 – 93.9)	91.5 (90.3 – 92.5)	91.0 (89.7 – 92.1)	90.6 (89.3 – 91.8)	90.6 (89.3 – 91.8)
DDKT	92.5 (91.1 – 93.7)	91.3 (89.8 – 92.6)	90.9 (89.4 – 92.3)	90.7 (89.0 – 92.1)	90.7 (89.0 – 92.1)

	1yr	2yr	3yr	4yr	5yr
Total	96.5 (95.9 – 97.0)	95.7 (95.1 – 96.3)	95.2 (94.4 – 95.8)	95.2 (94.4 – 95.8)	95.2 (94.4 -95.8)
LDKT	96.4 (95.6 – 97.1)	95.7 (94.9 – 96.5)	95.1 (94.1 – 95.9)	95.1 (94.1 – 95.9)	95.1 (94.1 – 95.9)
DDKT	96.7 (95.7 – 97.5)	95.8 (94.6 – 96.7)	95.4 (94.2 – 96.4)	95.4 (94.2 – 96.4)	95.4 (94.2 – 96.4)

Figure 5. Acute T-cell mediated rejection free- and acute antibody mediated rejection free- survival of Korean Organ Transplantation Registry

(A) Kaplan-Meier curve of acute T-cell mediated rejection-free survival (B) Kaplan-Meier curve of acute antibody mediated rejection-free survival

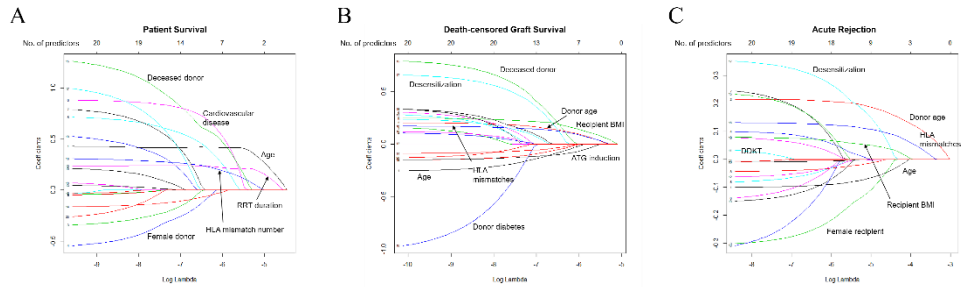


Figure 6. Variable selection and coefficient pathways in least absolute shrinkage and selection operator (LASSO) method for patient survival, death-censored graft survival, and acute rejection

(A) Coefficient path plot to patient survival (B) Coefficient path plot to death-censored graft survival (C) Coefficient path plot to acute rejection

Upper x-axis indicates included numbers of predictors in regularized LASSO models at certain log lambda values. Numeric lables indicate each predictors as follows: 1, recipient age; 2, donor age; 3, female recipient; 4, female donor; 5, diabetic recipient; 6, history of cardiovascular disease in recipient; 7, history of cancer in recipient; 8, systolic blood pressure in recipient; 9, body mass index in recipient; 10, donor diabetes; 11, donor hypertension; 12, duration of renal replacement therapy; 13, systolic blood pressure in donor; 14, body mass index in donor; 15, deceased donor; 16, HLA mismatch numbers; 17, desensitization; 18, anti-thymocyte globulin induction; 19, ever-smoker (recipient); 20, ever-smoker (donor)

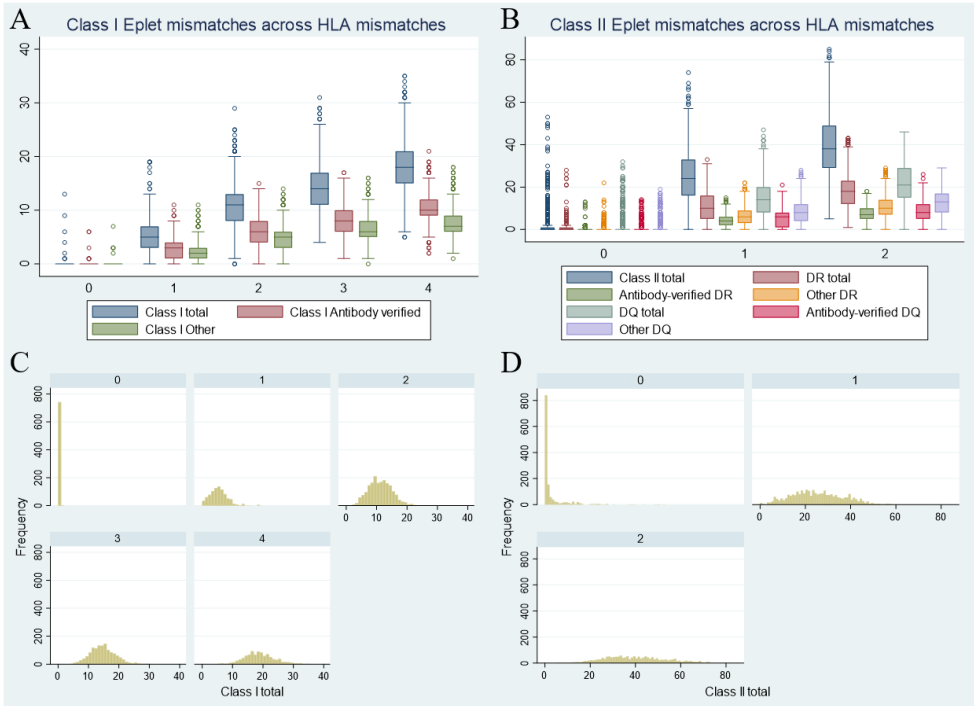


Figure 7. Distribution of eplet mismatches across human leukocyte antigen mismatches

(A) Box and Whisker plot of class I eplet mismatches across HLA class I mismatches (B) Box and Whisker plot of class II eplet mismatches across HLA class II mismatches (C) Histogram of eplet mismatch distribution across class I HLA mismatches (D) Histogram of eplet mistmach distribution across class II HLA mismatches

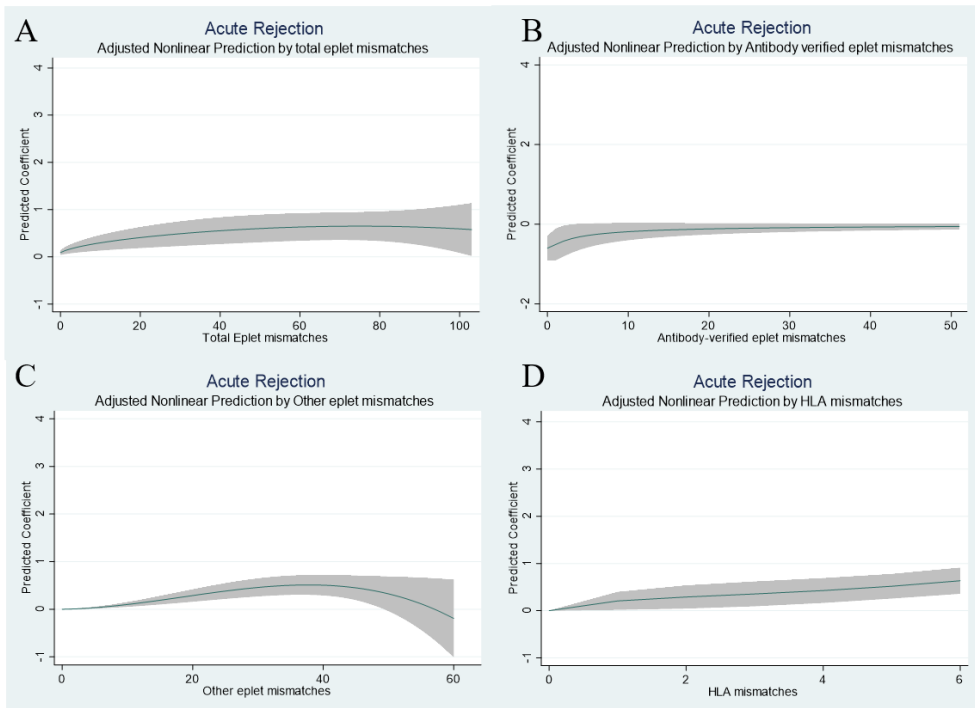


Figure 8. Adjusted risks of eplet mismatches or HLA mismatches to overall rejection (A) Non-linear risk of total eplet mismatch to acute rejection (B) Non-linear risk of antibody-verified eplet mismatch to acute rejection (C) Non-linear risk of non-antibody-verified eplet mismatch to acute rejection (D) Non-linear risk of HLA conventional genotype mismatch to acute rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta coefficient of target variable. Grey area indicate 95% confidence interval of

estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

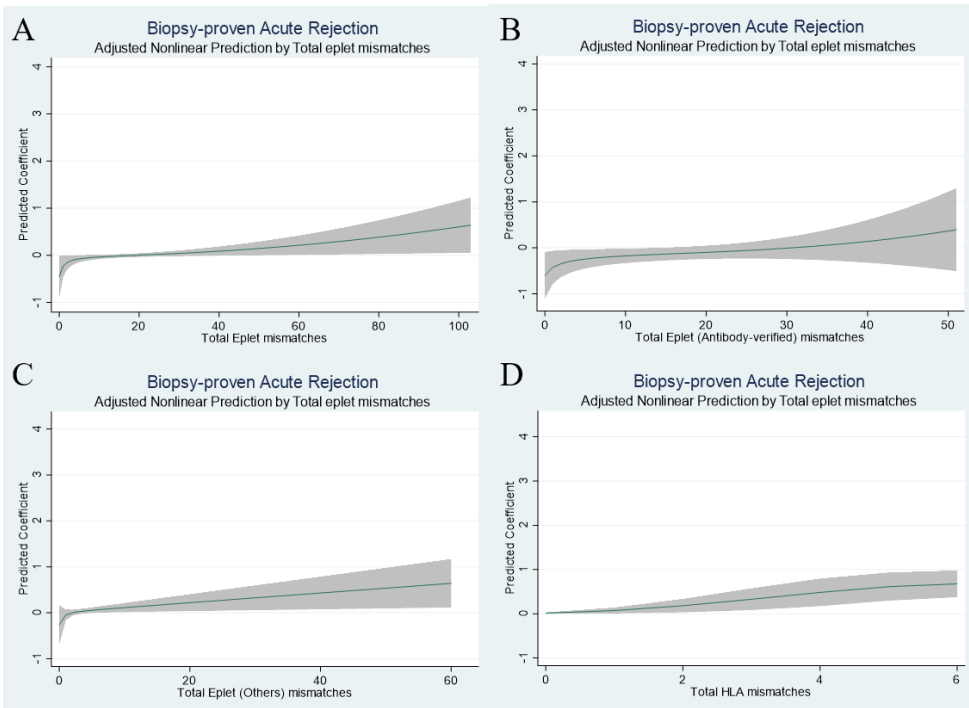


Figure 9. Adjusted risks of eplet mismatches or HLA mismatches to biopsy-proven rejection

(A) Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (B) Non-linear risk of antibody-verified eplet mismatch to biopsy-proven acute rejection (C) Non-linear risk of non-antibody-verified eplet mismatch to biopsy-proven acute rejection (D) Non-linear risk of HLA conventional genotype mismatch to biopsy-proven acute rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta

coefficient of target variable. Grey area indicate 95% confidence interval of estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

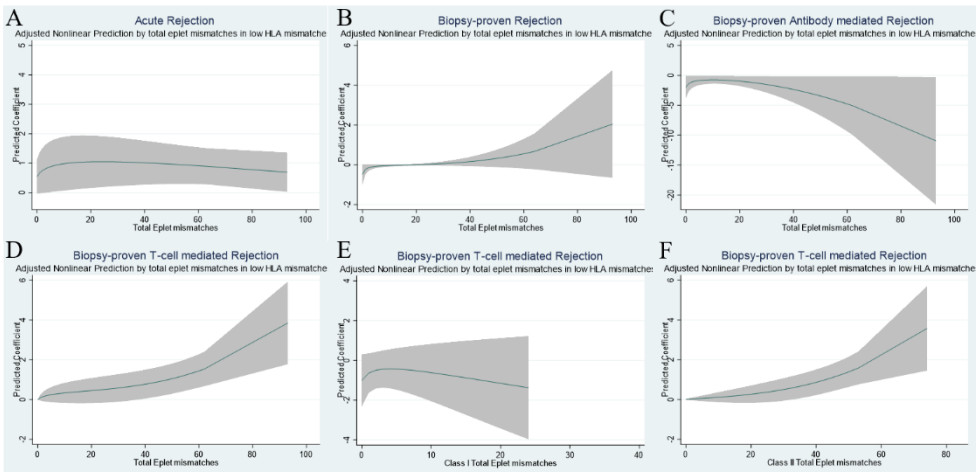


Figure 10. Adjusted risks of eplet mismatches to various rejection outcomes in low HLA mismatch settings (HLA mismatches < 3)

(A) Non-linear risk of total eplet mismatch to acute rejection (B) Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (C) Non-linear risk of total eplet mismatch to biopsy-proven antibody mediated rejection (D) Non-linear risk of total eplet mismatches to biopsy-proven acute T-cell mediated rejection (E) Non-linear risk of class I total eplet mistamches to biopsy-proven acute T-cell mediated rejection (F) Non-linear risk of class II total eplet mismatches to biopsy-proven acute T-cell mediated rejection

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension. Solid line indicates estimated beta coefficient of target variable. Grey area indicate 95% confidence interval of estimated beta. Statistical significance is achieved when the grey area do not cross horizontal zero-line at y-axis across included x-values.

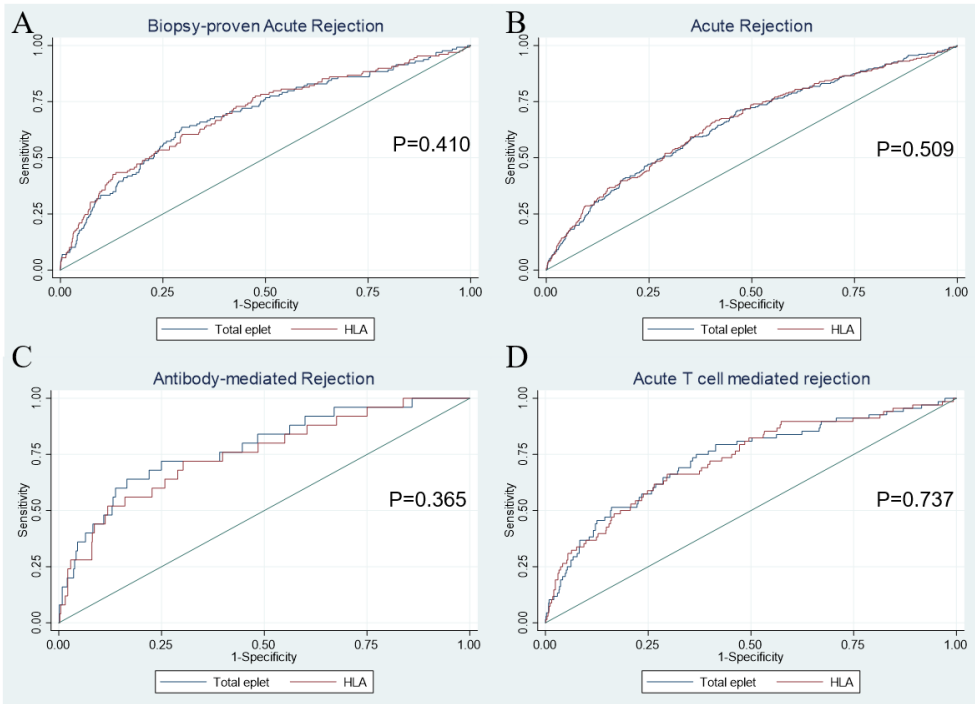


Figure 11. Comparisons of ROC curves of eplet mistmaches and human leukocyte antigen mismatches in low HLA mismatch settings (HLA mismatches < 3)

In these multivariable non-linear logistic regression models, simultaneously adjusted covariables include followings: recipient age, recipient sex, donor age, donor sex, deceased donor, anti-thymocyte globulin induction, ABO incompatibility, recipient diabetes, donor diabetes, donor hypertension.

(A) Area under curve of prediction model Non-linear risk of total eplet mismatch to biopsy-proven acute rejection (B) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute rejection (C) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute antibody-mediated rejection (D) Area under curve of prediction model Non-linear risk of total eplet mismatch to acute T-cell mediated rejection

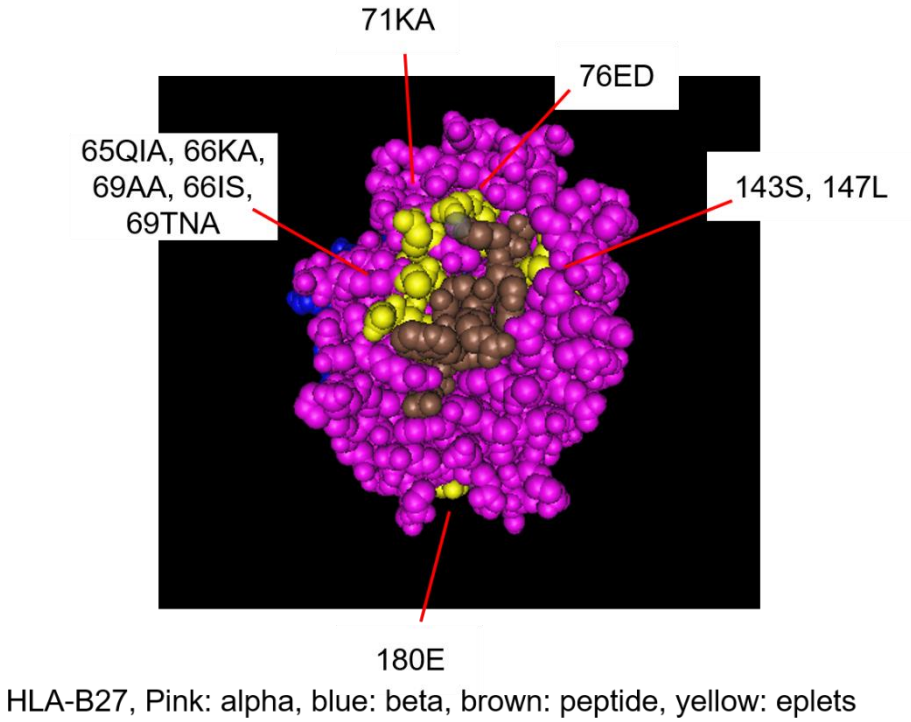


Figure 12. Graphical presentation of candidate eplets on three-dimensional HLA class I molecule (HLA-B27)

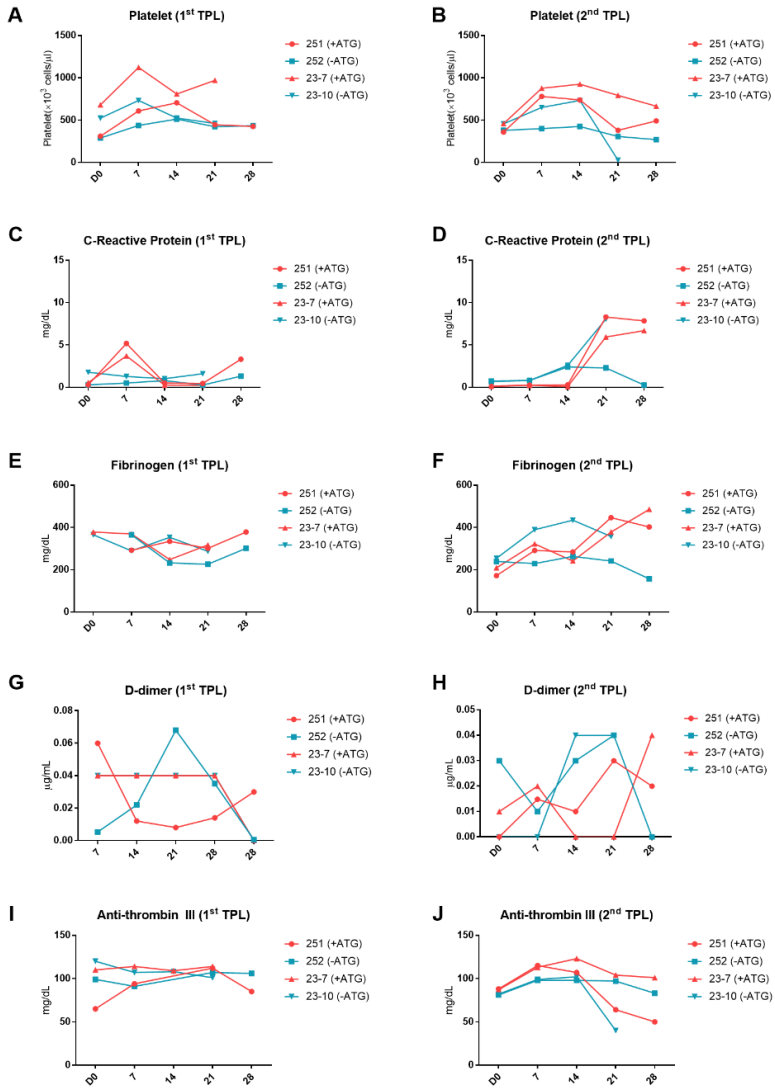


Figure 13. Platelet level and coagulation profiles after GTKO pig artery transplantation in Cynomolgus monkey

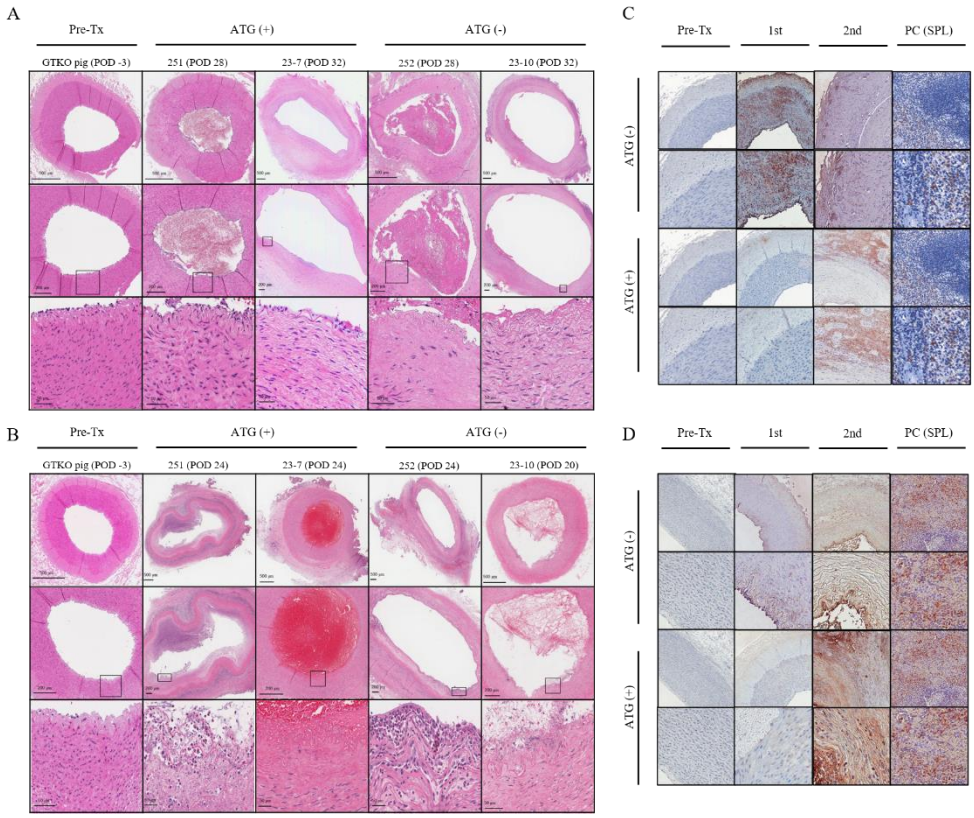


Figure 14. Histology and immunohistochemical stain of porcine vascular graft

(A) H&E stain of excised porcine vascular graft in 1st transplantation (B) H&E stain of excised porcine vascular graft in 2nd transplantation. Compared to 1st transplantation, more vigorous phenotype are observed (C) Immunohistochemical stain of anti-CD68 in 1st and 2nd transplantation (D) Immunohistochemical stain of anti-myeloperoxidase in 1st and 2nd transplantation

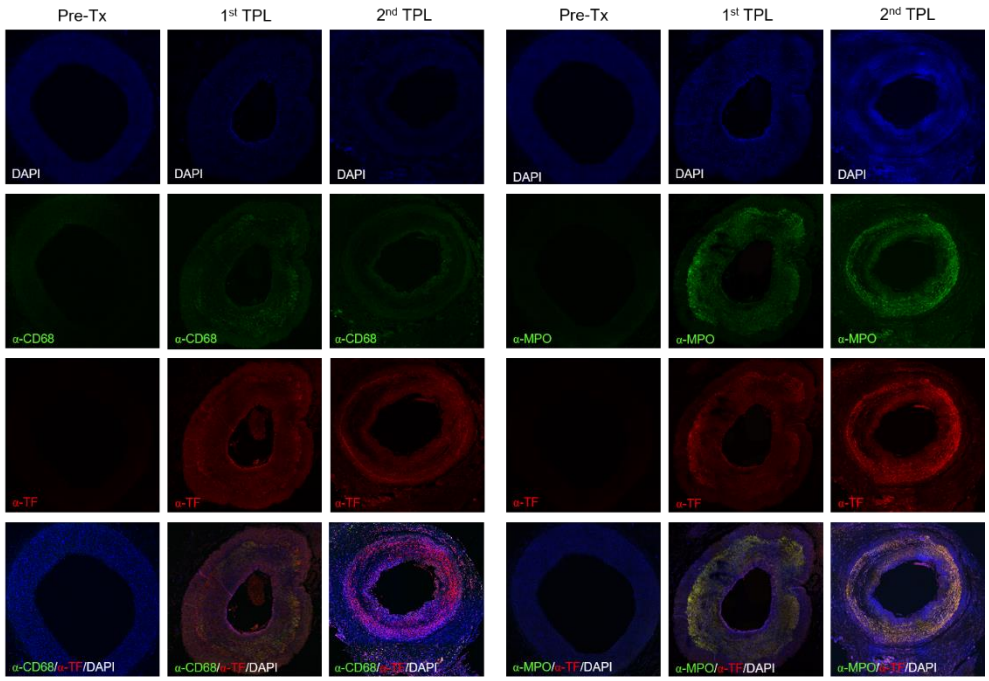


Figure 15. Immunofluorescence assay of CD68, myeloperoxidase, and tissue factor among 1st and 2nd xenograft.

Strong expression of myeloperoxidase and tissue factor are observed in tunica media and adventitia of excised porcine artery conduit xenograft

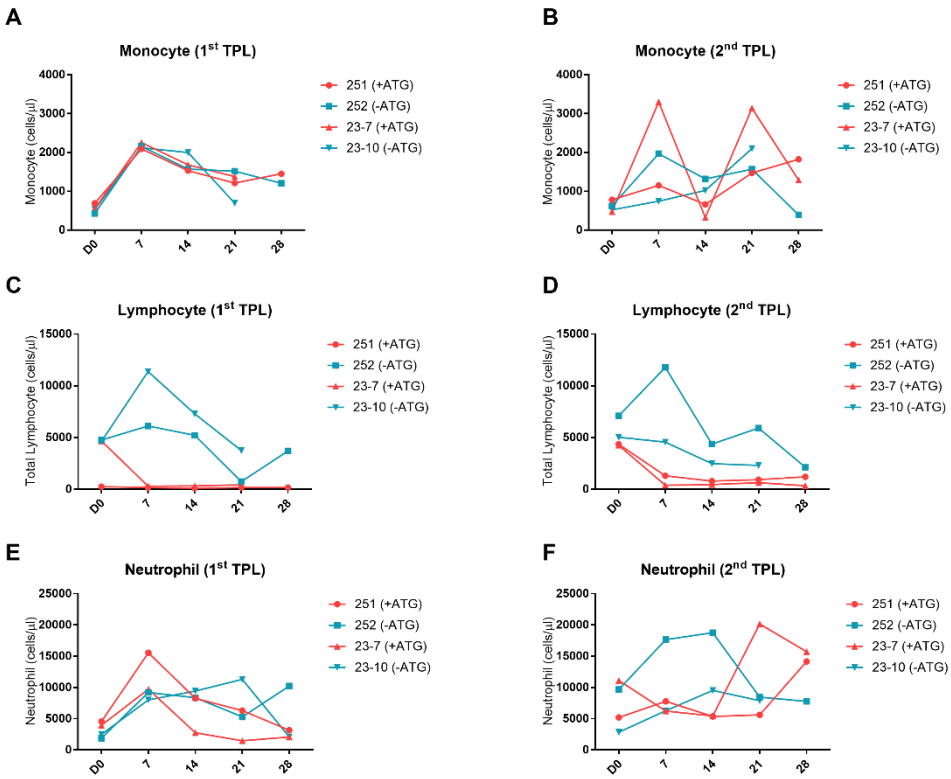


Figure 16. Peripheral circulating cell monitoring of GTKO pig artery transplantation in Cynomolgus monkey

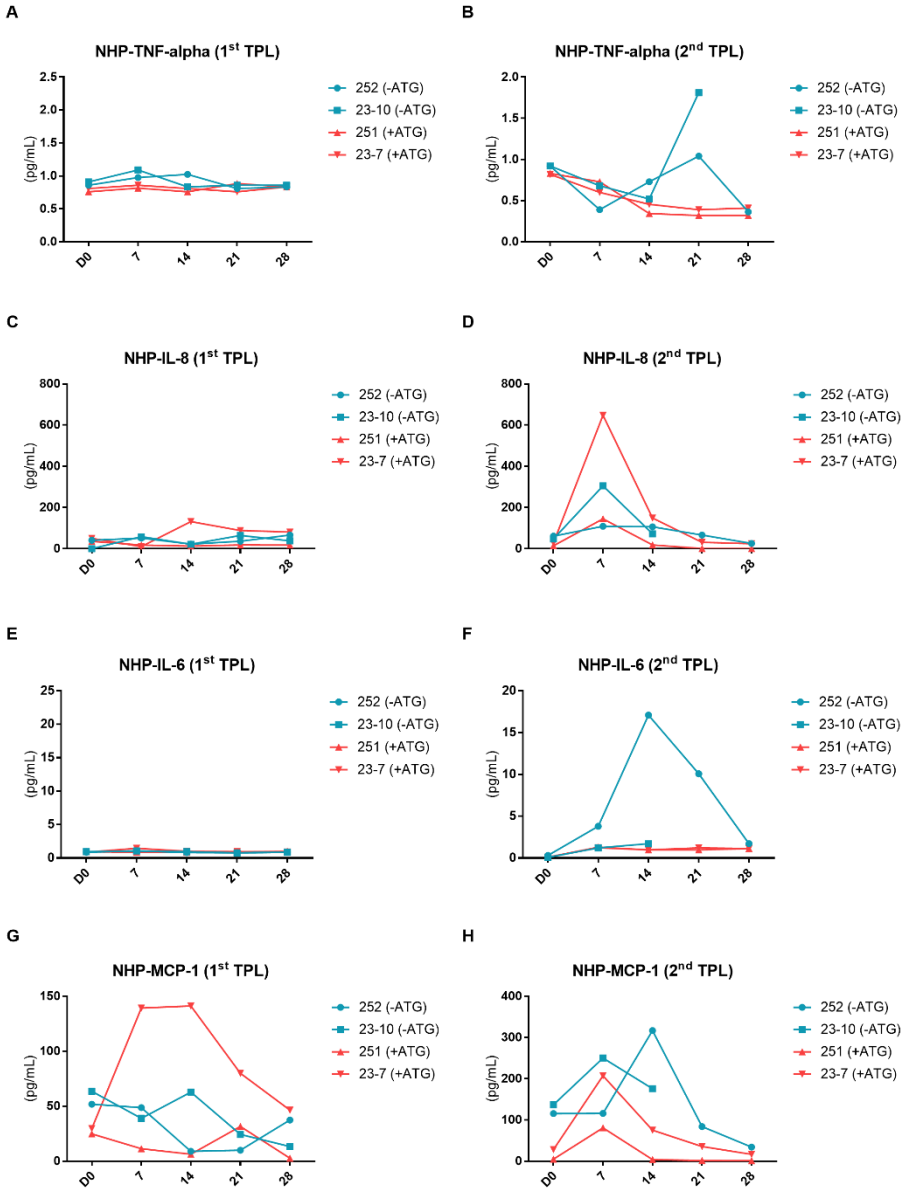


Figure 17. Longitudinal cytokine follow up of GTKO pig artery transplantation in Cynomolgus monkey

Statistical significance were achieved at day 14 of IL-6 in 2nd transplantation (P-value < 0.001) , and at at day 7 of IL-8 in 2nd transplantation. (P=0.017)

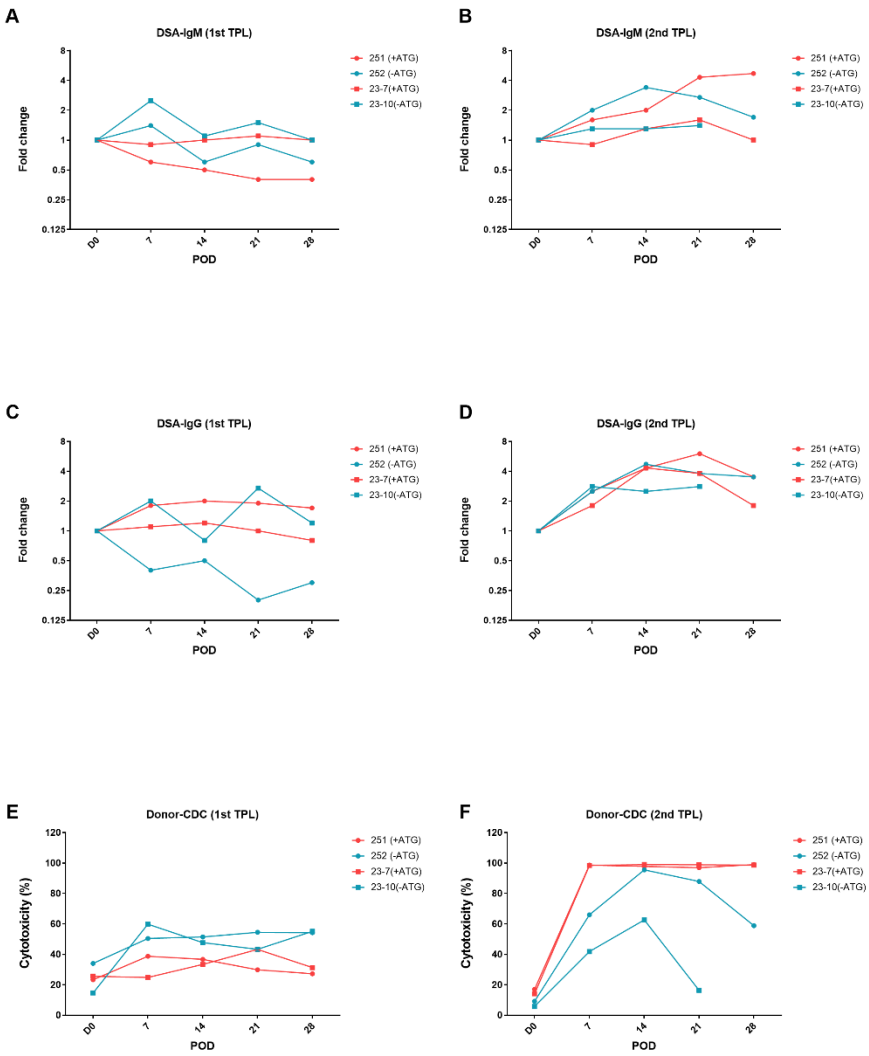


Figure 18. Trend of immunoglobulin M, immunoglobulin G and complement dependent cytotoxicity

Complement dependent cytotoxicity was strongly enhanced at the second transplantation.

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한국 장기이식 코호트 (KOTRY) 에서 신이식의 예후 분석 및 항체매개 거부반응의 영장류 혈관이식 모델 개발

배경

항체 매개 거부 반응은 동종 이식의 측면에서 장기 이식신 실패의 유의한 위험 요인이며, 고도 감작 이식에서의 극복해야할 대상이다. 한편, 신장이식의 경과를 통계학적으로 충분한 검정력을 가지고 파악하기에는 다기관 연구, 레지스트리 구축이 필요하다. 주조직항원의 에플렛은 항체매개 거부반응의 중요한 타겟 분자이다. 이종 이식에서 항체 매개 거부반응은 공여자 돼지의 유전자 조작을 통하여 극복되고 있으나, 자연 항체 및 유도 항체에 의한 면역의 감작 현상들은 여전히 중요한 도전과제이다. 영장류 이식 모델에서 항체 매개 거부반응을 연구하는데 한 가지 장애요인은 모델 형성의 기술적 난이도인데, 이는 현재 돼지 동맥 패치 모델을 통해서 개선되고 있다.

방법

이식 후 초기 성적의 예측 인자 연구 및 에플렛의 임상적 효용성 연구를 위해 한국 장기이식 코호트 (KOTRY) 의 자료가 이용되었다. 2014 년부터 2018 년까지 신장이식을 받은 환자가 등록되었다. 변수 선택법으로 라소 (LASSO), 후진 소거법을 이용하였고, 우세분석을 활용하여 선택 변수간의 상대적인 중요도를 서열화하여 우세 요인을 도출하였다. 에플렛 불일치는 HLA 하플로타입 분포의 매칭을 통하여여 HLA 4 자리수로 변경하여 추정하였다. 에플렛 불일치의 급성 거부반응과의 상관관계 모델링으로는 비선형 모형으로 분해 다항식 모형을 활용하였다. 항체매개성 거부반응의 기전 연구를 위하여 이종 이식

모형이 이용되었다. 이종재이식 모형으로 Gal 유전자 적중 돼지의 동맥편을 항-CD154 항체 및 면역억제제 3 제 요법 하에서 항-치모글로불린 유무에 따라 마카카 원숭이에 이식한 이종혈관이식 모형을 활용하였다.

결과

총 4,839 명의 한국장기이식코호트 신장 이식 수여자의 자료에서, 환자의 생존율은 각각 1 년째 98.4%, 3 년째 97.8%, 5 년째 97.6% 였다. 사망중도절단 이식신 생존율은 각각 1 년째 98.4%, 3 년째 97.0%, 5 년째 96.9% 였다. 생검으로 확인된 급성 거부반응이 없는 생존율은 각각 1 년째 90.3%, 3 년째 87.6%, 5 년째 87.3% 였다. T 세포 매개성 급성 거부반응이 없는 생존율은 각각 1 년째 92.8%, 3 년째 91.0%, 5 년째 90.6%였다. 급성 항체 매개성 거부반응이 없는 생존율은 각각 1 년째 96.5%, 3 년째 95.2%, 5 년째 95.2% 였다. 한국 장기이식 코호트에서 도출된 1 년 이내 초기 거부반응의 가장 우세한 예후 인자로서는 공여자 연령, HLA 불일치 개수였다. 1 년 이내 급성 항체매개성 거부반응의 가장 우세한 예후 인자로는 탈감작 여부, ATG 유도 요법, HLA 불일치 개수 였다. 60 개 이상의 에플렛 불일치는 HLA 유전자위 불일치가 2 개 이하로 적은 세부 그룹의 경우에 독립적인 급성 거부반응의 예측 인자였고, Class II 에플렛 불일치가 생검으로 확인된 급성 T 세포 매개 거부반응의 두드러진 유의한 예측인자였다. 돼지 동맥 패치 모델에 비해 본 돼지 동맥 혈관편 모델은, 혈관편의 기능 모니터링 (청진, 도플러 초음파)과 안전한 이식편 제거가 가능하여, 이종 이식에서 독특한 감작 연구를 가능하게 한다. 이종 혈관 재이식 모형에서 재이식 시의 혈중

인터류킨 6 의 상승, 보체 매개성 세포 독성의 증가와 이식 혈관편내의 조직 인자의 증가 및 이식 편의 증가된 거부반응을 관찰할 수 있었다.

결론

결론적으로, 본 연구에서는 한국장기이식코호트 자료를 활용하여 급성 거부 반응의 우세 인자를 확인하였고 에플렛 불일치의 임상적 효용을 확인하였다. 이종 재이식 모형을 통하여 감작 거부 반응에서의 항체 매개성 거부 반응의 증대 및 이의 보체 의존 세포독성, 말초 혈액 내 IL-6 의 상승, 이식 편 내의 조직인자 발현과의 관련성을 확인하였다.

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주요어: 장기이식 코호트, 장기이식 레지스트리, 항체매개성 거부반응, 신장이식, 영장류 혈관편 이식

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