STRATEGIES TO REDUCE SERIOUS INFECTIONS IN HIGH-RISK SOLID ORGAN TRANSPLANT RECIPIENTS

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ABSTRACT

Solid organ transplant (SOT) recipients endure frequent serious infections owing to multifactorial immunosuppression. This population, however, contains subgroups with distinctive immunodeficiencies including persons with HIV (PWH) and those with poor vaccine responses for whom infection risks may differ. This dissertation focuses on identifying high-risk phenotypes and strategies to reduce infections including immunosuppressive optimization and targeted prophylaxis.

The first work analyzes data from the HOPE in Action trials of HIV-donor-to-HIV-recipient SOT (Chapter 2) to elaborate the profile of donors with HIV. Certain coinfections (hepatitis B, syphilis, cytomegalovirus) were more prevalent among donors with HIV. Most donors were taking effective antiretroviral therapy (ART); 20% showed severe immunosuppression. Although HIV drug resistance mutations were frequent, resistance that might evade standard recipient ART was rare (2%). Overall, a minority of donors appeared high-risk for opportunistic infection transmission, though prophylaxis can be optimized.

The second work analyzes a national registry of 1225 PWH undergoing kidney transplant (HIV+ KT) (Chapter 3) evaluating the relationship between corticosteroid maintenance and organ rejection, treatment of which predisposes to infection. Early steroid withdrawal (ESW) varied widely among centers (0-90%), and was associated with increased rejection (18.4% vs 12.3% at one year; aHR:$_{1.02}$1.39$_{1.90}$, p=0.03); graft failure and mortality were not increased. Tailoring ESW to lower alloimmune risk PWH may mitigate the HIV+ KT infection-rejection cycle.

The final work (Chapter 4) is a clinical trial evaluating immunoprotection phenotypes following third SARS-CoV-2 mRNA vaccination in high-risk KT recipients. Preceding negative SARS-CoV-2-spike antibody (anti-RBD) after two-dose vaccination was strongly associated with poor responses to third doses; 45% remained seronegative, none showed Omicron variant neutralization. Immunogenicity varied considerably; 9% showed negative global (anti-RBD/T cell) responses, associated with high-dose mycophenolate use, while 40% showed global positive response; SARS-CoV-2-spike-specific CD4+ T cell expansion appeared necessary, but not sufficient for anti-RBD response. Breakthrough infections occurred in 16%, concentrated among poor anti-RBD responders, emphasizing need for alternative vaccine strategies to improve post-transplant COVID-19 immunoprotection.

These findings reinforce complementary roles of observational and trials datasets in understanding infection risks in SOT subpopulations. The COVID-19 pandemic highlights needed rigor for evaluating immunodeficiencies in generating personalized strategies to prevent serious post-transplant outcomes.

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LIST OF ABBREVIATIONS

Ab	antibody
Ab/Ag	antibody/antigen
aHR	adjusted hazard ratio
Anti-RBD	anti-receptor binding domain antibody
Anti-RBD^{LO}	low-titer anti-receptor binding domain antibody
Anti-RBD^{NEG}	negative anti-receptor binding domain antibody
aOR	adjusted odds ratio
AR	acute rejection
ART	antitretroviral therapy
ATG	anti-thymocyte globulin
AUC	area under the curve
CC	chemokine coreceptor
CMV	cytomegalovirus
cPRA	calculated panel reactive antibody
DAA	direct-acting antiviral
DCD	donation after circulatory death
DGF	delayed graft function
DRM	drug resistance mutation
ESRD	end-stage renal disease
ESW	early steroid withdrawal
FP	HIV false-positive donor
FSGS	focal segmental glomerulosclerosis
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV D+/R+	donor HIV+, recipient HIV+
HIV+	Human Immunodeficiency Virus-infected
HLA	human leukocyte antigen
IAS-USA	International Antiviral Society-USA
IL2R	interleukin 2 receptor
INSTI	integrase strand transfer inhibitor
IQR	interquartile range
KDIGO	Kidney Disease Improving Global Outcomes
KDPI	kidney donor profile index
KT	kidney transplant
KTR	kidney transplant recipient
LOS	length of stay
LT	live transplant
mAb	monoclonal antibody
MHC	major histocompatibility complex
MMF	mycophenolate
MSM	men who have sex with men
nAb	neutralizing antibody
NAT	nucleic acid testing
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor

OI	opportunistic infection
OPO	organ procurement organization
OPTN	Organ Procurement and Transplantation Network
PBMC	peripheral blood mononuclear cell
PI	protease inhibitor
PWH	people with HIV
RR	risk ratio
SC	steroid continuation
SRTR	Scientific Registry of Transplant Recipients
TAF	tenofovir alafenamide
TCR	T cell receptor
U/mL	units per milliliter
UNOS	United Network for Organ Sharing
VL	viral load

Chapter 1. Introduction

Solid organ transplantation (SOT) is a lifesaving procedure and the treatment of choice for irreversible end organ damage.[1] This practice is made possible through complex immunosuppressive regimens which protect against rejection of the transplanted organ, yet simultaneously impair multiple arms of the immune system[2] and thus increase the risk for serious infections. As a consequence, serious infections are a leading cause of morbidity and mortality after organ transplantation,[3,4] owing to both "typical" infections associated with health care exposure, as well as "opportunistic" infections that are related to unique deficiencies in an individual's immune armamentarium.[5]

The adage "an ounce of prevention is worth a pound of cure" holds true in the SOT population, who exist at the intersection of major medical comorbidities and multifactorial immunosuppression. Cornerstones of preventing serious infections in SOT recipients include understanding the landscape of serious infections across the population and identification of highest risk phenotypes. This facilitates targeted monitoring, antimicrobial prophylaxis, as well as the use of passive and active immunization (i.e., vaccination). Decades of clinical experience have formulated a framework for understanding the typical tempo and spectrum of infections following SOT and inform current guidance regarding prevention.[6,7]

Due to staggering advances in the science of transplantation, however, the breath of potential SOT candidates has expanded with respect to medical histories and

comorbidities now deemed appropriate. This has led to the development of subpopulations with distinctive immune profiles and deficits, with the potential for differing spectrum, timing, and consequences of serious infections after transplant. For these unique individuals, few and scattered among transplant centers, it is necessary to use national data to define risk factors and generate targeted guidance for best practices to prevent infectious complications. Creative analytical approaches using available observational data[8,9] and, if available, clinical trial data, are often required to quantify and visualize outcomes in these subgroups.

Transplant Recipients with Human Immunodeficiency Virus Infection (HIV+ SOT)

A group of particular interest is the growing population of people with HIV (PWH) who undergo solid organ transplantation (HIV+ SOT).[10] Before the era of effective antiretroviral therapy (ART), PWH were not considered candidates for transplant, despite suffering high rates of kidney failure.[11] This exclusion was due to the nearly universally fatal combination of uncontrolled HIV infection and transplant medication-related immunosuppression.[12,13] With later advances in HIV care, landmark trials in the early 2000s[14] showed that this practice was indeed feasible for PWH, and provided a significant survival benefit. Several barriers to optimal outcomes, however, did emerge, including complications related to viral coinfections, particularly hepatitis C,[15] as well as two-fold higher high rates of organ rejection,[16,17] the treatment of which leads to an intensified state of immunosuppression and increased risk for opportunistic infections with severe consequences. Immunosuppressive optimization in HIV+ SOT is therefore an attractive modifiable risk factor in reducing the morbid rejection-infection cycle.[18]

Moreover, due to impressive success of HIV+ SOT and the ongoing serious organ shortage,[19] use of organs from donors with HIV recipients with HIV (i.e., HIV-to-HIV transplantation) emerged as a promising frontier with an uncertain infectious risk profile.[20] In the United States, the HIV Organ Policy Equity (HOPE) Act was passed in 2013 and eventually enacted in 2015 to permit the use of organs from donors with positive HIV testing for recipients with HIV under research protocol.[21] This introduced yet another potential variable when assessing risks for serious infection after transplant, due to the potential for transmission events between immunocompromised donor and recipient. HIV-to-HIV transplantation is currently under study through the national HOPE in Action trials group (ClinicalTrials.gov NCT02602262; NCT03500315; NCT03734393).

Despite major potential differences in the landscape of serious infections in HIV+ SOT recipients, current best practices for monitoring and prevention are primarily derived from experience with PWH without transplant, or standard prophylactic and monitoring protocols for the general SOT population.[22] This disconnect underlies the rationale for dedicated study of risk factors for serious infection in HIV+ SOT recipients as means to counsel patients and providers as well as tailor prophylactic protocols.

Frame Shift: The Specter and Reality of Coronavirus Disease 2019 (COVID-19) in SOT Recipients

Like much of medicine and broader society, discussions of infectious complications and risk thereof changed fundamentally in the era of the Coronavirus Disease 2019 (COVID-19)

pandemic. SOT recipients have been described as the "sentinel chicken" for serious infectious diseases in the community[23] and this was indeed the case during the COVID-19 pandemic; although the risk of many common infectious complications remained, COVID-19 had an outsized impact on the practice of transplantation as well as profound loss of life in the SOT population, with case fatality rates approaching 20%.[24,25]

With the advent of highly immunogenic and effective Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) vaccines in late 2020 and early 2021, SOT recipients and their providers finally saw a path toward reduction of infection and severe COVID-19. Unfortunately, it soon became evident that the vaccines were significantly less immunogenic (i.e., produced lower antibody and cellular responses) among some SOT recipients; antibody (anti-spike or anti-receptor binding domain [RBD]) seroconversion rates were only 55% following the two-dose mRNA vaccine series,[26] accompanied by nearly 500-fold increase in clinically-significant vaccine breakthrough versus rates reported in clinical trials.[27] Accruing data indicated that additional vaccine doses might ameliorate some of these vaccine responses,[28] yet among SOT recipients who demonstrated poor response to the initial series, antibody response varied[29,30] and levels of immunoprotection were uncertain.

Amid the ongoing global health emergency, the National Institutes of Health (NIH) deemed it a priority to explicitly examine the degree to which alternate SARS-CoV-2 vaccines strategies could augment immune responses in highest-risk SOT recipients, leading to development of the COVID-19 Protection After Transplant (CPAT) Trials (launched in August 2021)[31] (NCT04969263; NCT05077254).

Scope of the Dissertation: Strategies to Reduce Serious Infections in High-Risk SOT Recipients

The paradigm of delineating high-risk phenotypes and tailoring interventions to reduce infection burden holds across different SOT subgroups and is the focus of this dissertation. The outlined works use a combination of observational and trials data to assemble and study cohorts of SOT recipients with unique immune profiles, namely PWH (Chapters 2 and 3) and poor antibody responders to SARS-CoV-2 vaccines (Chapter 4), in assessing infection risks and strategies to reduce serious outcomes.

Specifically, Chapter 2 leverages the national multicenter HOPE in Action trial of HIV-to-HIV transplantation to assess the potential unique infectious risks associated with receipt of an organ from a donor with positive HIV testing. As complement, Chapter 3 details a national transplant registry analysis of HIV+ SOT recipients to specifically assess whether a common immunosuppressive strategy, the withdrawal of corticosteroids early after transplant, is associated with increased kidney rejection. Chapter 4 addresses the central impact of the COVID-19 pandemic on SOT recipients in detailing the CPAT pilot trial of an additional (third) dose of SARS-CoV-2 mRNA vaccine in KT recipients who demonstrated poor response to the two-dose series, defining phenotypes of vaccine response and assessing immunoprotection.

Chapter 2. National Landscape of Human Immunodeficiency Virus-Positive Deceased Organ Donors in the United States

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ABSTRACT

Background: Organ transplantation from donors with HIV to recipients with HIV (HIV D+/R+) presents risks of donor-derived infections. Understanding clinical, immunologic, and virologic characteristics of HIV+ donors is critical for safety.

Methods: We performed a prospective study of donors with confirmed HIV-positive (HIV+) and HIV false-positive (FP) testing within the HOPE in Action studies of HIV D+/R+ transplantation (ClinicalTrials.gov NCT02602262; NCT03500315; NCT03734393). We compared clinical characteristics in HIV+ versus FP donors. We measured CD4+ T cells, HIV viral load (VL), drug resistance mutations (DRMs), co-receptor tropism, and serum antiretroviral therapy (ART) detection using mass spectrometry in HIV+ donors.

Results: Between 03/2016-03/2020, 92 donors (58 HIV+, 34 FP), representing 98.9% of all US HOPE donors during this period, donated 177 organs (131 kidney, 46 liver). Each year the number of donors increased. Prevalence of hepatitis B (16% vs. 0%), syphilis (16% vs. 0%), and cytomegalovirus (91% vs. 58%) was higher in HIV+ versus FP donors; hepatitis C viremia was similar (2% vs. 6%). Most HIV+ donors (71%) had known HIV diagnosis, of whom 90% were prescribed ART and 68% had VL <400 copies/mL. Median CD4 count was 194 cells/uL (IQR=77-331); median CD4% was 27.0 (IQR=16.8-36.1). Major HIV DRMs were detected in 42%, including non-nucleoside reverse transcriptase inhibitors (33%), integrase strand transfer inhibitor (INSTI,
4%), and multiclass (13%). Serum ART was detected in 46% and matched ART by history.

Conclusion: Utilization of HIV+ donor organs is increasing. HIV DRMs are common, yet resistance that would compromise INSTI-based regimens is rare, which is reassuring regarding safety.

INTRODUCTION

Transplantation of organs from donors with HIV to recipients with HIV (HIV D+/R+) was pioneered in South Africa in 2008.[32] The 2013 HIV Organ Policy Equity (HOPE) Act permitted HIV D+/R+ in the United States, with an initial focus on liver and kidney transplantation.[21,33,34] Theoretical risks associated with this practice include donor-derived opportunistic infection (OI) and superinfection with drug-resistant HIV.[35,36] These complications have not been observed in South Africa, however this was in the setting of a primarily antiretroviral therapy (ART)-naïve donor population (87%), and recipients were generally on protease inhibitor (PI)-based recipient ART which may overcome HIV drug resistance mutations (DRMs).[37] In the US, the risk profile of HIV+ donors may be higher given differing HIV acquisition behaviors associated with transmitted drug resistance (e.g., men who have sex with men [MSM]),[38] more prevalent coinfections (e.g., hepatitis C [HCV]),[39,40] and higher ART utilization and circulating DRMs (<10% in South Africa versus 20-40% in the US).[41-46] Transient detection of donor HIV strains, including presence of DRMs, has been described in cases of HIV D+/R+ transplantation in high-income countries.[47,48]

Understanding the risk profile of the US HIV+ donor population is critical for transplant providers who make time-constrained, point-of-care decisions to accept organs for HIV+ candidates and may not have access to immunologic data or HIV genotypes for individual donors. The objective of this study was to describe clinical, virologic, and immunologic characteristics of donors under the HOPE Act, with focus on ART use and HIV DRMs, to characterize the safety profile of donors in HIV D+/R+ transplantation.

METHODS

Study Population

Deceased donors with reactive HIV tests or HIV history who had organs recovered for transplantation to HIV+ recipients from 03/01/2016-03/15/2020 within the HOPE in Action studies (ClinicalTrials.gov: NCT02602262; NCT03500315; NCT03734393) were included. HIV screening assays were performed per Organ Procurement and Transplantation Network (OPTN) protocols[49,50]: HIV antibody (Ab), antibody/antigen (Ab/Ag), nucleic acid testing (NAT).

Donors could not have active OI and the transplant team had to anticipate an "effective, safe, and tolerable" recipient ART regimen.[51] There were no restrictions on donor HIV VL or CD4. Donors with discordant Ab/NAT testing and no known history of HIV infection were suspected to have false-positive HIV tests ("FP donors").[52] For these donors, confirmatory testing was performed by the OPO or research team using Western blot or fourth-generation HIV Ag/Ab (if Ab+) and/or quantitative HIV viral load (VL) (if NAT+).

This study was considered exempt from Human Subjects Research by the Johns Hopkins Institutional Review board as it only included data and biospecimens from decedents. Authorization for donation including collection of biospecimens for research purposes was confirmed by Organ Procurement Organizations (OPOs), in accordance with federal regulations.

National Enumeration of Donors with Reactive HIV testing

To determine the total number of donors with reactive HIV testing who completed organ donation during the study time period, we used the Scientific Registry of Transplant Recipients (SRTR) which includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by members of the OPTN. The Health Resources and Services Administration, U.S. Department of Health and Human Services provides oversight to OPTN and SRTR contractors. Within limited datasets released by OPTN/SRTR, each donor is assigned an anonymous identifier; the record includes infection serostatus. Donors with reactive HIV tests were identified as having "positive" HIV Ab and/or HIV NAT. SRTR identifiers were compared to those in the HOPE in Action database to confirm a match. HIV history and FP confirmatory assay results were not in SRTR.

Donor Characteristics

OPOs collected demographics, comorbidities, social history, serologies, and administered medications. An additional HIV Medical History Form was completed to obtain (if available) HIV diagnosis date, acquisition risk(s), ART experience, OI history, and laboratory data (e.g., CD_4, VL, genotype/phenotype). HIV provider notes were also obtained if available.

Laboratory Testing

All donors had serology testing per OPTN[50] for hepatitis B (surface Ag, core Ab), hepatitis C (Ab, NAT), and HIV (Ab, Ab/Ag, NAT). Donors were predominantly screened using anti-HIV I/II Abs (enzyme-linked immunosorbent or chemiluminescent assay) and

multiplex qualitative NAT testing for HIV/HCV/HBV (Supplemental Table 1). Additional serologies included cytomegalovirus (IgG), Epstein-Barr virus (IgM/IgG/EBNA), syphilis (RPR), and toxoplasma (IgG). These results were available to providers during donor evaluation.

For HOPE donors, 100cc of blood was collected to measure CD4 cells, HIV VL (Abbott RealT*ime* HIV-1 assay, limit of detection 40 copies/mL), sequencing for DRMs (GenoSure PRIme®/Archive® assays), and chemokine coreceptor (CC) tropism (Trofile® RNA assay) (Monogram Biosciences, San Francisco, CA). These results were not available in SRTR and were performed within the HOPE in Action studies. They were also not available to providers in real time to inform clinical care.

Major DRMs were defined per the International Antiviral Society-USA (IAS-USA)[53] and Stanford University HIV Drug Resistance Database.[54] Multiclass resistance was defined as ≥1 major DRM per IAS-USA versus >1 drug class.

To detect serum ART, liquid chromatography-tandem mass spectrometry (QExactive; Thermo Fisher Scientific, San Jose, CA) was performed for 22 drugs[55]: abacavir, amprenavir, atazanavir, darunavir, dolutegravir, efavirenz, elvitegravir, emtricitabine, indinavir, lamivudine, lopinavir, maraviroc, nelfinavir, nevirapine, raltegravir, rilpivirine, ritonavir, saquinavir, stavudine, tenofovir, tipranavir, zidovudine (limit of detection: 10 ng/mL). Bictegravir, cobicistat, and doravirine were not assayed.

Statistical Analysis

Demographic and transplant factors were summarized and compared between HIV+ and FP groups. Summary statistics were expressed as median (interquartile range) for continuous variables and counts (percent) for categorial variables. Continuous variables were compared using Wilcoxon rank-sum testing and categorical variables compared using Chi-squared or Fisher's exact testing. Analyses were performed using Stata/MP2_v16.1 (StataCorp, College Station, TX).

RESULTS

Counts and Regional Distribution of HOPE Donors

Ninety-two donors (58 HIV+, 34 FP) donated 177 organs (131 kidney, 46 liver) to HIV+ recipients at 24 transplant centers. During the study period, OPTN/SRTR reported 92 donors with any reactive HIV test, of which 91 were confirmed in our cohort. One FP donor in our cohort was identified by medical history from next of kin yet had negative HIV testing, whereas one donor in SRTR with reactive HIV testing was transplanted outside of the HOPE in Action Consortium. Thus, this series encompassed 98.9% of the US HIV+/FP donor total.

The number of donors increased each year (Figure 1a). There was ≥1 HIV+ donor in each of the 11 United Network for Organ Sharing (UNOS) regions, including 26 donors (18 HIV+, 8 FP) in southeast regions 3 and 11 (Figure 1b).

Donor Characteristics: HIV+ versus False Positive

Donor characteristics are shown in Table 1. Median age was 36 years (IQR 29-46) in HIV+ versus 31 years (IQR 23-41) in FP donors (p=0.01). Sex was 76% male in HIV+ versus 65% in FP (p=0.25); race was 37% white in HIV+ versus 44% in FP (p=0.21). Median BMI was 25.2 (IQR 22.7-29) in HIV+ and 28.4 (IQR 22.1-34.8) in FP (p=0.11). Drug intoxication was the cause of death in 26% of HIV+ and 12% of FP (p=0.11). HCV NAT was positive in 2% of HIV+ and 6% of FP (p=0.11). Hepatitis B (HBV) core Ab was positive in 16% of HIV+ and 0% of FP (p=0.02). Cytomegalovirus IgG was positive in 91% of HIV+ and 58% of FP (p<0.001). Syphilis (RPR) was positive in 16% of HIV+ and 0% of FP (p=0.02). Median kidney donor profile index (KDPI), a marker of organ quality with lower number signifying better quality, was 41 (IQR 30-63) in HIV+ and 36 (IQR 21-60) in FP, (p=0.2). There were fewer donations after circulatory death (7% vs. 21%, p=0.05) and more corticosteroid administration (72% vs. 55%, p=0.08) in HIV+ versus FP donors, respectively.

Donor HIV Testing

All HIV+ donors had reactive anti-HIV Ab at donation; 69% had reactive qualitative NAT (Table 2). FP donors predominantly had isolated reactive anti-HIV Ab (79%), whereas 15% had isolated reactive multiplex NAT. One FP donor had a false-positive Ab/Ag test. The FP donor identified by erroneous medical history had both negative HIV Ab and NAT. All FP donors, by definition, had negative confirmatory testing (Supplemental Table 1).

Donor HIV History, Risk Factors, and ART experience

Of HIV+ donors, 71% had prior known HIV infection, versus 24% discovered at admission (Table 2). HIV acquisition risk factors included men who have sex with men (43%),

injection drug use (22%), and heterosexual sex (28%). OI history was unknown in 67%. Three donors had prior OIs: complicated HSV infection (acyclovir-resistant genital ulcers) and cryptococcosis; pneumocystosis; cytomegalovirus disease and HSV esophagitis.

Most HIV+ donors (64%) were prescribed ART (Table 3a), including 90% of those with known HIV diagnosis. ART history was unavailable for six (10%) donors, two of whom had undetectable VLs. The most common regimens included two nucleoside reverse transcriptase inhibitors (NRTIs) plus an integrase strand transfer inhibitor (INSTI) (65%). Overall, 28 (77%) of donor regimens included INSTIs, whereas eight (22%) included protease inhibitors (PIs), and three (8%) included non-nucleoside reverse transcriptase inhibitors (NNRTIs).

HIV Viral Control

Quantitative HIV VL was performed on all 58 HIV+ donor samples (Table 2). Overall, median VL was 882 copies/mL (IQR <40-20,417) and 47% of donors had VL<400 copies/mL.

Stratifying by ART treatment status, median VL was <40 copies/mL for those on ART versus median HIV VL 20,417 copies/mL for those not on ART. Among those on ART, 27% had VL>1000 copies/mL; compared to those with suppressed VL, clinical characteristics were similar (median age 36 vs. 43 years; p=0.38; male sex 83% vs. 72%, p=0.45; Black race 42% vs. 48%, p=0.6), apart from a trend towards more MSM (67% vs. 36%, p=0.08).

CD4 T Cell Counts and Percentages

CD4 T cell measurements were performed on 53 HIV+ donor samples. Overall, median CD4 count was 194 cells/uL (IQR 77-331) and 51% of donors had absolute CD4<200 cells/uL (Table 2). Median CD4% was 27.0% (IQR 16.8-35.4) and 22% of donors had CD4%<14. Historical CD4% was strongly correlated with donation CD4% (r=0.72, Supplemental Figure 1a), whereas historical absolute CD4 count was moderately correlated (r=0.43, Supplemental Figure 1b).

Stratifying by treatment status, median CD4 count was 262 cells/ul for those on ART and 118 cells/uL for those not on ART (p<0.01); median CD4% was 29.9% for those on ART and 17.2% (p=0.02) for those not on ART (p=0.02).

There were 11 donors with CD4%<14, all of whom were viremic with median VL 83,770 copies/mL (IQR 2,238-380,736). This included five donors with newly diagnosed HIV infection and three donors prescribed ART (VLs 1905; 2111; 51827 copies/mL). Geography, demographics, and acquisition risks were indistinguishable in these donors with low CD4% versus other donors (data not shown).

Discordance between Absolute and Percent CD4, Corticosteroid Administration

Notably, 59% of donors with CD4 count<200 cells/mL had discordant CD4%≥14 (Figure 2, upper left quadrant); no donor with CD4%<14 had CD4 count>200 cells/mL (Figure 2, lower right quadrant). In-hospital corticosteroid administration was higher in donors with CD4<200 cells/mL versus ≥200 cells/uL (81% vs 62%, p=0.11).

ART Detection by Mass Spectrometry

Mass spectrometry was performed on 54/58 (93%) HIV+ donor samples with detection of ≥1 ART drug in 25 samples (46%) (Table 3b). All samples with detectable ART were from donors documented as prescribed ART (25/34, 74%). Of the nine donors reported as prescribed ART in whom no ART was detected, four had HIV VL>10,000 copies/mL and the remainder had a median length of stay (LOS) of ten days before donation (IQR 6-11). ART was not detected in any of the seven donors reported as either not prescribed ART or unknown ART history. Percent agreement of historical versus laboratory ART detection was 83% (95% CI:49-86; kappa 0.67).

HIV Drug Resistance Mutations

HIV genotypes were successfully performed on 47 donation samples (81%); 11 (19%) assays failed, nine among donors with HIV VL<40. In addition to genotypes performed on blood at the time of organ donation ("laboratory genotypes"), we also collected prior genotype reports from the medical record which was available in 14 (24%) donors, including one for whom the laboratory genotyping failed.

Of donors with any genotype data, 20/48 (42%) had ≥1 major DRM (Table 4); eight donors had at least one historical DRM and 15 had at least one laboratory DRM detected. There were no significant differences between donors with and without DRMs in demographics, HIV acquisition risk factors, HIV VL, CD4 count/%, or ART exposure (data not shown). NNRTI resistance was common (33%), most frequently substitutions at the K103 position of the reverse transcriptase (RT) gene. Three donors had historical NNRTI DRMs, identical to those detected on laboratory genotyping.

NRTI mutations were detected in 19%, particularly M184V/I (10%). One donor had historical NRTI DRMs, confirmed on laboratory genotyping. Thymidine analogue mutations were detected in three donors (6%). One donor had multi-NRTI resistance (A62V+K65N).

INSTI mutations were seen in two donors (4%) on historical genotypes, only (T66I+E92Q; Y143C). Multiclass DRMs were detected in six donors (13%), all with NRTI resistance plus a second class, commonly NNRTIs. These donors were all reported as prescribed ART, typically with NRTI+INSTI or NRTI+PI backbone, and four (66%) were virologically suppressed. One donor with perinatal HIV acquisition demonstrated DRMs versus three drug classes (including INSTIs), with HIV VL<40 at donation on an NRTI+INSTI+PI regimen.

Viral Coreceptor Tropism

CC tropism testing was performed in 50 donors; the assay failed in 22 including in 16 donors with VL<40 copies/mL. Of 28 with reportable results, 19 (68%) showed R5 and nine (32%) mixed R5-X4 tropism. Among those with mixed tropism, median VL was 83,770 (IQR 15,488-410,407), CD4 count 80 (IQR 42-176), and CD4% 12.9 (IQR 4.6-20.9). No donor was reported as taking maraviroc.

DISCUSSION

In four years of the HOPE in Action studies, there were 92 deceased donors with reactive HIV screens who donated 177 organs to recipients with HIV. Among donors, 37% had FP

tests and 24% had new HIV diagnoses (24%) and of those with known HIV infection, most (90%) were prescribed ART. Major HIV DRMs were frequent, yet INSTI and multiclass DRMs were rare. Overall, this should be reassuring to providers who aim to minimize the risk of HIV breakthrough in potential recipients due to donor-derived INSTI or multiclass DRMs.

In contrast to South African HIV D+/R+ data where 8% of donors were ART-experienced and circulating DRMs ≤10%,[36,41] most (64%) US donors were ART-experienced and 42% had ≤1 DRM. NNRTI DRMs were common (33%), including mutations affecting second-generation drugs such as rilpivirine. This concords with transmitted NNRTI drug resistance patterns in the US, >10% in some populations,[46] as well as rising community prevalence of DRMs against this class (e.g., 23% in black MSM).[42,43,56] Thus, relying on NNRTIs as empirical primary backbone in US HIV D+/R+ transplantation appears unfavorable. Doravirine, however, may maintain activity against most detected NNRTI DRMs. Otherwise, multi-NRTI DRMs and TAMs were only seen in 8%, maintaining this class as active.

As in the greater US HIV population,[57,58] INSTI DRMs were uncommon in HOPE donors. Moreover, none were predicted to affect later-generation INSTIs such as dolutegravir or bictegravir. Multiclass DRMs were only detected in six donors (13%), two of whom were virologically suppressed on NRTI+INSTI regimens, and all of whom, based on available genotypes, would likely have achieved viral suppression on such regimens. One donor with perinatal HIV acquisition and extensive ART exposure demonstrated DRMs against three drug classes (including INSTIs) and had viral suppression on an NRTI+INSTI+PI

regimen. This rare case highlights the need for thorough HIV history ascertainment and indicates that broadly active ART (e.g., INSTI+PI) may be required in select circumstances. Finally, the frequency of R5-X4 tropic virus (32%) among HIV+ donors may limit CCR5 inhibitors as post-transplant ART in HIV D+/R+ transplantation.

Other features among HIV+ donors included frequent HBV, CMV, and syphilis seropositivity, approaching rates seen in the North American population of PLWH,[59,60] and higher than in FP donors or the general donor population.[49,61] Additionally, CD4 count/% discordance was observed in 31% of donors, potentially related to corticosteroid administration (73%), previously reported to be associated with CD4 lymphopenia in HIV-uninfected donors without affecting CD4%.[62]

Detailed HIV history was an important adjunct to OPTN questionnaires and serologies. A considerable proportion of donors had historical ART (64%) and genotype (24%) data, key to informing post-transplant ART selection. Mass spectrometry for ART exposure was largely concordant with medical record, showing 83% agreement, particularly in the setting of VL suppression. Of the nine donors prescribed ART who had no ART detected in serum, four had VL>10,000 copies/mL, consistent with non-adherence. The remainder had prolonged LOS, which, if holding enteral ART during critical illness, may have led to washout of drugs with short elimination half-life such as TAF,[63] which was rarely detected even in donors with VL suppression. Overall, medication ascertainment by OPO staff and transplant providers was a very good point-of-care metric to determine ART exposure and emphasizes the critical role of infectious diseases providers in risk stratifying donors in HIV D+/R+ transplantation.[64]

This study had several limitations. Donors were carefully selected given novelty of HIV D+/R+ transplantation and may not reflect the greater potential HIV+ US donor population. Similarly, there were only 92 donors over four years, far lower than projections (350-600/year),[65,66] possibly reflecting measured adoption of a new practice by OPOs and transplant centers, as well as stigma surrounding donor HIV disclosure and lagging registration for organ donation despite high willingness.[67] Regardless, annual donations quadrupled over time and occurred across all UNOS regions, with concentration in the southern US, overlapping the current HIV epicenter[68] and consistent with estimated HIV+ deceased donor distribution.[69] Due to missing historical genotype information, technical assay failure, and imperfect sensitivity of laboratory genotypes, we may have underestimated DRM prevalence in HIV+ donors. Additionally, we were unable to correlate donor DRMs with HIV breakthrough in HOPE recipients as the blinded studies are ongoing. Reassuringly, however, early studies of the South African[37] and US HIV D+/R+ cohorts[70] have not revealed conclusive donor HIV superinfection and the HIV D+/R+ pilot study noted only one case of HIV viremia, attributed to ART interruption rather than resistant donor virus.

This report highlights the promise of organ donation from deceased donors with HIV. As HIV D+/R+ transplantation expands, further characterization of HIV donors will focus on facilitating risk stratification to identify donors with problematic drug resistance (INSTI, multiclass DRMs) and permit post-transplant ART optimization for recipients.

Table 1: HOPE donor characteristics, compared between HIV+ and false-positive (FP) donors.

Donor Factor	Total n=92	HIV+ n=58	FP n=34	*p*-value[a]
Age, years	33.0 (28.0, 44.0)	36.0 (29.0, 46.0)	31.0 (23.0, 41.0)	0.01
Male	67 (72)	44 (76)	22 (65)	0.25
Race				
White	37 (41)	22 (38)	15 (44)	0.21
Black	37 (40)	27 (47)	10 (29)	
Other	18 (19)	9 (16)	9 (26)	
BMI, kg/m^2	26.1 (22.7, 30.5)	25.2 (22.7, 29.0)	28.4 (22.1, 34.8)	0.11
Diabetes	9 (10)	5 (9)	4 (12)	0.64
Hypertension	25 (27)	16 (28)	9 (26)	0.87
CAD	3 (3)	1 (2)	2 (6)	0.29
Cause of Death				
Anoxia	38 (41)	27 (47)	11 (32)	0.53
Cerebrovascular	24 (26)	14 (24)	9 (26)	
Head Trauma	28 (30)	15 (26)	13 (38)	
Other	3 (3)	2 (3)	1 (3)	
Mechanism of Death				
Drug Intoxication	19 (21)	15 (26)	4 (12)	0.11
Cardiovascular	12 (13)	9 (16)	3 (9)	0.36
Suicide	9 (10)	7 (12)	2 (6)	0.33
Screening Serologies				
HCV Ab+	4 (4)	1 (2)	3 (9)	0.11
HCV NAT+[b]	3 (3)	1 (2)	2 (6)	0.56
HBV Ab+	9 (10)	9 (16)	0 (0)	0.02
CMV IgG+	72 (79)	53 (91)	19 (58)	<0.001
Toxoplasma IgG+	5 (6)	3 (6)	2 (7)	0.84
Syphilis (RPR)	9 (10)	9 (16)	0 (0)	0.02
Type of Organ Donated				
Kidney (≥1)	77 (84)	46 (79)	31 (91)	0.14
Liver	46 (52)	34 (59)	12 (35)	0.03
Kidney and Liver	31 (34)	22 (38)	9 (26)	0.17
KDPI, %	40 (28, 62)	41 (30, 63)	36 (21, 60)	0.20
DCD	11 (12)	4 (7)	7 (21)	0.05
Steroid given	60 (66)	42 (72)	18 (55)	0.08
PHS Increased Risk	62 (67)	47 (81)	15 (44)	<0.001

Continuous values reported as median (IQR) and categorical/binary variables reported as N (%)

[a] Comparisons are unadjusted.

[b] Two other FP donors had false-positive HCV screens by multiplex HIV/HCV/HBV NAT, with negative confirmatory quantitative PCR (omitted from table)

Abbreviations: Ab antibody, BMI body mass index, CAD coronary artery disease, CMV cytomegalovirus, DCD donation after circulatory death, HBV hepatitis B core Ab, HCV hepatitis C virus, IgG immunoglobulin G, KDPI kidney donor profile index, NAT nucleic acid test, PHS Public Health Service

Table 2: Donor HIV history, screening, and biology.

HIV Factor	HIV+ n=58	FP n=34
Reactive HIV Screening Assay[a] (n, %)		
Anti-HIV I/II Ab	58 (100)	27 (79)
HIV Qualitative NAT	40 (69)	5 (15)
Ab/Ag+	-	1 (3)
Confirmatory Rule-Out Assay (n, %)[b]		
Western Blot	-	25 (74)
Ag/Ab (4^{th} gen)	-	7 (21)
Quantitative PCR	-	4 (12)
Time of HIV Discovery (n, %)		
Prior knowledge	41 (71)	-
At admission	14 (24)	-
Unknown	3 (5)	-
HIV Acquisition Risk[c] (n, %)		
MSM	25 (43)	-
IVDU	13 (22)	-
Heterosexual sex	16 (28)	-
Perinatal	1 (2)	-
Other or unknown	16 (28)	-
Reported ART Use (n, %)		
Yes	37 (64)	-
No	15 (26)	-
Unknown	6 (10)	-
CD4 at Donation[d]		
Median CD4 count (cells/uL) (IQR)	194 (77, 331)	-
Median CD4% (IQR)	27.0 (16.8, 36.1)	-
CD4<200 (n, %)	27 (51)	-
CD4%<14 (n, %)	11 (22)	-
HIV VL at Donation[e]		
Median HIV VL (copies/mL) (IQR)	882 (<40, 20417)	-
Median Log HIV VL (IQR)	2.9 (1.0, 4.3)	-
HIV VL <400 (n, %)	27 (47)	-
CC Tropism[f] (n, %)		
R5	19 (68)	
Dual R5-X4	9 (32)	-
X4	0 (0)	-

[a] No FP donor had >1 positive screening assay and one screened positive by medical history only. All NAT+ FP donors were by multiplex qualitative assay (HIV/HBV/HCV). One FP donor had reactive HIV Ag (negative Ab.
[b] See Supplemental Table 1 for additional information; multiple rule-out assays were utilized.
[b] Risk categories not mutually exclusive. "Heterosexual sex" includes sex work and intercourse with sex workers.
[c] Donation CD4 counts available for 53 donors (CD4% for 51).
[d] VL <40 copies/mL was set at 10 copies/mL (1 log) for analysis.
[e] HIV tropism results among 38 donors with successful assays.

Table 3: Antiretroviral therapy regimens (A) reported for HOPE donors at donation and (B) detected by serum mass spectrometry.

Reported ART Regimen (n=37)	**n (%)**
2 NRTI + INSTI	**24 (65%)**
TAF/FTC/EVG/c	9
ABC/3TC/DTG	7
TAF/FTC/BIC	5
TAF/FTC + DTG	2
TDF/FTC/EVG/c	1
2 NRTI + NNRTI	**4 (11%)**
TAF/FTC/RPV	3
TDF/FTC + ETR	1
2 NRTI + PI	**3 (8%)**
TAF/FTC + ATV/c	1
TDF/FTC + ATV/c	1
TDF/FTC + DRV/c	1
Other	**6 (16%)**
FTC + DTG	1
3TC + DTG + DRV/r	1
TDF/FTC + DTG + ATV/r	1
ABC/3TC + TDF + DRV/c	1
d4t + TAF/FTC + DTG + DRV/c	1
TDF/FTC/EFV + DRV/r	1

Abbreviations: 3TC lamivudine, ABC abacavir, ART antiretroviral therapy, ATV atazanavir, BIC bictegravir, c cobicistat, d4t stavudine, DTG dolutegravir, EFV efavirenz, ETR etravirine, EVG elvitegravir, FTC emtricitabine, INSTI integrase strand transfer inhibitor, NRTI nucleoside reverse transcriptase inhibitor, NNRTI nonnucleoside reverse transcriptase inhibitor, PI protease inhibitor, r ritonavir, RTV ritonavir, TAF tenofovir alafenamide, TDF tenofovir disoproxil fumarate, VL viral load

Individual ART Drug	Detected in Serum[b] (Detected/Total)	Detected in Serum if HIV VL<400[c] (n=23) (Detected/Total)
NRTI		
3TC	7/8	6/6
ABC	2/6	1/5
d4T	0/1	0/1
FTC	15/26	11/17
TAF	1/20	1/14
TDF	3/6	2/3
NNRTI[a]		
RPV	0/2	0/2
PI[a]		
ATV	3/3	2/2
DRV	5/5	4/4
RTV	3/4	2/2
INSTI[a]		
EVG	6/10	4/5
DTG	9/11	8/9

[a] BIC, ETR, and cobicistat were not assayed by mass spectrometry
[b] Mass spectrometry unavailable for 3/37 donors with reported ART use, whose drug regimens are excluded from this table: ABC/3TC/DTG (1), TAF/FTC/RPV (1), TAF/FTC/BIC (1)
[c] HIV viral load (VL) available for 33/34 donors with mass spectrometry data and known ART regimens, including 23 with VL<400

Abbreviations: 3TC lamivudine, ABC abacavir, ART antiretroviral therapy, ATV atazanavir, BIC bictegravir, c cobicistat, d4t stavudine, DTG dolutegravir, EFV efavirenz, ETR etravirine, EVG elvitegravir, FTC emtricitabine, INSTI integrase strand transfer inhibitor, NRTI nucleoside reverse transcriptase inhibitor, NNRTI nonnucleoside reverse transcriptase inhibitor, PI protease inhibitor, r ritonavir, RTV ritonavir, TAF tenofovir alafenamide, TDF tenofovir disoproxil fumarate, VL viral load

Table 4: (A) Major and (B) multiclass drug resistance mutations among HIV+ HOPE donors.

Donors with Major DRMs (n, %)				
Total 20 (42%)[a]	**NRTI** 9 (19%)	**NNRTI** 16 (33%)	**PI** 1 (2%)	**INSTI** 2 (4%)
All Detected DRMs[b]	M184V/I (5) D67N/G/E/H/S/T (2) M41L A62V K65R/N/E L74V/I T215Y/F/C/D	K103N/S/H/T/R/Q/E (8) V179D/E/F/I/L/T (6) V108I (2) L100I/V K101E/H/P/Q/R/N V106A/M/I Y181C/I/V/S/G	L90M	T66A/I/K E92Q/G/V Y143Y/C

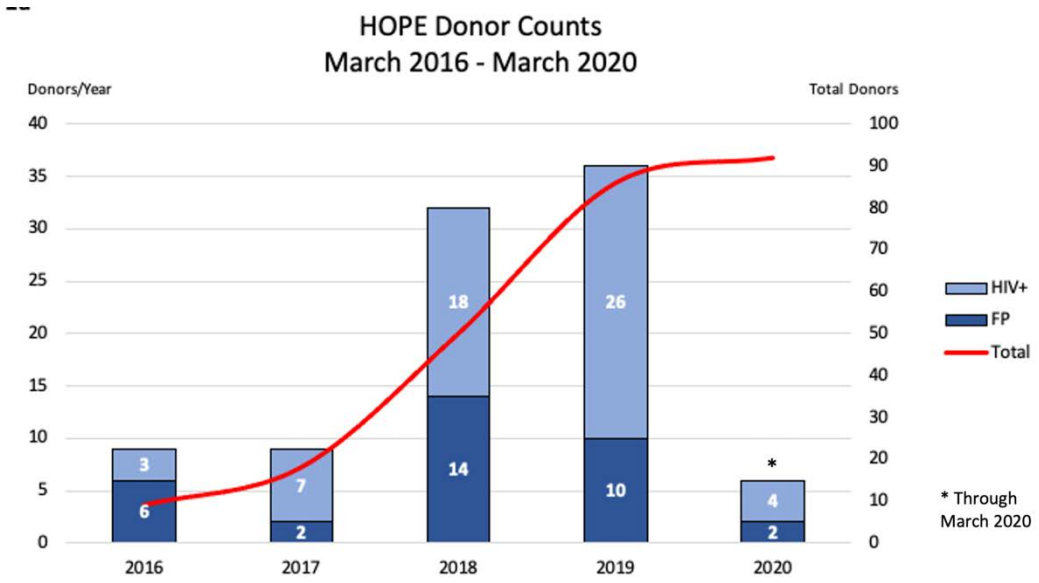
[a] 48 HIV+ donors had available genotype data: 47 interpretable laboratory genotypes and 14 historical genotypes, which were combined.
[b] Mutations were reported on only one donor genotype unless frequency otherwise specified in parentheses.

Abbreviations: ABC abacavir, ART antiretroviral therapy, ATV atazanavir, BIC bictegravir, c cobicistat, DRM drug resistance mutation, DRV darunavir, DTG dolutegravir, EFV efavirenz, EVG elvitegravir, FTC emtricitabine, INSTI integrase strand transfer inhibitor, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside reverse transcriptase inhibitor, PI protease inhibitor, r ritonavir, TAF tenofovir alafenamide, TDF tenofovir disoproxil fumarate, VL HIV viral load

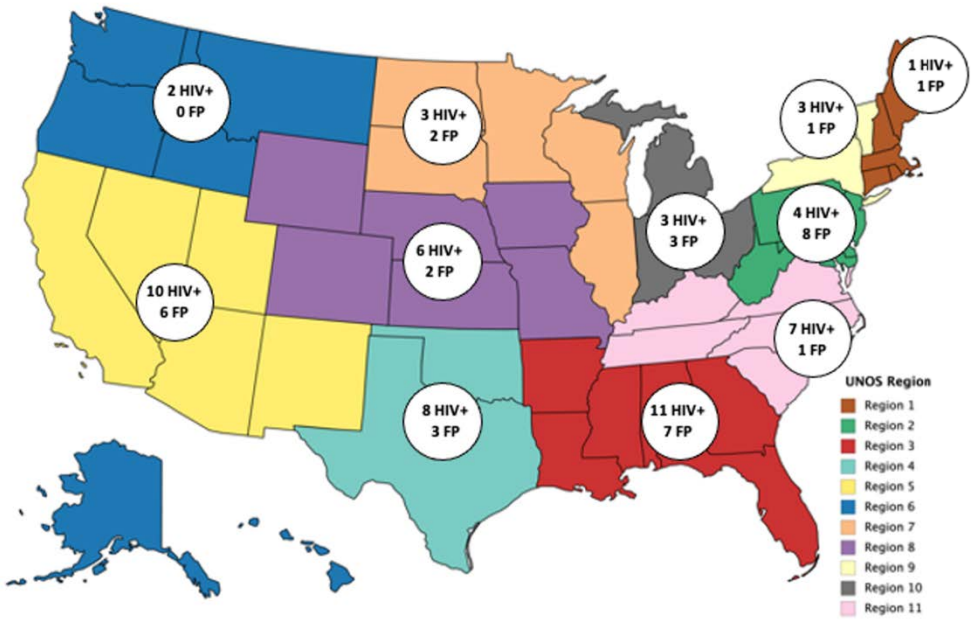
Donors with Multiclass DRMs 6 (13%)	**NRTI**	**NNRTI**	**PI**	**INSTI**
Donor 1 ART: ABC/3TC/DTG VL: <40	A62V K65N	L100I V108I V179I	-	-
Donor 2 ART: TAF/FTC/EVG/c VL: 51827	M184I	K103N	-	-
Donor 3 ART: TAF/FTC + DRV/c VL: <40	M184V	V179I	L90M	-
Donor 4 ART: TDF/FTC + DTG + ATV/r VL: <40	M41L M184V T215C/Y	K103N	-	Y143C
Donor 5 ART: TDF/FTC/EFV + DRV/r VL: 1905	M184V/I	-	-	T66I E92Q
Donor 6 ART: TAF/FTC/BIC VL: <40	D67N	K103N	-	-

Abbreviations: ABC abacavir, ART antiretroviral therapy, ATV atazanavir, BIC bictegravir, c cobicistat, DRM drug resistance mutation, DRV darunavir, DTG dolutegravir, EFV efavirenz, EVG elvitegravir, FTC emtricitabine, INSTI integrase strand transfer inhibitor, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside reverse transcriptase inhibitor, PI protease inhibitor, r ritonavir, TAF tenofovir alafenamide, TDF tenofovir disoproxil fumarate, VL HIV viral load

Figure 1: (A) Number of HOPE organ donors (both HIV+ and false-positive [FP]) who donated kidneys and/or livers during the study period, by calendar year. (B) National distribution of HOPE donors during the study period.

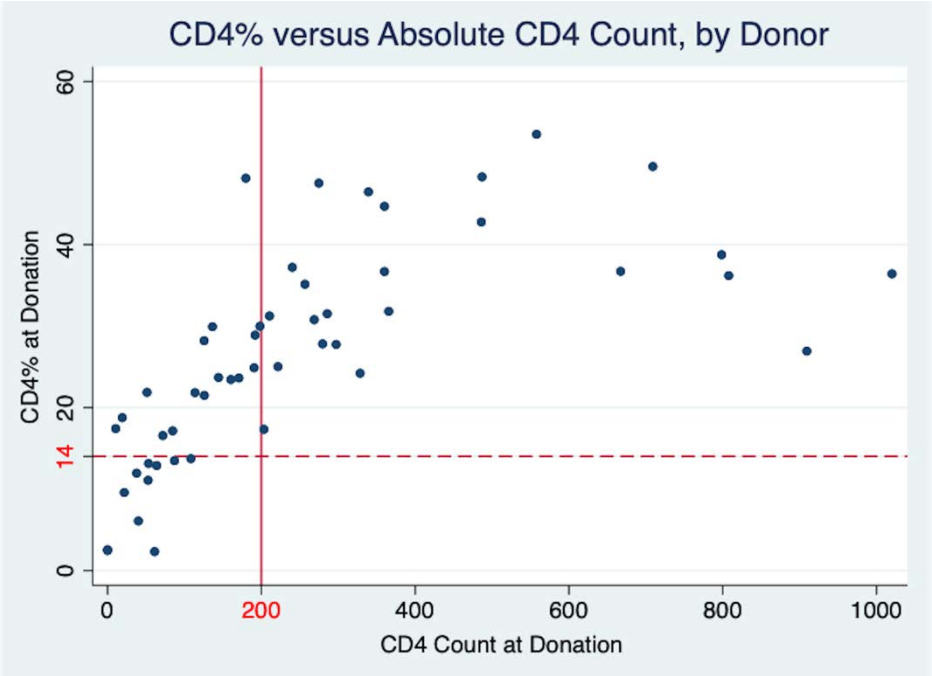


Donations increased significantly in 2018 and continued upward through March of 2020.



At least one HOPE donation took place in each of the 11 UNOS regions.

Figure 2: Donor CD4% versus absolute CD4 count at donation for 51 HIV+ donors.



The solid vertical line corresponds to CD4 count of 200 and the dashed horizontal line corresponds to CD4% of 14 (i.e., AIDS-defining thresholds). Of these 51 donors, 27 (53%) had donation CD4<200, yet 16 (59%) of these donors had a preserved CD4%>14 (left upper quadrant). In contrast, zero donors with CD4%<14 had a CD4 count>200 (bottom right quadrant). Included donors were those with available absolute count and percentage at donation.

Chapter 3. Early Steroid Withdrawal in HIV-infected Kidney Transplant Recipients: Utilization and Outcomes

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Werbel WA, Bae S, Yu S, Al Ammary F, Segev DL, Durand CM. Early steroid withdrawal in HIV-infected kidney transplant recipients: Utilization and outcomes. Am J Transplant. 2021;21(2):717-26. Epub 2020/07/19. doi: 10.1111/ajt.16195. PubMed PMID: 32681603; PMCID: PMC7927911.

ABSTRACT

Kidney transplant (KT) outcomes for HIV-infected (HIV+) persons are excellent, yet acute rejection (AR) is common and optimal immunosuppressive regimens remain unclear. Early steroid withdrawal (ESW) is associated with acute rejection (AR) in other populations, but its utilization and impact are unknown in HIV+ KT. Using SRTR, we identified 1225 HIV+ KT recipients between 1/1/2000-12/31/2017 without AR, graft failure, or mortality during KT admission, and compared those with ESW versus steroid continuation (SC). We quantified associations between ESW and AR using multivariable logistic regression and interval-censored survival analysis, as well as with graft failure and mortality using Cox regression, adjusting for donor, recipient, and immunologic factors. ESW utilization was 20.4%, with more zero HLA mismatch (8% vs 4%), living donors (26% vs 20%), and lymphodepleting induction (64% vs 46%) compared to the SC group. ESW utilization varied widely across 129 centers, with less use at high versus moderate volume centers (6% vs 21%, p<0.001). AR was more common with ESW by one year (18.4% vs 12.3%; aOR:$_{1.08}1.61_{2.41}$, p=0.04) and over the study period (aHR:$_{1.02}1.39_{1.90}$, p=0.03), without difference in death-censored graft failure (aHR $_{0.60}0.91_{1.36}$, p=0.33) or mortality (aHR:$_{0.75}1.15_{1.77}$, p=0.45). To reduce AR after HIV+ KT, tailoring of ESW utilization is reasonable.

INTRODUCTION

Patient and graft survival among HIV+ kidney transplant (KT) recipients is excellent[14], and focus has shifted toward reducing post-transplant morbidity. An important opportunity to improve care is through mitigation of acute rejection (AR), as HIV+ KT recipients experience 2-3-fold higher rates than in the general KT population[71] for uncertain reasons. One modifiable risk factor for AR is optimization of maintenance immunosuppression. Current guidelines have not established ideal strategies for HIV+ KT recipients[22] and practices may vary among centers.

Early steroid withdrawal (ESW) is an approach utilized in 30% of all KTs to limit corticosteroid exposure, and is an attractive strategy to reduce associated cardiometabolic and infectious complications in at-risk patients.[72] Several early trials in select populations such as living donor recipients and recipients of lymphodepleting antibody induction did not find significant increases in serious AR or graft failure with ESW.[73,74] In contrast, ESW use in immunologically higher risk populations such as black recipients not receiving lymphodepleting induction[75], and those with delayed graft function,[76] showed associations with increased AR and graft failure. Systematic reviews and meta-analyses studying the total KT population have indicated 1.56-1.77-fold increased risk of AR with ESW,[77,78] while noting decreased burden of cardiovascular disease and death with a functioning graft.[79] In HIV+ KT recipients, ESW data are limited to two small, single-center retrospective series that observed one-year AR rates ranging from 9% in one study of 11 patients[80] to 54% in another study of 13 patients;[81] as such, national data are critical.

The objectives of our study were to use national registry data to (i) describe ESW utilization in HIV+ KT recipients over time and across transplant centers and (ii) compare characteristics and outcomes between HIV+ KT recipients undergoing ESW versus those treated with steroid continuation (SC), with a focus on AR.

METHODS

Data source

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

Study population

We identified 1437 HIV+ KT recipients aged≥18, undergoing transplantation between January 1, 2000 and December 31, 2017. We excluded recipients with incomplete immunosuppressive exposure and outcome data (n=135), rejection, graft failure, or death, or length of stay >90 days during index transplant hospitalization (n=132), or with prior KT or multiorgan transplant (n=37) (n=212 total excluded, Figure 1), for a study population of n=1225. We defined the early steroid withdrawal (ESW) group as those discharged from

index transplant hospitalization without a corticosteroid maintenance drug, and the steroid continuation (SC) group as those discharged on any corticosteroid medication. Demographics and immunologic factors were compared between ESW and SC groups via Fisher's exact and chi-square testing as appropriate for categorial variables, and via Student's t-test and Wilcoxon rank-sum testing for continuous variables.

National and center-level ESW utilization

ESW utilization (proportion of HIV+ KT recipients undergoing ESW) was presented by calendar year, starting in 2004 when >20 HIV+ KTs were performed, through 2017. Individual center-level ESW utilization during the study period was calculated and displayed for those centers performing 20-40 HIV+ KTs ("moderate volume centers") and those performing >40 HIV+ KTs ("high volume centers"). Median ESW utilization between groups was compared via Wilcoxon rank-sum testing.

Outcome definitions

The primary outcome was acute rejection (AR), defined as first event recorded during follow up, irrespective of need for biopsy or treatment, comparing ESW and SC groups. Secondary outcomes included (i) recipient mortality and (ii) death-censored graft failure (DCGF), defined as graft failure, retransplantation, or resumption of maintenance dialysis prior to recipient death. All outcomes were compared using Fisher's exact and chi-square testing as appropriate, while change in AR incidence over time was assessed using non-parametric test of trend (extension of Wilcoxon rank-sum testing).

Multivariable model

Analyses tested for associations of the primary exposure, ESW, with the primary outcome (AR) and secondary outcomes (recipient mortality, DCGF), adjusting for possible confounders including: donor factors (age, living donation), recipient factors (age, black race, hepatitis C [HCV] antibody status), immunologic factors (calculated panel reactive antibody [cPRA] at KT, human leukocyte antigen [HLA] zero mismatch on A, B, and DR loci, anti-thymocyte globulin [ATG] induction, delayed graft function [DGF]), and transplant era (2000-2007 [reference], pre-HIV integrase strand transfer inhibitor [INSTI] era; 2008-2013, INSTI era; and 2014-2017, INSTI + HCV direct-acting antivirals [DAA] era). Recipients missing covariable data (n=53) were excluded from the final model (Figure 1).

Logistic regression

Multivariable logistic regression was used to assess for associations of ESW with AR by one year (i.e., reported on 3, 6, or 12-month follow-up forms, within 365 days of KT), using the above model. Additional analyses included evaluation for effect measure modification, i.e., whether the effect of ESW on AR varied by level of other key factors, via interaction terms and likelihood ratio testing of nested models informed by Akaike information criteria. This included interactions between ESW and ATG induction, recipient black race, transplant era, and living donation. Additionally, we performed subgroup analyses to assess adjusted odds ratios (aORs) for populations of interest, restricting upon recipients coadministered mycophenolate derivatives plus tacrolimus (n=1022) as well as those undergoing KT during the INSTI and INSTI+DAA eras (n=1099). As a sensitivity analysis, we explored the impact of transplant center volume during the study period by addition of a factor variable for low (<20 HIV+ KTs), moderate (20-40 HIV+ KTs), or high (>40 HIV+ KTs) volume centers. Finally, we explored inverse probability of treatment weighting

(IPTW; a form of propensity analysis) to balance observed and unobserved confounding and assess for changes in the association between ESW and AR. Covariable balance was assessed to ensure standardized differences <0.1, and density of the predicted probabilities assessed to ensure no violation of the overlap assumption.

Survival analyses

For AR, an interval-censoring approach was used[82] because OPTN does not capture the precise date of rejection events after KT, instead recording the dates of serial patient follow-up form submissions containing updated outcome information. This permits definition of an interval between the last follow-up form reporting no rejection ("left time"), and the first follow-up form to report a rejection event ("right time"), during which a rejection event has occurred. A Weibull parametric proportional hazards model was selected to estimate the hazard of AR over time, with fit confirmed by plotting Cox-Snell residuals versus the estimated cumulative hazard function. The hazard ratio (HR) for ESW was calculated adjusting for identical donor, recipient, and immunologic variables as in the logistic regression model. The impact of transplant center volume during the study period was also explored.

For mortality and death-censored graft failure (DCGF) between ESW and SC groups, Cox proportional hazards regression was used to calculate the aHR for ESW, adjusting for identical factors as in the logistic regression and interval-censoring survival analysis models. Unadjusted survival curves, the complements of DCGF and mortality, were plotted using the Kaplan-Meier method and functions were compared using log-rank testing. The proportional hazards assumption was examined via log-log plot of survival

curves over time. We explored center-level effects in each Cox model by performing a sensitivity analysis accounting for random effects common to individuals at each center (a shared frailty model).

Statistical analyses

All analyses were performed using Stata/SE 15.1 for Mac (College Station, Texas). Confidence intervals for aORs were presented per the method of Louis and Zeger.[83] Significance level for all tests was set at a two-sided alpha <0.05.

RESULTS

Population characteristics

Among 1225 HIV+ KTs, 1099 (90%) occurred following the advent of HIV INSTIs (2008-2017), and 661 (54%) in the INSTI + HCV DAA era (2014-2017) (Figure 2). There was a sharp increase in transplant volume beginning in 2015, with an average of 180 HIV+ KTs performed per year from 2015-2017 (n=542, 44% of total). ESW was utilized in 250 patients (20.4%) during the study period. ESW utilization ranged from 10-26% per year from 2004-2017, and remained fairly stable from 2008-2017 (median 20%, IQR 19-23), without a clear temporal trend.

Donor and recipient characteristics were largely similar between ESW and SC groups (Table 1). There were more living donors in the ESW group (26% vs 20%, p=0.03) and shorter median cold ischemia time (12.2 vs 14.3 hours, p=0.02), though median KDPI was nearly identical (44 vs 44, p=0.7). Notable recipient characteristics included high

proportion of black patients (70% vs 76%, p=0.27), with low proportion of diabetes (19% vs 16%, p=0.29) and HCV coinfection (18% vs 19%, p=0.8). Etiology of end-stage renal disease (ESRD) was similar between groups, with two-thirds requiring KT for either HIV-associated nephropathy or hypertension. Immunologic characteristics were also similar, including cPRA>30% (20 vs 17% p=0.44), although zero HLA mismatch was more common in the ESW group (8% vs 4%, p=0.02). Notably, lymphodepleting induction was used more often in the ESW group (64% vs 46%, p<0.001), with less use of anti-IL2 receptor blockade (32% vs 48%, p<0.001). Both groups were frequently coadministered mycophenolate and tacrolimus (87% vs 86%, p=0.82).

Center-level ESW utilization

During the study period, 129 centers performed at least one HIV+ KT (median n=23 KTs per center, IQR 10-48). Among moderate volume centers, there was wide variation in ESW utilization (median 21%, IQR 5-74%) (Figure 3). ESW utilization was lower, and more consistent, at the six highest volume centers (median 6%, IQR 2-14%; p<0.001 versus moderate volume centers). When contrasting patient composition at moderate versus high volume centers, however, there were many similarities: 78% vs 80% black recipients (p=0.52), 19% vs 23% living donors (p=0.21), 44% vs 41% ATG induction (p=0.32), and 17% vs 15% diabetic recipients (p=0.49). Otherwise, although cPRA profiles were very similar (data not shown), there was somewhat more zero HLA mismatch (7% vs 4%, p=0.054) and more DGF (35% vs 25%, p<0.01) among recipients at moderate volume centers versus at high volume centers.

Association of ESW with AR

The cumulative incidence of AR by 1 year was 18.4% in the ESW group (46 events) versus 12.3% in the SC group (120 events), a 1.5-fold increase in the ESW group (p=0.04). AR seemed to decrease across transplant eras (15.9% pre-INSTI, 15.1% INSTI, 12.1% INSTI + DAA), yet the trend did not reach statistical significance (p trend=0.12). When stratifying by steroid maintenance strategy, there remained no significant decrease in AR among the ESW group across transplant eras (16.0%, 19.6%, 18.1%, p trend>0.9), albeit a stronger pattern of decrease in the SC group (15.8%, 13.9%, 10.6%, p trend=0.069).

After adjustment for donor, recipient, and immunologic factors, ESW was associated with 1.61-fold higher odds of AR (aOR) by one year ($_{1.08}1.61_{2.41}$, p=0.02). The association between ESW and AR at one year did not vary by recipient race (p interaction>0.9), donor type (p interaction>0.9), induction (p interaction=0.14), or transplant era (p interaction=0.21).

When restricting to HIV+ KT recipients receiving mycophenolate and tacrolimus maintenance (n=1022), the point estimate for odds of one-year AR with ESW did not appreciably change (aOR $_{1.03}1.60_{2.49,}$ p=0.04). Restricting to the INSTI and INSTI + DAA eras (post 2007, N=1064), the ESW aOR remained statistically significant (aOR $_{1.16}1.75_{2.67}$, p<0.01). When adjusting for center volume, ESW aOR was $_{1.14}1.72_{2.60,}$ p=0.01; center volume itself was not significantly associated with AR (data not shown). Similarly, using IPTW, the average treatment effect of ESW was similar with aOR $_{1.08}1.49_{2.05}$, p=0.02.

In interval-censored survival analysis, unadjusted estimated AR survival curves separately quickly after KT in favor of SC (Figure 4). AR was more common at one, three, and five years in the ESW vs the SC group (15.6%, 23.6%, 28.3% versus 12.6%, 19.2%, 23.2%; crude

HR $_{0.93}1.26_{1.71}$, p=0.12). This pattern was more prominent in adjusted analysis, where ESW was associated with a 1.39-fold higher hazard of AR (aHR $_{1.02}1.39_{1.90}$, p=0.03; Table 2, Supplemental Figure 1). In multiple secondary analyses, the aHR for ESW was largely unchanged: restricting to INSTI and INSTI+DAA eras (aHR $_{1.08}1.49_{2.06}$), restricting to tacrolimus plus MMF maintenance (aHR $_{1.03}1.46_{2.07}$), and assessing for center effects by center volume category (aHR $_{1.04}1.43_{1.96}$).

Graft Failure and Recipient Mortality

Death-censored graft failure (DCGF) did not significantly differ between ESW and SC groups at one, three, or five years (2.2%, 7.6%, 13.3% versus 2.8%, 7.7%, 13.8%), log-rank p=0.31 (Figure 5a). There was no significant association between ESW and graft failure, aHR $_{0.60}0.91_{1.36}$ (p=0.33). Similarly, recipient mortality did not differ between ESW and SC groups at one, three, or five years (1.7%, 7.2%, 10.7% versus 1.9%, 5.2%, 8.0%), log-rank p=0.19 (Figure 5b). There was no significant association between ESW and mortality, aHR $_{0.75}1.15_{1.77}$ (p=0.45). When accounting for center-level effects, the point estimates for DCGF (aHR $_{0.59}0.91_{1.36}$) and patient survival (aHR $_{0.75}1.15_{1.77}$) were essentially identical.

DISCUSSION

In this national study, we found that 20.4% of HIV+ KT recipients were treated with ESW. ESW utilization varied widely across US centers, but was consistently lower at centers with a higher volume of HIV+ KT. AR was more common in those undergoing ESW (18.4%) than those undergoing SC (12.3%) by 1 year post KT, with a 39% higher estimated

hazard after adjustment for donor, recipient, and immunologic factors. DCGF and mortality were similar between groups at one, three, and five years post KT.

Our finding of 20% ESW utilization in HIV+ KT recipients is lower than utilization in the general KT population.[72] In keeping with KDIGO recommendations,[84] this difference may be due more immunologically high-risk characteristics in HIV+ KT versus HIV- KT, such as higher proportion of black recipients (75% vs 27%) and less use of lymphodepleting induction therapy (50% vs 65%). Otherwise, there was lower prevalence of diabetes in the HIV+ KT population (17% vs 37%), which may further influence risk-benefit calculus regarding ESW.

There is significant center-level variability in ESW utilization, particularly among moderate-volume HIV+ KT centers, while this approach was 3.4-fold less common at the six highest volume centers (median 21% vs 6%, $p<0.001$). Although some variability may be related to differences in patient factors among centers, many important characteristics appear similar across both moderate and high-volume centers (e.g., recipient black race, living donation, cPRA, ATG induction). Therefore, some of this observed variability is likely related to local provider preference, further emphasizing the need for evidence-based guidelines to inform immunosuppressive selection in this unique population.

Our finding of 13.6% AR at one year was consistent with prior registry studies of HIV+ KT[85,86] and supports the paradigm that AR remains a significant issue in this population. Reasons for elevated AR risk in HIV+ KT recipients are not fully elucidated, but include: drug interactions with HIV protease inhibitors and calcineurin inhibitors[86] most common

in the pre-INSTI era, reluctance to use lymphodepleting induction therapy[18], HCV coinfection,[87] as well as immune dysregulation and possible HIV infection of the graft itself.[88] Optimizing immunosuppressive regimens remains a priority in order to reduce AR, subsequent immunosuppressive intensification, and associated opportunistic infections.[5] Our study suggests a potential contribution of ESW in worsening AR risk.

It is notable that the subgroup with the lowest AR rate (10.6%) was the SC group undergoing KT during the most recent transplant era (when HIV INSTIs and HCV DAAs were available), a rate similar to that reported for HIV- KT recipients per OPTN[72]. This may indicate a potential added approach toward normalizing AR rates among HIV+ KT recipients in the modern era and reducing associated complications.

There are several limitations of this work. Regarding ESW exposure, we defined this as discharge without corticosteroid, assuming it was a deliberate management strategy and defining an "intention-to-treat" population. We were not, however, able to confirm decision-making for medication selection including whether this was made in response to events occurring during index hospitalization (e.g., uncontrolled hyperglycemia, infection, wound-healing concerns) than may predispose to downstream sequela. That said, median length of stay for the analytic cohort was 5 days (IQR 4-7), consistent with the recommended timing for ESW per KDIGO[84] and employed in prior clinical trials (≤ 7 days).[74] Regardless, we were most interested in the primary outcome of incident AR following decision to pursue ESW, irrespective of rationale. Underlying basis for reinstitution of corticosteroids (e.g., incident AR, resolution of preceding infection, improvement in glucose control, etc) was not available and thus limits conclusions.

It is also possible that not all rejection episodes were captured in the SRTR[89]. The observed AR rate of 13.6% by one year in this study is lower than that reported in some clinical trials of HIV+ KT.[71] Several of these series, however, predate the eras of HIV INSTIs and HCV DAAs and may be less representative of HIV+ KT in the modern age. Regardless, we do not suspect differential reporting of rejection in the SRTR based upon corticosteroid exposure, so bias in our particular inferences is unlikely. Additionally, as in most registry analyses, factors such as medication adherence, calcineurin inhibitor trough levels, preformed donor specific antibodies, and Banff classification of rejection were not available. Otherwise, details on HIV control and biology e.g., longitudinal viral loads and CD4 T-cell counts were unavailable in the SRTR, which could impact post-KT outcomes. That said, HIV+ KT recipients are a highly select group with median CD4 T-cell counts typically >400 cells/uL along with durable viral suppression before transplant.[14] In fact, lymphodepleting induction, recorded in SRTR, is likely a major arbiter of CD4 lymphopenia after transplant,[90] while viral breakthrough is uncommon and typically low level.[91]

Overall, this is the largest study of US HIV+ KT recipients to date, detailing important clinical characteristics and outcomes with key emphasis on the modern antiviral era. Additionally, it is the first dedicated study to explore associations of steroid maintenance strategy with AR following HIV+ KT, which is an important step toward development of evidence-based optimization strategies for post-transplant immunosuppression. Future investigations should focus on steroid-associated side effects post HIV+ KT to more fully

inform the risk/benefit calculus for ESW in this complex and expanding patient population that may be at elevated risk.[92-94]

Table 1: Demographic and immunologic characteristics of donors and recipients, by steroid maintenance strategy.

Recipient Factor	**ESW (N=250)**	**SC (N=975)**	**Total (N=1225)**	***p* value**
Age, med (IQR)	50 (42, 56)	49 (42, 55)	49 (42, 55)	0.43
Male, %	78	76	76	0.60
Black, %	70	76	75	0.27
HCV Antibody +, %[a]	18	19	19	0.8
Diabetes, %	19	16	17	0.29
BMI ≥ 30, %[a]	20	22	22	0.42
Etiology of ESRD, %				0.15
HIV Nephropathy	30	35	35	
Hypertension	35	33	33	
Diabetes	16	12	13	
Other FSGS	5	6	6	
Glomerulonephritis	5	7	7	
Other	8	7	7	
cPRA, %[a]				0.44
0%	64	65	64	
0.01-29.9%	17	18	18	
30-80%	17	13	14	
>80%	3	4	4	
HLA Mismatch, med (IQR)[a]	5 (4, 5)	5 (4, 5)	5 (4, 5)	0.58
DGF, %	27	28	28	0.8
Induction, %				
Lymphodepletion	64	46	50	<0.001
ATG	53	43	45	<0.01
Alemtuzumab	8	2	3	<0.001
Anti-IL2R	32	48	45	<0.001
Maintenance, %				
Tacrolimus	89	89	89	0.79
Mycophenolate	95	95	95	0.69

Values are presented as percent (%) for categorical variables and median, interquartile range [med (IQR)] for continuous variables.

[a] **Missing data**: recipient HCV status (31), BMI (44), HLA mismatch on A, B, DR loci (6), cPRA (25), cold ischemia time (38)

Abbreviations: ATG=anti-thymocyte globulin, BMI=body mass index, cPRA=calculated panel reactive antibody, DCD=donation after circulatory death, DGF=delayed graft function, ESRD=end-stage renal disease, ESW=early steroid withdrawal, FSGS=focal segmental glomerulosclerosis, HCV=hepatitis C virus, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IL2R=interleukin-2 receptor, KDPI=kidney donor profile index (deceased donors), SC=steroid continuation

Donor Factor	ESW (N=250)	SC (N=975)	Total (N=1225)	*p* value
Age, med (IQR)	38 (26, 51)	38 (26, 48)	38 (26, 49)	0.52
Male, %	54	59	58	0.11
Black, %	26	24	24	0.88
KDPI, med (IQR)	44 (25, 67)	44 (27, 64)	44 (27, 64)	0.70
Living donor, %	26	20	21	0.03
Cold ischemia time, med (IQR)[a]	12 (6, 20)	14 (8, 22)	14 (8, 22)	0.02
DCD, %	15	14	14	0.64

Values are presented as percent (%) for categorical variables and median, interquartile range [med (IQR)] for continuous variables.

[a] **Missing data**: recipient HCV status (31), BMI (44), HLA mismatch on A, B, DR loci (6), cPRA (25), cold ischemia time (38)
Abbreviations: ATG=anti-thymocyte globulin, BMI=body mass index, cPRA=calculated panel reactive antibody, DCD=donation after circulatory death, DGF=delayed graft function, ESRD=end-stage renal disease, ESW=early steroid withdrawal, FSGS=focal segmental glomerulosclerosis, HCV=hepatitis C virus, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IL2R=interleukin-2 receptor, KDPI=kidney donor profile index (deceased donors), SC=steroid continuation

Table 2: Association of early steroid withdrawal with post-KT outcomes.

Outcome	Number of Events[a] ESW vs SC		Crude ESW HR (95% CI)	*p* value	Adjusted ESW HR[b] (95% CI)	*p* value
Acute rejection	54	177	$_{0.93}1.26_{1.71}$	0.14	$_{1.02}1.39_{1.90}$	0.03
Death-censored graft failure	29	143	$_{0.55}0.81_{1.21}$	0.31	$_{0.60}0.91_{1.36}$	0.33
Recipient mortality	30	92	$_{0.87}1.31_{1.98}$	0.20	$_{0.75}1.15_{1.77}$	0.45

Point estimates are flanked by subscripts indicating lower and upper bounds of the 95% confidence interval.

[a] N=1172 HIV+ KT recipients (232 ESW, 940 SC) included in survival analyses.

[b] Multivariable models adjusted for donor age, living donation, recipient age, recipient black race, recipient HCV antibody status, calculated panel reactive antibody at KT, human leukocyte antigen zero mismatch on A, B, DR loci, anti-thymocyte globulin induction, delayed graft function, and transplant era (2000-2007, pre-HIV integrase strand transfer inhibitor [INSTI] era; 2008-2013, INSTI era; 2014-2017, INSTI + HCV direct-acting antivirals [DAA] era).

Figure 1: Study flow diagram

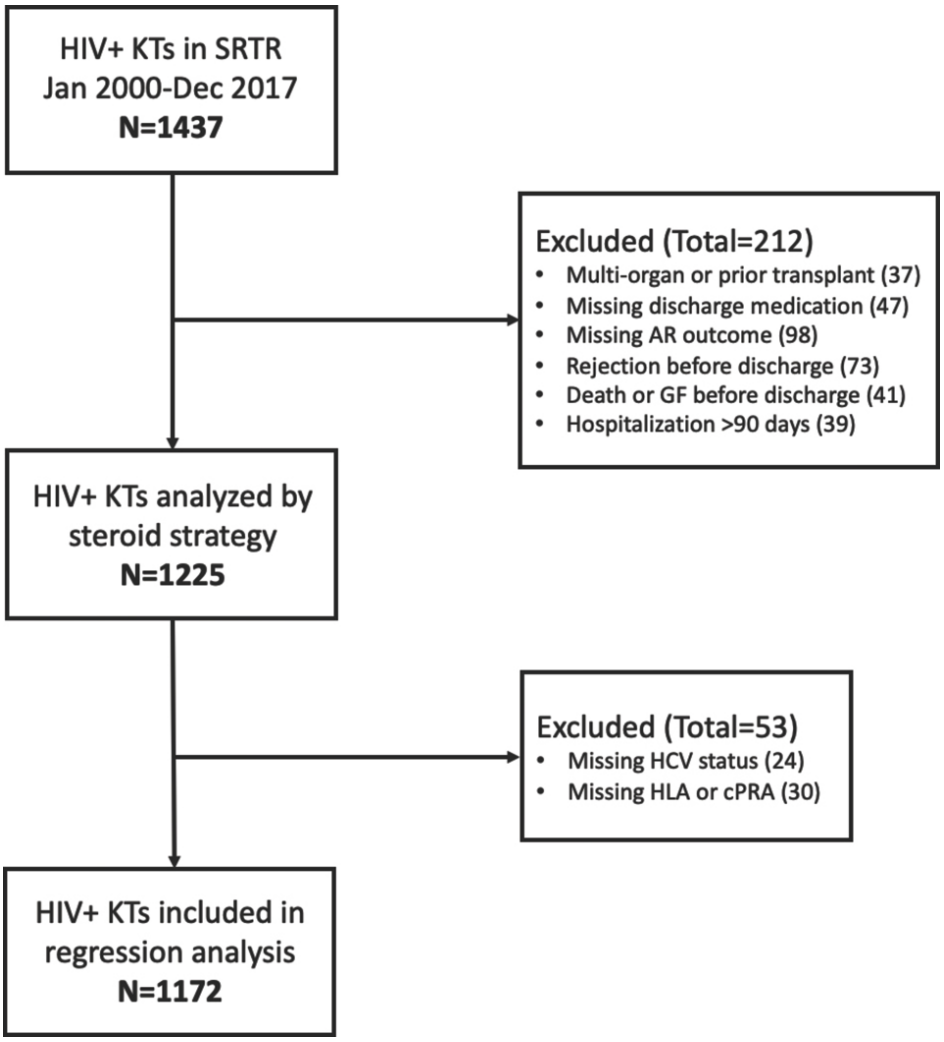
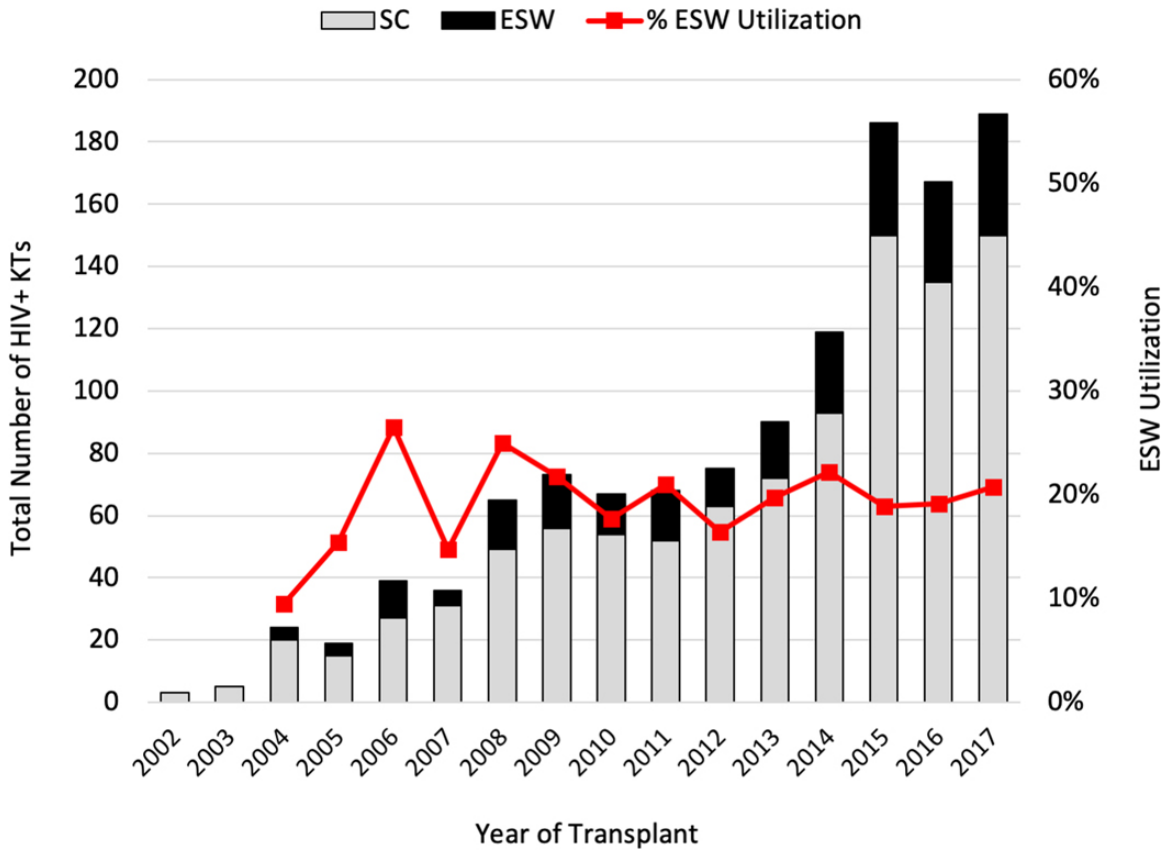
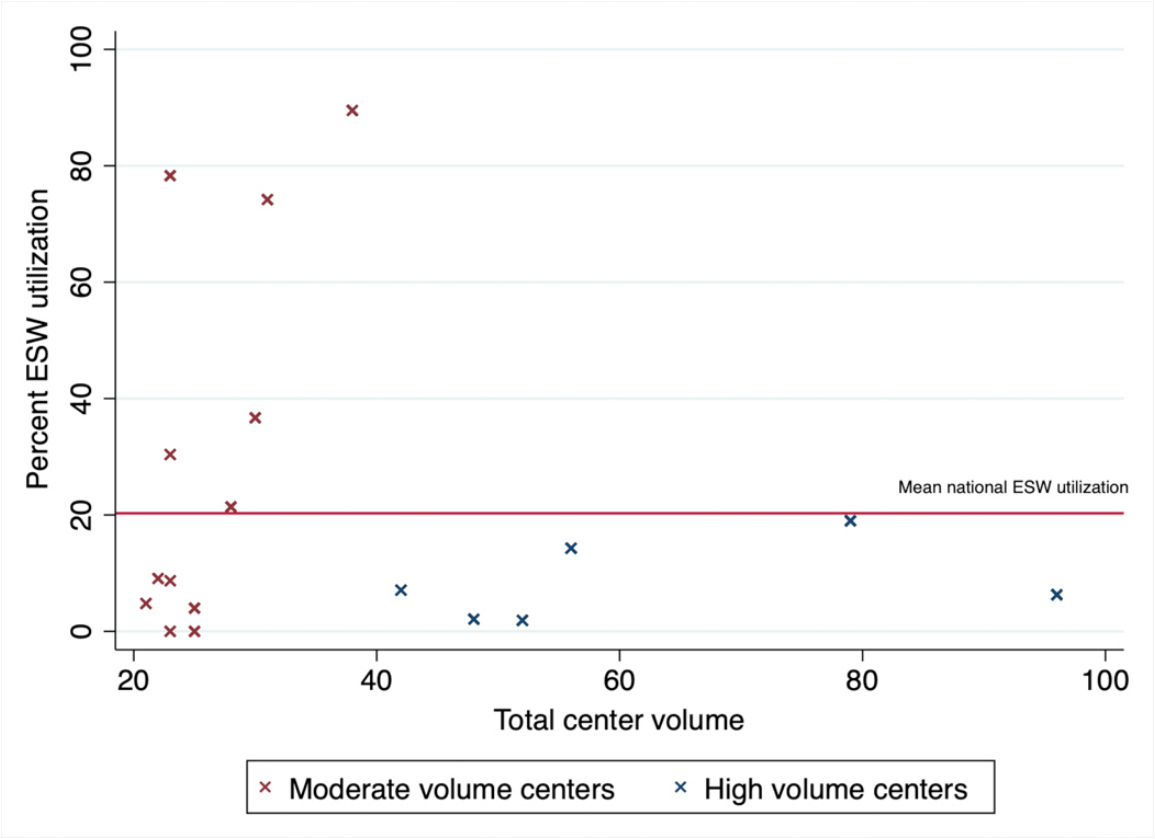


Figure 2: Early steroid withdrawal utilization among HIV+ KT recipients



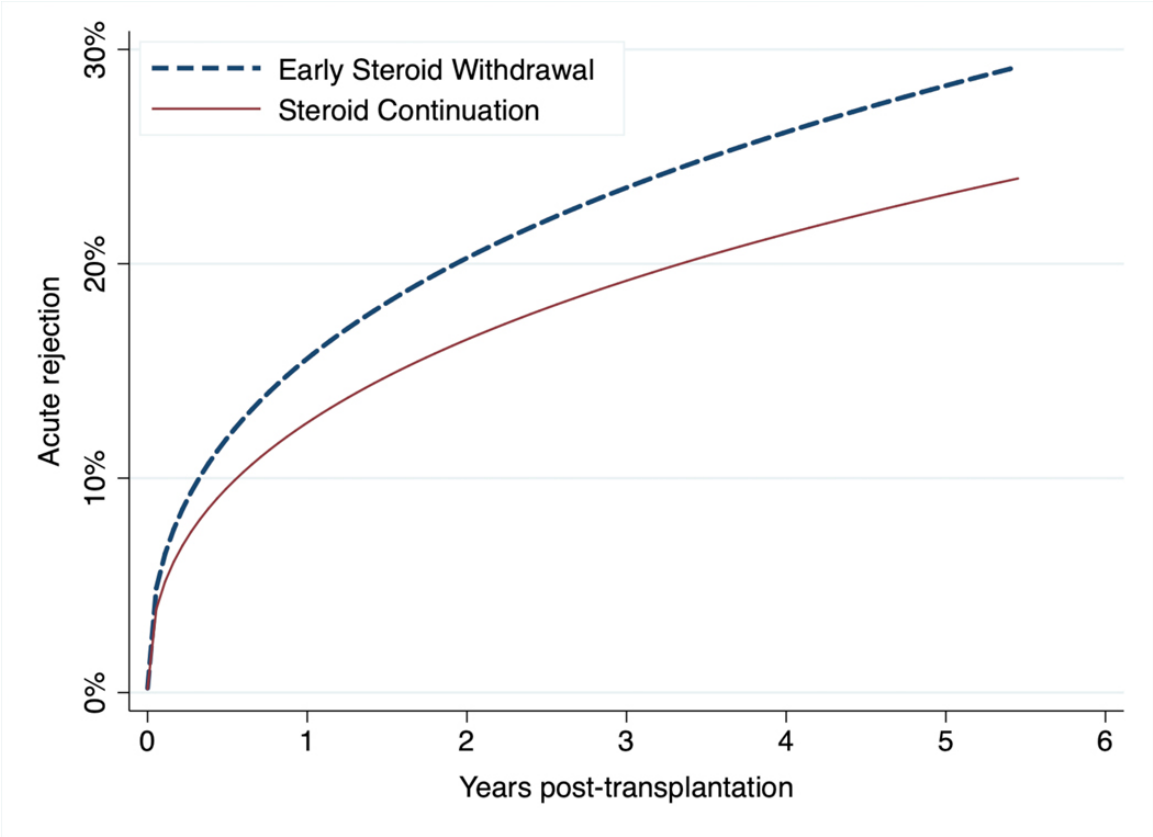
Dark bars denote the number of HIV+ KT recipients undergoing ESW each year in the study population (N=1225). Percent yearly ESW utilization, displayed by the red line, was approximately stable from 2004–2017 (median 20.3% KTs).

Figure 3: Early steroid withdrawal utilization across transplant centers.



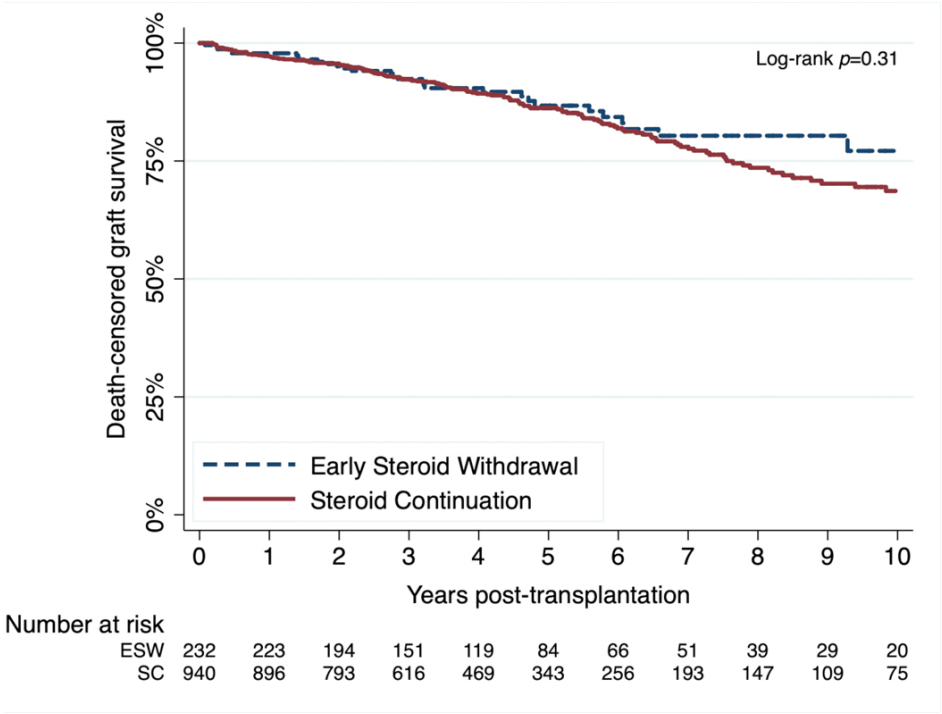
Each "x" represents centers performing ≥20 HIV+ KTs during the study period (n=18), with the y axis denoting the percent ESW utilization at each center. The red line denotes overall national ESW utilization (20.4%). Among moderate volume centers (20–40 KTs), ESW utilization varied greatly (median 21%, IQR 5–74%). Among high volume centers (>40 KTs), there was more uniformity in practice and less ESW utilization (median 6%, range 2–14).

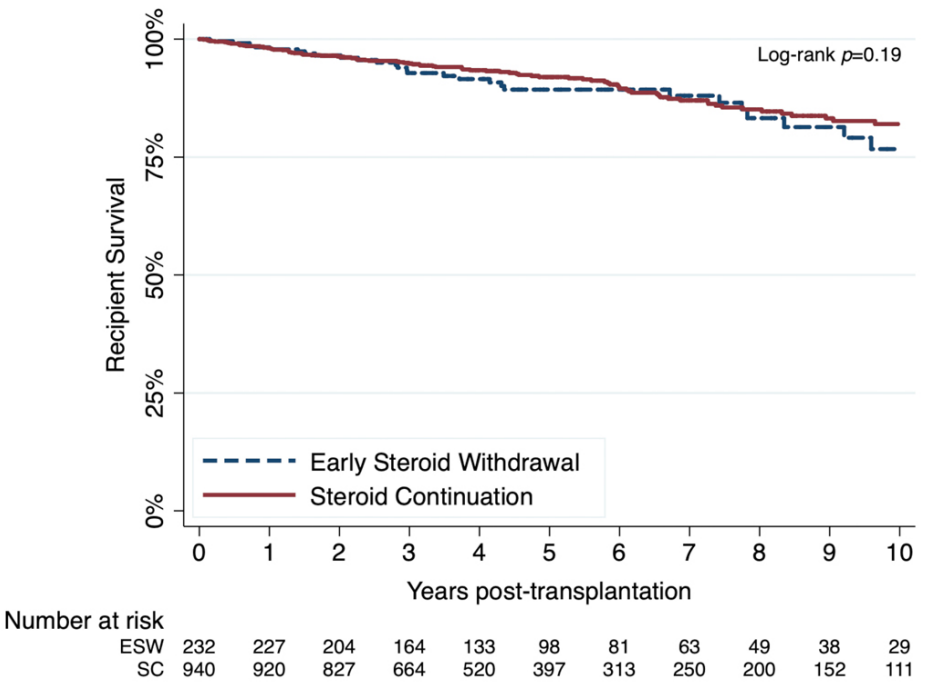
Figure 4: Unadjusted interval-censored survival curves for acute rejection in early steroid withdrawal vs steroid continuation groups.



Dashed lines represent the ESW group and solid lines represent the SC group.

Figure 5: Unadjusted Kaplan-Meier survival curves for (A) graft survival, censored for death and (B) recipient survival.





Dashed lines represent the ESW group and solid lines represent the SC group.

Chapter 4. Persistent SARS-CoV-2-specific immune defects in kidney transplant recipients following third mRNA vaccine doses

AUTHORS

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ABSTRACT

While kidney transplant recipients (KTRs) respond poorly to 2 doses of SARS-CoV-2 mRNA vaccines, outcomes and immune phenotypes following a third dose are ill-defined. We administered third mRNA vaccines to 81 KTRs with no detectable (anti-RBD^{NEG}, n=40) or low-titer [anti-RBD^{LO}, n=41, (<50U/mL]) baseline anti-RBD, measuring day 30 anti-RBD, Omicron BA.1 neutralization, spike-specific CD8+%, and SARS-CoV-2-reactive repertoires [T cell receptor (TCR) sequencing (T-MAP classifier)]. Day 30 anti-RBD was >200-fold lower in anti-RBD^{NEG}, 45% of whom remained seronegative. Only 8% developed Omicron nAb>1:20, all anti-RBD^{LO}. Day 30 spike-specific CD8+% was undetectable in 65%, without correlation to baseline anti-RBD. Breakthrough infections occurred in 16% including 2 hospitalizations; none showed pre-infection Omicron neutralization. Spike-specific SARS-CoV-2-reactive $CD4^+$ TCR repertoires increased from 21% to 50% and associated with high-dose mycophenolate ($aRR=_{0.02}0.06_{0.20}$;p<0.001) and $CD4^+$ TCR breadth ($aRR=_{1.10}2.12_{4.11}$;p=0.026); $CD8^+$ expansion was modest. Day 30 global negative response (-)anti-RBD/(-)T-MAP occurred in 9% and global positive response (+)anti-RBD/(+)T-MAP in 40%; 42% were (+)anti-RBD/(-)T-MAP and 9% were (-)anti-RBD/(+)T-MAP. Baseline anti-RBD status and $CD4^+$ breadth were highest in (+)anti-RBD/(+)T-MAP, yet TCR expansion varied widely. $CD4^+$ expansion appears necessary, but insufficient for high-level anti-RBD response and only <10% KTRs neutralized Omicron. Development of more effective vaccine strategies are critical for KTRs. (NCT04969263)

INTRODUCTION

Kidney transplant recipients (KTRs) demonstrate poorer humoral[26] and cellular immunogenicity[95,96] following primary mRNA SARS-CoV-2 vaccination and endure higher rates of vaccine breakthrough.[97] Neutralizing antibody is the best current correlate of protection against SARS-CoV-2 infection, which is approximated by the clinically-accessible biomarker of anti-receptor binding domain (anti-RBD) binding antibody.[98] High levels of anti-RBD are required for KTRs to neutralize Omicron variants.[99,100] Associations with anti-RBD response in KTRs are well defined, including the negative impact of immunosuppressive regimens containing mycophenolate.[101-103] Preceding anti-RBD level has also emerged as a powerful predictor of antibody response to additional and booster vaccine doses,[29,104,105] with the potential for early identification of subgroups at higher risk for COVID-19 breakthrough[106-108] and in need of targeted immunoprophylactic interventions.

In contrast, determinants and clinical impact of T cell responses induced by SARS-CoV-2 vaccines in KTRs, including correlation with the anti-RBD biomarker, are less well delineated. This is in part due to use of varying research assays and metrics to describe response across studies, often in the setting of cohorts heterogenous in organ type, immunosuppressive regimen, history of SARS-CoV-2 infection, and vaccines received. Among prior studies, discordance between antibody and T cell response has been reported in 0-50% of transplant recipients,[109-112] though phenotypes of humoral and/or cellular anti-SARS-CoV-2 immune responses and their determinants are incompletely characterized. It

is therefore uncertain whether T cell immunoprotection against COVID-19 is achieved among KTRs who do not develop high-level anti-RBD responses.

Given these knowledge gaps, we enrolled a homogenous cohort of KTRs with poor anti-RBD response following two-dose mRNA vaccination in a rigorous clinical trial setting to determine the effects of a third vaccine dose on (i) anti-RBD response and neutralizing capacity of variants of concern including Omicron, (ii) SARS-CoV-2-specific T cell expansion using two complementary assays, and (iii) phenotypes of each compartment of immune response. Clinical and immunological associations with vaccine breakthroughs were also recorded.

MATERIALS AND METHODS

2.1 Study Background and Design

The COVID-19 Protection After Transplant (CPAT) trials are a National Institutes of Health-funded effort to investigate safety and immunogenicity of SARS-CoV-2 vaccination strategies in solid organ transplant recipients (Supplement). The study described herein began 8/10/2021, as a single-arm, open-label trial to test humoral and cellular responses to additional (third) homologous mRNA vaccination in KTRs who failed to respond to two prior mRNA vaccine doses. "Failure to respond" was defined as negative (<0.8 units/mL, anti-RBD^{NEG}) or low-titer (0.8-50 units/mL, anti-RBD^{LO}) on the Roche Elecsys anti-SARS-CoV-2 S assay; this threshold was chosen given low probability of neutralizing ancestral SARS-CoV-2 variants.[113,114]

Participants included adult, kidney-only recipients on stable calcineurin-inhibitor-based immunosuppression, without major graft dysfunction or organ rejection within 6 months of screening; full study criteria are available at ClinicalTrials.gov (NCT04969263, study flow diagram in Supplemental Figure 1). The primary immunogenicity outcome was day 30 anti-RBD response, stratified by baseline anti-RBD titer (anti-RBD^{NEG} or anti-RBD^{LO}, on day 0) given anticipated differential responses.[29,30] Secondary immunogenicity outcomes included neutralization of SARS-CoV-2 variants as well as dimensions of cellular response, measured at days 14, 30, 90, 180, and 365. Safety outcomes included reactogenicity, serious adverse events, and alloimmune events including serial measurement of donor specific antibody. There was serial monitoring for SARS-CoV-2 infection via polymerase chain reaction testing of nasal swabs and anti-nucleocapsid testing at days 30, 90, 180, and 365; symptom screening occurred at each study visit and continuous for-cause testing was performed via clinical teams. This trial was approved by the Johns Hopkins University IRB (IRB00288774) and participants provided written informed consent.

2.2 Antibody and Neutralization Assays

Anti-Receptor Binding Domain Antibody

Anti-RBD response was measured using the semi-quantitative Roche Elecsys® Anti-SARS-CoV-2 S, a US FDA emergency use authorized pan-immunoglobulin electrochemiluminescence immunoassay, at each time point. Units/milliliter (U/mL) are ~1:1 to World Health Organization binding antibody units, correlating with neutralizing activity against SARS-CoV-2 variants. Per manufacturer recommendation, <0.8U/mL was reported as negative, with lower limit of quantification 0.4U/mL (set as 0.2U/mL for

analysis). Values ≥250U/mL were diluted in replicate until highest and most recent dilution signals were equivalent.

ACE2 Inhibition Assays (Surrogate Neutralization)

The Meso Scale Discovery (MSD) ACE2 inhibition assay was used as a surrogate measure of neutralization, quantifying the plasma inhibition of ACE2 binding to full-length SARS-CoV-2 spike protein. ACE2 MSD V-PLEX SARS-CoV-2 Panel 13 and 23 plates pre-coated with spike proteins expressing mutations corresponding to variants of concern were incubated with participant plasma and then human ACE2 protein conjugated with a light-emitting label was added. If plasma fully bound the coated spike protein and blocked ACE2 binding, no light was emitted during the stimulation phase of the assay, corresponding to 100% inhibition (i.e., full neutralization). Whereas, if there was no effective binding of spike by plasma, then ACE2 fully bound the coated spike protein and illuminated during plate activation, corresponding to 0% inhibition (i.e., no neutralization). Data in vaccinated SOT recipients have indicated ≥20% ACE2 inhibition on this high-throughput assay is associated with measurable live virus neutralizing antibody, including versus variants of concern.[115]

Live Virus Neutralization

Neutralization of live ancestral (SARS-CoV-2/USA-WA1/2020), Delta (hCoV19/USA/MD-HP05660/2021, EPI_ISL_2331507) and Omicron BA.1 (hCoV19/USA/MD-HP20874/2022, EPI_ISL_7160424) variants were assessed. Briefly, VeroE6-TMPRSS2 cells were cultured and incubated with viral transport media from SARS-CoV-2 infected patients.[116] SARS-CoV-2 RNA was verified using a Qiagen extraction kit, with variant confirmation by

quantitative RT-PCR and sequencing. Viral titer was then determined on VeroE6-TMPRSS2 cells using a 50% tissue culture infectious dose ($TCID_{50}$) assay as previously described[117]. Live virus neutralizing antibody (nAb) levels were determined using twofold dilutions of plasma (starting at 1:20)[118] with addition of infectious virus at 1x10^4 $TCID_{50}$/mL. Samples were incubated at 37˚C for 2 days or until complete cytopathic effect was visible in wells exposed to virus, alone. The cells were then fixed, further incubated, and stained with Napthol Blue Black (MilliporeSigma).

nAb titer (NT_{50}) was calculated as highest serum dilution that eliminated cytopathic effect in 50% of wells, after which area under the curve (AUC) values were calculated using GraphPad Prism; AUC provides a continuous measure of nAb for data visualization. Minimal nAb was defined as (+)<1:20 NT_{50}, whereas high-level nAb was defined as >1:160 NT_{50}. For participants without live virus nAb assayed against Omicron BA.1 at each time point, missing values were imputed based upon anti-RBD and neutralization data (Supplement). Specifically, persons with negative nAb versus ancestral and/or Delta variants at a timepoint were imputed as having negative nAb versus Omicron given need for 10-20-fold higher titers for neutralization.[118] Negative nAb titers were imputed at subsequent timepoints (i.e., last value carried forward) if there was no (i) intercurrent COVID-19 by clinical syndrome, PCR testing, or anti-nucleocapsid seroconversion, (ii) receipt of antibody product, or (iii) receipt of additional SARS-CoV-2 vaccination.

2.3 Cellular Analyses and Methodology

SARS-CoV-2 Spike-specific $CD8^+$ Memory T cell Response

Peripheral blood mononuclear cells (PBMCs) from HLA-A*02:01+ KTRs (n=33) were isolated and analyzed by flow cytometry for SARS-CoV-2 spike-specific CD8+ T cell responses using HLA-peptide pentamers (see Supplement). Briefly, cells were washed and stained with four biotinylated MHC class I pentamers corresponding to immunodominant SARS-CoV-2 spike protein epitopes (ProImmune Ltd.: FIAGLIAIV, LITGRLQSL, YLQPRTFLL, RLQSLQTYV).[119,120] The frequency of spike-specific CD8+ T cells (staining positive for at least one spike-specific epitope) was evaluated out of total memory CD8+ T cells (gated on $CD3^+CD4^-CD8^+$ cells, excluding naïve $CCR7^+CD45RA^+$ T cells). The threshold for positive spike-specific $CD8^+$ T cell response was ≥0.009% (above background).

Immunosequencing of SARS-CoV-2 Associated T cell Repertoires:

We utilized the Adaptive Biotechnologies (Seattle, WA) immunoSEQ Assay® to measure changes in the broader SARS-CoV-2-associated T cell repertoire via TCR sequencing.[121,122] PBMCs were isolated at day 0 and day 30, frozen, and then sent for high-resolution immunosequencing to quantify absolute abundance of each unique TCRβ CDR3 sequence (i.e., define the overall TCR repertoire, SARS-CoV-2-associated and non-SARS-CoV-2-associated). The binary T-MAP™ COVID classifier, which leverages the same machine-learning algorithm as the clinically-available US FDA T-Detect COVID Test (https://www.fda.gov/media/146481/download), was applied to map participant TCR repertoires against a library of TCRs determined to react to SARS-CoV-2, reporting whether a participant had a "positive," "negative," or "indeterminate" SARS-CoV-2-reactive repertoire before and after vaccination.

TCR repertoire components were separately analyzed and included: (i) *breadth*, the proportion of unique clonal rearrangements that react to SARS-CoV-2 of all unique TCRs (i.e., describing diversity of the SARS-CoV-2-reactive clones) and (ii) *depth*, the proportion of all productive TCR templates that react to SARS-CoV-2 of all detected TCRs (i.e., enumerating the total number of SARS-CoV-2-reactive clones). These metrics were reported for $CD4^+$ and $CD8^+$ compartments, as well as for spike-specific and non-spike cognate regions. TCRs for non-spike regions would not be expected to expand following vaccination with SARS-CoV-2 spike mRNA, whereas $CD4^+$ and $CD8^+$ spike-specific TCRs would be expected to expand as markers of vaccine-associated T cell response.

2.4 Statistical Analysis

Characteristics were compared among anti-RBD^{NEG} versus anti-RBD^{LO} by Fisher's exact and Wilcoxon rank-sum for categorical and continuous variables, respectively. For immunogenicity measures, participants who developed incident COVID-19 (for all outcomes) or received monoclonal antibody (mAb) (for humoral outcomes) were excluded from statistical analyses, yet were included in data visualization as triangles (developed COVID-19) or open circles (received mAb).

Characteristics of anti-RBD^{NEG} participants who remained seronegative versus those who seroconverted at day 30 were compared using Fisher's exact and Wilcoxon rank-sum. Anti-RBD half-life among those with anti-RBD≥500 at day 30 was estimated via exponential decay modeling. Associations with day 30 anti-RBD were assessed using (i) Poisson regression with robust variance estimator for the outcome of anti-RBD >2500U/mL (potential minimum threshold associated with Omicron sublineage

neutralizing capacity[99,123]) and (ii) negative binomial regression for continuous anti-RBD titer. Multivariable models incorporating mycophenolate use and dosage ("high-dose" as >1000mg mycophenolate mofetil or >720mg mycophenolic acid, daily), time since transplant, and TCR responses were built based on exploratory data analysis.

Associations between SARS-CoV-2-specific $CD8^+$ response by MHC-pentamer staining at day 14 and clinical and laboratory factors were assessed using Poisson regression with robust variance estimator. The proportion of participants with SARS-CoV-2-reactive repertoires ([+]T-MAP™) at day 0 and day 30 was compared used Fisher's exact test. Associations of baseline characteristics with (+)TMAP™ at day 30 were assessed using Poisson regression with robust variance estimator; persons with indeterminate repertoires were excluded from comparative analyses. Differences in TCR breadth and depth from day 0 to day 30 were analyzed by Wilcoxon rank-sum testing. Associations between day 30 spike-specific TCR expansion and day 30 anti-RBD were assessed by linear regression, and Spearman rank. Participants with undetectable ("0") SARS-CoV-2 TCRs were assigned a value of $1x10^{-6}$ for analytical and visualization purposes, and as sensitivity analysis were excluded from regression analyses. Participants were then phenotyped across two binary dimensions of immune response at day 30, defined as (+)/(-)anti-RBD and (+)/(-)T-MAP™, with comparison of clinical and transplant characteristics among groups.

Point estimates and 95% confidence intervals were reported in the style of Louis and Zeger,[83] $_{\text{lower 95\% CI}}$Point Estimate$_{\text{upper 95\% CI}}$. Two-sided α 0.05 was used to determine statistical significance. Statistical analyses were performed using Stata/SE 17.0.

RESULTS

3.1 Study Population

After screening, n=81 KTRs (n=40 anti-RBD^{NEG}, n=41 anti-RBD^{LO}) were enrolled and a third, homologous vaccine dose was administered (22 mRNA-1273, 59 BNT162b2) at median (IQR) 167 (149, 177) days post dose 2. Demographics and transplant factors were similar between anti-RBD^{NEG} and anti-RBD^{LO} participants, as were the vaccines administered, though the anti-RBD^{LO} group had slightly longer time since last vaccination (Table 1). One participant who developed COVID-19 and one treated with casirivimab/imdevimab (monoclonal antibody) (both anti-RBD^{LO}) were excluded from primary day 30 analyses; see *Breakthrough Infections*, Section 3.5. Three additional participants who developed COVID-19 were excluded from day 90 analyses.

3.2 Antibody and Neutralization

Binding Antibody Responses

Among 79 participants, median (IQR) day 30 anti-RBD titer was 386 (9-2331)U/mL (Figure 1). Day 30 median (IQR) anti-RBD titer was >200-fold higher in anti-RBD^{LO} versus anti-RBD^{NEG} participants: 2331 (712-4390)U/mL versus 10 (<0.4-132)U/mL ($p<0.001$), respectively. Among participants with anti-RBD≥500 U/mL at day 30, median titers decreased 31% by day 90, with estimated half-life of 65 days per exponential decay modeling.

Among anti-RBD^{NEG} participants, 18/40 (45%) remained anti-RBD negative at day 30. Demographic, immunosuppressant, and vaccination factors were similar among those

who did versus did not seroconvert (Table S1). Persistent negative anti-RBD participants demonstrated lower median (IQR) total immunoglobulin levels (779 [684, 881] versus 978 [849, 1128] mg/dL, p=0.002) and trend toward lower absolute lymphocyte counts (0.77 [0.59, 1.36] versus 1.16 [0.93, 1.57] K/mm^3, p=0.052) despite similar CD4+ T cell counts (Table S1).

Among 79 participants, longer time since transplant was associated with increased relative risk (RR) of developing high-level anti-RBD (>2500U/mL) response at day 30 ($RR=_{1.02}1.27_{1.58}$ [per 5 years], p=0.029) (Table 2), but not participant age or use of the mRNA-1273 vaccine (versus BNT162b2). Anti-RBD titer was lower among participants using high-dose mycophenolate ($RR=_{0.11}0.36_{1.14}$, p=0.08), yet this did not reach statistical significance. Multivariable modeling demonstrated statistically significant negative association of high-dose MMF ($aRR=_{0.02}0.06_{0.20,}$ p<0.001) and positive association of day 30 spike-specific CD4+ T cell breadth ($aRR=_{1.10}2.12_{4.11}$ [per 1 log], p=0.026) with increased anti-RBD response, after accounting for time since transplant. On sensitivity analysis excluding participants with 0 TCR breadth, the point estimate for high-dose MMF was similar ($aRR=_{0.02}0.07_{0.21,}$ p<0.001), while the aRR for $CD4^+$ breadth increased ($aRR=_{3.99}15.12_{57.34}$ [per 1 log],p<0.001).

Neutralization

Among 79 participants, day 30 surrogate neutralization (%ACE2 inhibition) increased against the ancestral strain from median (IQR) 4.8% (3.3-6.1%) to 13.1% (6.3%-30.3%);p<0.001, and the Delta variant from median (IQR) 3.1% (1.7%-4.7%) to 9.9% (5.2%-18.0%);p<0.001 (Figure S2). These increases were largely observed in anti-RBD^{LO} participants (Table S2). In contrast, there was minimal increase in Omicron BA.1 surrogate

neutralization in either group by day 30, from median (IQR) 3.0% (0.3%, 5.4%) to 5.4% (2.8%, 10.4%); p<0.001; only three (4%) participants demonstrated ≥20% Omicron spike inhibition at day 30 (0 anti-RBD^{NEG}) (Figure 2).

These findings were corroborated by live virus testing, where among anti-RBD^{NEG} versus anti-RBD^{LO}, 20% vs 82% developed nAb versus the ancestral variant, 0% vs 56% versus the Delta variant, and 3% vs 38% versus Omicron BA.1 at day 30 (Figure 3, Figure S2). Only six (8%) participants (0 anti-RBD^{NEG}) showed more than minimal nAb versus Omicron BA.1. Neutralization decreased by day 90, with only 4/75 (11%) of participants (0 anti-RBD^{NEG}) showing any nAb versus Omicron BA.1. Notably, history of prior COVID-19 prior to vaccination was not associated with augmented neutralization.

3.3 Cellular Analyses

SARS-CoV-2 Spike-specific CD8$^+$ T Cell Response

Among HLA-A*02 participants studied by flow cytometry, 18/33 (55%) had spike-specific CD8$^+$ T cell response at day 0 (59% anti-RBD^{NEG} vs. 50% anti-RBD^{LO}). Median spike-specific CD8$^+$ T cell percentage transiently increased from day 0 to day 14 (0.008% to 0.01%, p=0.005), decreasing by day 30 (0.008%, p=0.28 versus day 0). CD8$^+$ responses were negative in 13/32 (41%) at day 14 and 20/32 (63%) at day 30 (Figure 4). CD8$^+$ T cell response neither correlated with anti-RBD level (Figure S3), nor differed by day 0 anti-RBD group (data not shown). In univariable analysis, there were no statistically significant associations between demographic or transplant characteristics and CD8$^+$ T cell response (Table S3).

SARS-CoV-2 T cell Repertoire Analysis (TCR sequencing)

SARS-CoV-2-reactive TCR repertoire (i.e., [+]T-MAP^{TM}) was detected in 10 (19%) at day 0 and increased to 28 (50%) by day 30 (p=0.001), after excluding participants with indeterminate repertoires; reactive repertoires were >2-fold more frequency in anti-RBD^{LO} participants 18/29 (62%) versus anti-RBD^{NEG} 10/27 (37%). Clinical and transplant factors were similar among participants with (+) versus (-)T-MAP^{TM} at day 30, apart from longer time since transplant in those with (+)T-MAP^{TM} (median [IQR] 8.1 [4.9, 13.3] vs 4.9 [2.2, 8.8] years, p=0.04, Table S4). In univariable analysis, no demographic, transplant, or laboratory factors were statistically significantly associated with T-MAP^{TM} positivity at day 30, though (+)T-MAP^{TM} at day 0 was associated with increased RR of (+)T-MAP^{TM} at day 30 ($RR_{1.78}^{2.56}{}_{3.68}$, p<0.01, Table S3).

Median spike-specific TCR breadth ("clonal diversity," from $1.76x10^{-5}$ to $3.97x10^{-5}$; p<0.001) and depth ("total clones," from $9.66x10^{-6}$ to $2.53x10^{-5}$; p<0.001) increased from day 0 to day 30 (Table S5); these measures were highly correlated (Figure S4). TCR expansion was prominent in the spike-specific $CD4^{+}$ compartment, with increased $CD4^{+}$ breadth from $1.41x10^{-5}$ to $2.75x10^{-5}$; p<0.001. Spike-specific $CD8^{+}$ breadth expansion was limited, from $<1.0x10^{-6}$ to $2.85x10^{-6}$; p=0.01 (Table S5). Notably, all dimensions of the spike-specific TCR repertoire at day 30 were 2-3-fold greater in anti-RBD^{LO} versus anti-RBD^{NEG} participants, e.g., spike-specific $CD4+^{+}$ breadth of $3.68x10^{-5}$ vs. $1.57\ x10^{-5}$ (p=0.038). As expected, there was no statistically significant increase in non-spike TCRs by day 30. TCR dimensions were similar after inclusion of participants with indeterminate repertoires (data not shown).

3.4 Immune Phenotypes after Full Vaccination: Correlating Humoral and Cellular Responses

Categorization of anti-RBD and T cell Responses

Immune phenotypes of day 30 vaccine response were characterized using dichotomous categories of (+)/(-)anti-RBD and (+)/(-)T-MAP^{TM}); n=8 participants with indeterminate T-MAP^{TM} were excluded. Global negative response (-)anti-RBD/(-)TMAP^{TM} was seen in 5 (9%) participants versus global positive response (+)anti-RBD/(+)TMAP^{TM} in 22 (40%). Discordant responses were seen in 28 (51%) participants: 23 (42%) with (+)anti-RBD/(-)TMAP^{TM} and 5 (9%) with (-)anti-RBD/(+)TMAP^{TM} (Table S6). High-dose mycophenolate was used in 4/5 (80%) with global negative responses, as compared to 9-27% of participants with other phenotypes (Table S6, p=0.008). Age and other demographic features were similar across phenotypes.

Association of TCR Repertoire Expansion and anti-RBD Response

Among participants with (+)anti-RBD at day 30, there was a positive linear correlation between higher spike-specific $CD4^+$ TCR breadth and higher anti-RBD at day 30 (Figure 5, ρ=0.33 by Spearman rank); a similar association was observed with spike-specific $CD4^+$ T cell depth (Figure 5, ρ=0.35). Correlations with $CD4^+$ depth (ρ=0.35) and breadth (ρ=0.37) were similar on sensitivity analysis excluding participants with 0 SARS-CoV-2-reactive TCRs. In contrast, among participants with (-)anti-RBD at day 30, spike-specific $CD4^+$ TCR responses varied widely. Additionally, median spike-specific $CD4^+$ TCR breath at day 30 was similar between anti-RBD^{NEG} participants who did versus did not seroconvert (p=0.27,

data not shown). There was no statistically significant association between day 30 $CD8^+$ TCR breadth or depth and anti-RBD level (Figure 5).

Participants with global positive responses at day 30 had median (IQR) anti-RBD 1499 (118-4314)U/mL, including 10 (45%) participants with anti-RBD>2500U/mL and 4 (18%) demonstrating Omicron BA.1 nAb>1:20. In contrast, participants with (+)anti-RBD/(-)T-MAP™ (discordant phenotype) demonstrated median (IQR) anti-RBD 386 (22-1096)U/mL (p=0.03 versus global positive), including only 1 (4%) with anti-RBD>2500U/mL and Omicron BA.1 nAb>1:20. Overall, anti-RBD>2500U/mL was achieved in 37% T-MAP™ positive versus 4% T-MAP™ negative participants (p=0.002).

3.5 Breakthrough Infections

There were 13 SARS-CoV-2 infections (16%) at median 99 days (range 13-141) after third vaccination (Table 3). Four participants were infected before day 90, during the US Delta wave, whereas most (88%) late infections occurred during the Omicron BA.1 wave. Nearly all cases (92%) were symptomatic and 2 (15%) required hospitalization for moderate disease, without need for intensive care. Median (IQR) anti-RBD level pre-infection was 91 (16-429)U/mL, including 3 (23%) with negative titers; none displayed pre-infection neutralizing capacity of Omicron BA.1, though 2 showed capacity to neutralize Delta (1 received prior active mAb).

Post-infection antibody and neutralizing responses for the 4 participants infected before day 90 were augmented (triangles, Figures 1-3), above that of nearly all other participants; two of these participants were the only KTRs to demonstrate high-level Omicron BA.1 nAb

at day 90. Neutralizing capacity after Omicron infections was variable, including 3 participants showing Omicron ACE2 inhibition<20% post infection. Of 10 participants with pre-infection SARS-CoV-2 T cell data, 5/6 (83%) had negative CD8+ response by MHC-pentamer staining and 3/7 (43%) had (-)T-MAP^{TM}; one participant with (+)T-MAP^{TM} pre-infection required hospitalization.

DISCUSSION

In this clinical trial specifically designed to deeply characterize immunogenicity of third mRNA vaccine doses in poor anti-RBD responders, we demonstrated substantial SARS-CoV-2-specific immune deficits despite full vaccination. The findings confirm the major impact of anti-RBD serostatus on subsequent anti-RBD responses, with nearly half anti-RBD^{NEG} failing to seroconvert. Although some participants with anti-RBD^{LO} attained high anti-RBD titers, Omicron BA.1 neutralization was <10%, overall. SARS-CoV-2-specific CD4^{+} responses as measured by TCR sequencing improved with vaccination, dovetailing with higher-level anti-RBD, to define a phenotype of global positive responders (40% of cohort). Yet, even in these participants, SARS-CoV-2-specific CD8^{+} responses measured by both MHC-pentamer staining and TCR sequencing were limited. Breakthrough infections were common and concentrated among poor anti-RBD responders without clear relation to measures of T cell reactivity.

This trial further supports the paradigm that high-dose mycophenolate use is negatively associated with humoral vaccine response,[98,102,103] suggesting heavier lymphocyte impairments. This association strengthened in multivariable modeling after accounting

for $CD4^+$ TCR breadth and time since transplant. Given suboptimal immune responses in KTRs, the potential for peri-vaccination mycophenolate reduction among low alloimmune risk KTRs is of great interest, having shown promise and safety in small observational studies,[124] and now the focus of a multicenter CPAT trial (NCT05077254) to confirm potential benefits and risk.

Interestingly, although persistent anti-RBD negativity was common, there were no clear association with clinical or transplant characteristics, with many participants showing $CD4^+$ expansion equivalent to that of anti-RBD responders. In other words, $CD4^+$ expansion appeared necessary, but not sufficient for high-level anti-RBD responses. This constellation, coupled with lower absolute lymphocyte counts and lower gamma globulin levels in persistent negative anti-RBD participants (despite comparable $CD4^+$ counts), suggests B cell dysfunction as a contributor to poor antibody response.[96] These includes quantitative or qualitative deficits such as metabolic dysfunction related to mycophenolate,[125] with possibly contribution of ineffective $CD4^+$ T cell function and/or co-stimulation.[98,110] Investigating the metabolic state of B and T cells in KTRs with poor humoral response despite evidence of T cell reactivity is a potential avenue to understand mechanisms of poor vaccine response and target strategies for augmentation.

Although breakthrough infections were common, they were concentrated among those with poor plasma neutralizing capacity, and in the era of active therapeutics (mAb) there were no cases of severe disease. Impressive humoral responses were elicited following infection with the Delta variant including cross-variant neutralization of Omicron BA.1, yet immunogenicity following Omicron BA.1 infection was variable, echoing findings in

other populations potentially related in part to high antigenic distance from the vaccine strain.[126] Notably, several participants showed SARS-CoV-2 T cell reactivity prior to infection, including in one participant who required hospitalization, suggesting that unlike neutralizing capacity, cellular markers may not correlate with protection against COVID-19. Given overall poor $CD8^+$ response and lack of correlation with anti-RBD, it is not currently possible to presume T cell immunoprotection in the absence of high-level antibody response.

Strengths of this study include explicit focus on high-risk poor anti-RBD responders, using clinically available biomarkers as well as investigation of their association with gold standard nAb and deep evaluation of the SARS-CoV-2-associated T cell compartments. Additionally, breakthrough ascertainment was robust, with serial assessment of pre- and post-infection antibody and T cell responses. Limitations of this study include smaller sample size, resulting from strict inclusion criteria and availability of third vaccines outside of the trial setting during enrollment; this reduced power to detect associations with immunological outcomes. Additionally, due to HLA restrictions as well as PBMC availability, in-depth T cell analyses were not performed on all participants. Although the broader SARS-CoV-2-reactive T cell repertoire was interrogated in this study, the functional capacity and metabolic state of these cells were not explicitly evaluated, which is the focus of ongoing investigation and will better elucidate degrees of cellular immunoprotection.

In summary, a third mRNA vaccine dose augmented anti-RBD titers in KTRs with prior detectable antibody after a two-dose series, yet nearly half of negative anti-RBD

participants remained seronegative and <10% overall demonstrated Omicron neutralization. Spike-reactive $CD4^+$ T cell repertoires after vaccination correlated with highest-level anti-RBD response, yet did not alone discriminate antibody responders from those remaining seronegative. High-dose mycophenolate significantly impaired anti-RBD response, potentially due to B cell dysfunction and/or ineffective $CD4^+$ help. The relative paucity of neutralization and $CD8^+$ response on two detailed assays suggest vulnerability to infection in the majority of these high-risk vaccinees in the Omicron era. Alternative vaccination strategies are needed to enhance immunoprotection in KTRs, particularly those with negative anti-RBD levels, such as targeted immunosuppression reduction[124,127] or potentially use of platforms with differential immunogenicity including adjuvanted vaccines.

Table 1: Demographic and transplant characteristics of trial participants, by day 0 anti-RBD level.

	Total (N=81)	anti-RBD^{NEG} (N=40)	anti-RBD^{LO} (N=41)	p-value^{+}
Demographics				
Age (years), median (IQR)	66 (57, 73)	66 (56.5, 73)	66 (57, 74)	0.86
Female sex, no. (%)	26 (32)	16 (40)	10 (24)	0.16
Race, no. (%)				0.71
White	49 (60)	22 (55)	27 (66)	
Black/African American	24 (30)	13 (33)	11 (27)	
Asian	7 (9)	4 (10)	3 (7)	
Hispanic/Latino ethnicity, no. (%)	3 (4)	2 (5)	1 (2)	0.62
BMI (kg/m^2), median (IQR)	25.9 (23, 31.3)	27.4 (23.2, 31.9)	25.9 (23, 31.1)	0.64
Medical comorbidities				
Diabetes, no. (%)	26 (32)	14 (35)	12 (29)	0.64
HCV infection, no. (%)	4 (5)	1 (3)	3 (7)	0.62
Lung disease, no. (%)	16 (20)	8 (20)	8 (20)	>0.99
Cardiovascular disease, no. (%)	72 (89)	34 (85)	38 (93)	0.31
Autoimmune disease, no. (%)	8 (10)	5 (13)	3 (7)	0.48
Transplant history and immunosuppression				
Years since transplant, median (IQR)	5.4 (2.1, 10.5)	5.2 (2, 9.3)	5.7 (3.2, 10.7)	0.32
Indication for most recent kidney transplantation, no. (%)				
Diabetes	12 (15)	6 (15)	6 (15)	>0.99
Hypertension	30 (37)	17 (43)	13 (32)	0.36
FSGS	6 (7)	5 (13)	1 (2)	0.11
Glomerulonephritis	1 (1)	1 (3)	0 (0)	0.49
Cystic kidney disease	11 (14)	3 (8)	8 (20)	0.19
Living donor, no. (%)	35 (43)	14 (35)	21 (51)	0.18
DSA positive at baseline, no. (%)[*]	15 (19)	8 (20)	7 (18)[a]	>0.99
Baseline Immunosuppressant, no. (%)[b]				
Mycophenolate Mofetil	55 (68)	27 (68)	28 (68)	>0.99
Total daily dose (mg), median (IQR)	1000 (500, 1000)	1000 (500, 1000)	1000 (500, 1000)	0.71
Mycophenolic Acid	9 (11)	6 (15)	3 (7)	0.31
Total daily dose (mg), median (range)	720 (500, 900)	810 (540, 1440)	540 (270, 810)	0.20
High dose mycophenolate	14 (22%)	9 (27%)	5 (16%)	0.37
Prednisone	75 (93)	37 (93)	38 (93)	>0.99
Total daily dose (mg), median (IQR)	5 (5, 5)	5 (5, 5)	5 (5, 5)	0.38
Tacrolimus	74 (91)	38 (95)	36 (88)	0.43
Cyclosporine	5 (6)	1 (3)	4 (10)	0.36
Triple IS, no. (%)	57 (70)	29 (70)	28 (68)	0.81
COVID-19 and vaccination history				
Prior SARS-CoV-2 infection[^], no. (%)	4 (5)	3 (8)	1 (2)	0.36
Days between 2nd and 3rd dose, median (IQR)	167 (149, 177)	158.5 (139.5, 174)	170 (154, 182)	0.047
Vaccine manufacturer, no. (%)				0.46

Pfizer-BioNTech (BNT162b2)	59 (73)	31 (78)	28 (68)	
Moderna (mRNA-1273)	22 (27)	9 (23)	13 (32)	
Laboratory results				
Creatinine (mg/dL), median (IQR)				
Day 0 (Baseline)	1.2 (1, 1.5)	1.15 (1, 1.5)	1.2 (1.1, 1.5)	0.51
Day 30	1.2 (1, 1.5)	1.25 (1, 1.5)	1.2 (1.1, 1.5)	0.62
Estimated GFR (ml/min/1.73m^2), median (IQR)				
Day 0 (Baseline)	58 (46, 73)	57.5 (49.5, 74.5)	59 (46, 73)	0.91
Day 30	59 (46, 72)	55.5 (48, 72)	60 (45, 71)	0.94
Baseline ALC (K/cu mm), median (IQR)	1.01 (0.69, 1.47)	1.03 (0.68, 1.52)	0.97 (0.7, 1.37)	0.73
Baseline Total IgG (mg/dL), median (IQR)	849 (737, 1031)	872 (760, 1033)	815 (732, 1031)	0.57
Baseline CD4+ T cell count, median (IQR)[++]	171 (114, 225)	172 (119, 225)	170 (114, 220)	0.57

[*] One participant had unavailable HLA donor type.
[**] Any combination of three immunosuppressants at Day 0.
[^] By positive prior molecular testing or reactive anti-nucleocapsid antibody at enrollment.
[+] Continuous outcomes compared by Wilcoxon rank sum testing and categorical variables were compared by Fisher's exact testing.
[++] T cell subtyping performed on n=34 participants (17 negative, 17 low-titer)

BMI, body mass index; IQR, interquartile range; FSGS, focal segmental glomerulosclerosis; DSA, donor-specific antibody; IS, immunosuppressant; GFR, glomerular filtration rate; ALC, absolute lymphocyte count; IgG, Immunoglobulin G; HCV, hepatitis C virus.

Table 2: Associations between clinical factors and Day 30 Anti-RBD level.

Factor	>2500 U/mL IRR	p-value (crude)	continuous titer RR	p-value (crude)	continuous titer aRR	p-value (adj)
Age (per 10 yr)	0.64 0.90 1.26	0.53	0.68 0.92 1.25	0.61		
Female sex	0.49 1.18 2.84	0.72	0.43 1.14 2.98	0.79		
mRNA-1273 vaccine	0.43 1.08 .2.72	0.87	0.33 0.91 2.46	0.85		
Mycophenolate (n=78)	0.29 0.77 2.05	0.61	0.33 0.64 1.27	0.20		
High-dose mycophenolate (n=79)	0.04 0.29 2.04	0.21	0.11 0.36 1.14	0.081	**0.02 0.06 0.20**	**<0.001**
Triple Immunosuppression	0.31 0.75 1.80	0.53	0.25 0.68 1.81	0.44		
Transplant vintage (per 5 yr)	**1.02 1.27 1.58**	**0.029**	0.85 1.16 1.60	0.35	0.79 0.95 1.15	0.59
Lymphocyte <1000 cell/uL	1.00 2.59 6.70	0.050	0.76 1.85 4.51	0.18		
Absolute CD4+ count (per 100) (n=33)	0.20 0.52 1.34	0.18	0.58 0.95 1.56	0.85		
Day 0 CD4+ breadth (per 10-fold) (n=63)	**1.13 3.94 13.70**	**0.031**	0.72 1.51 3.20	0.28		
Positive day 0 T-MAP™ (n=52)	**1.15 3.11 8.38**	**0.025**	0.48 1.74 6.34	0.40		
Day 30 CD4+ breadth (per 10-fold)	0.56 1.86 6.18	0.31	0.83 1.62 3.14	0.16	**1.10 2.12 4.11**	**0.026**
Positive day 30 T-MAP™ (n=55)	**1.40 10.37 77.00**	**0.31**	**1.11 3.06 8.46**	**0.031**		

Crude univariable associations are presented for the outcomes of high-titer anti-RBD response (>2500U/mL) and continuous anti-RBD level at day 30. An adjusted multivariable model for continuous anti-RBD response is also presented. All analyses excluded n=1 participant with incident COVID-19 and n=1 participant who received monoclonal antibody, whereas the mycophenolate analysis excluded n=1 additional participant with inconsistent medication use peri-vaccination (not prescribed high-dose mycophenolate). Bolded values represent statistical significance at the p<0.05 level.

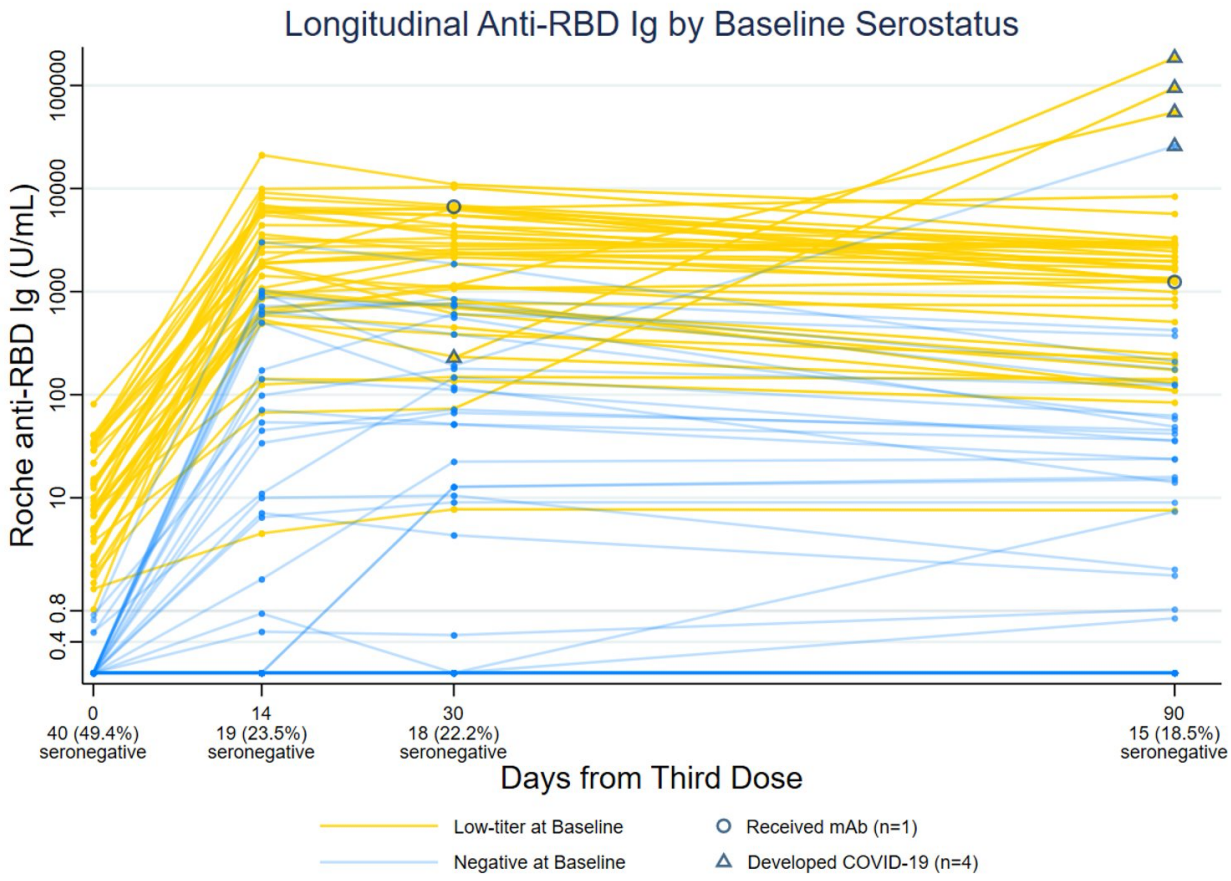
Table 3: Clinical and Immunological Characteristics of 13 Breakthrough SARS-CoV-2 Infections.

Age	Sex	Days since D3[*]	Variant Wave[**]	Day 0 Anti-RBD	Pre-Anti-RBD	Pre-Delta % ACE2i	Pre-Omicron % ACE2i	Pre-T-MAP	Days until post sample	mAb	Post-Anti-RBD	Post-Delta % ACE2i	Post-Omicron % ACE2i	C-19[+] Severity
50-59	M	13	Delta	LO	22	9.0%	1.3%	NEG	18	..	227.3	>99%	98.1%	Mild
30-39	M	74	Delta	LO	1145	11.8%	3.4%	POS	15	C/I	54850	>99%	22.0%	Mild
40-49	F	74	Delta	NEG	191	33.3%	4.8%	..	22	..	25760	72.9%	19.7%	Mild
50-59	M	76	Delta	LO	73.1	8.8%	6.8%	POS	16	C/I	94440	>99%	72.0%	Moderate
70-79	M	95	Omicron	LO	1983	13.5%	3.0%	NEG	86	..	7155	98.3%	71.5%	Mild
60-69	M	98	Omicron	NEG	123	4.5%	5.7%	..	77	ST	1469	73.6%	11.9%	Mild
70-79	M	98	Delta	NEG	<0.8	4.5%	8.1%	..	86	B/E	4091	90.5%	0%	Mild
40-49	F	99	Omicron	NEG	<0.8	4.5%	0.0%	..	..	..	..	..	..	Moderate
60-69	M	122	Omicron	LO	1348	6.3%	2.2%	..	51	ST	35680	90.4%	62.9%	Mild
50-59	F	126	Omicron	LO	1240[^]	98.0%	0.0%	POS	66	ST	10910	..	..	Mild
60-69	F	127	Omicron	NEG	<0.8	6.4%	9.0%	POS	61	ST	2603	39.3%	12.5%	Mild
70-79	M	128	Omicron	NEG	24	9.6%	6.6%	..	58	..	44617[+]	>99%	>99%	Mild
50-59	M	141	Omicron	LO	110	6.6%	2.7%	NEG	46	ST	..	53.1%	15.2%	Mild

[*] Date of PCR confirmation was used for 12 participants, and date of symptom onset was used in 1 participant without PCR confirmation.
[**] Delta wave defined as confirmed infection between August 1st and December 1st, 2021. Omicron wave (BA.1) defined as confirmed infection occurring between December 24th, 2021 and February 1st, 2022; there were no infections during the overlapping period of Delta and Omicron co-circulation December 1st-December 24th, 2021). Confirmatory sequencing was not performed.
[^] Received C/I on day 16 post vaccination.
[^^] Received a fourth vaccine dose (mRNA booster) before post-infection sampling.
[+] Mild disease defined as not requiring hospitalization and moderate disease defined as requiring hospitalization. One mild case was detected on asymptomatic screening while both moderate cases required supplemental oxygen by nasal cannula.

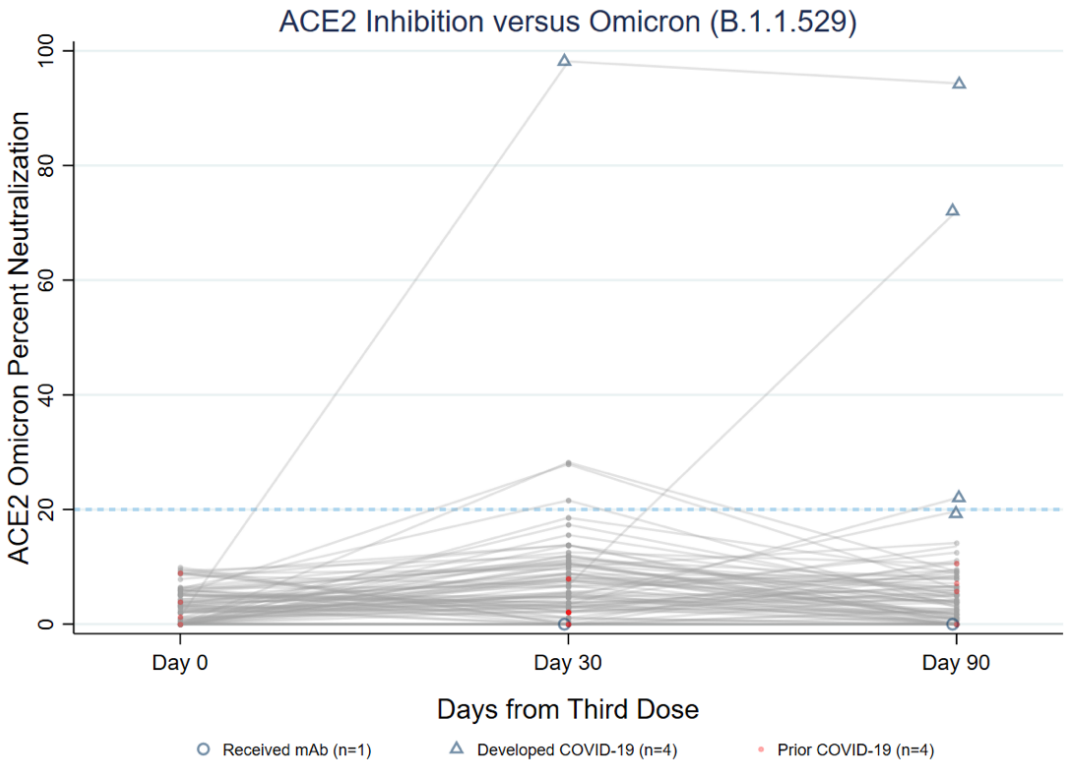
Abbreviations: %ACE2i percent ACE2 inhibition, B/E bamlinivimab/etesevimab, C/I casirivimab/imdevimab, C-19 COVID-19, D3 third mRNA vaccine dose, LO anti-RBD<50U/mL, mAb monoclonal antibody, NEG anti-RBD<0.8U/mL, ST sotrovimab

Figure 1: Anti-receptor binding domain (anti-RBD) titers following a third mRNA vaccine dose, stratified by day 0 anti-RBD level.



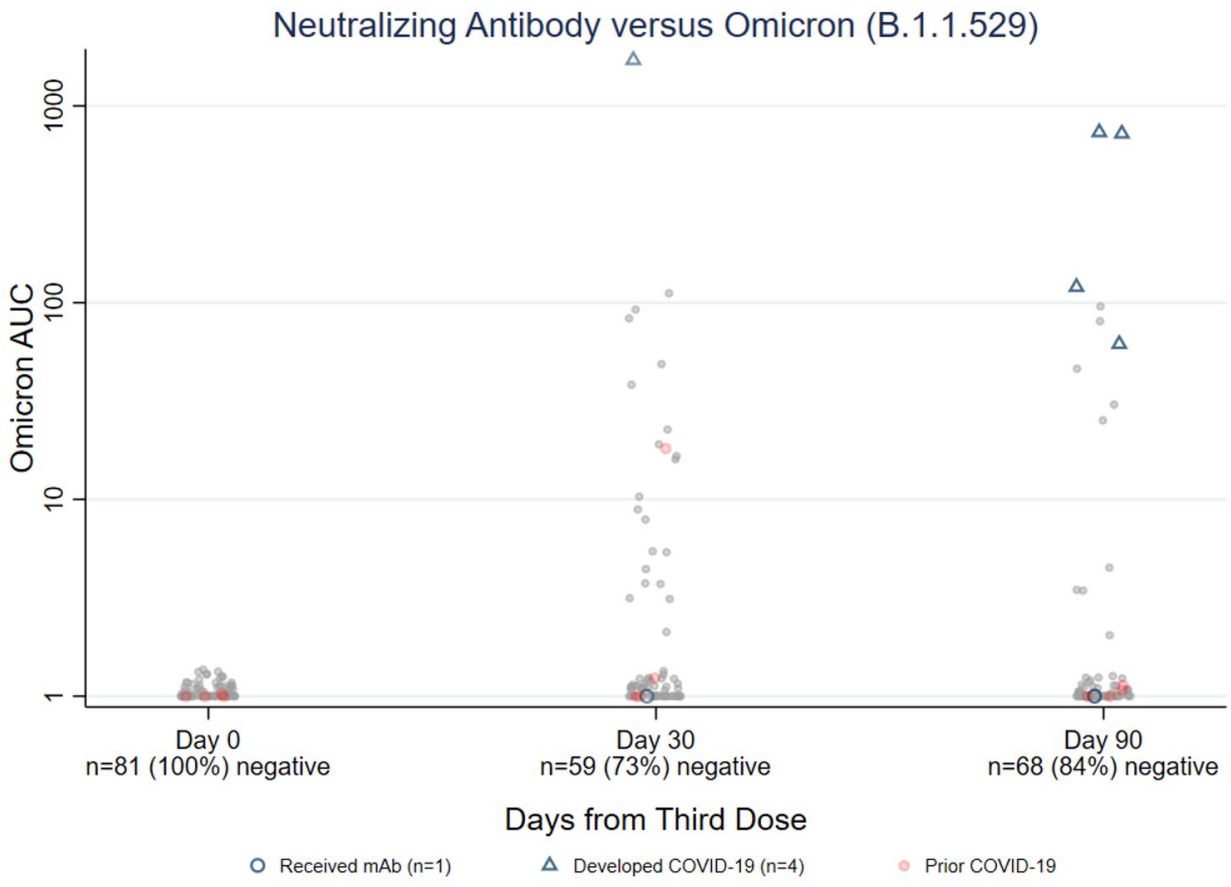
Blue trajectories represent anti-RBD^{NEG} (n=40) and yellow trajectories represent anti-RBD^{LO} low-titer (n=41). Anti-RBD titers are represented in U/mL on the logarithmic scale. Triangles represent participants who developed incident Covid-19 (n=4) and circles represent participants receiving monoclonal antibody (mAb) (n=1).

Figure 2: Surrogate plasma neutralizing capacity of Omicron BA.1 spike protein following a third mRNA vaccine dose, over time.



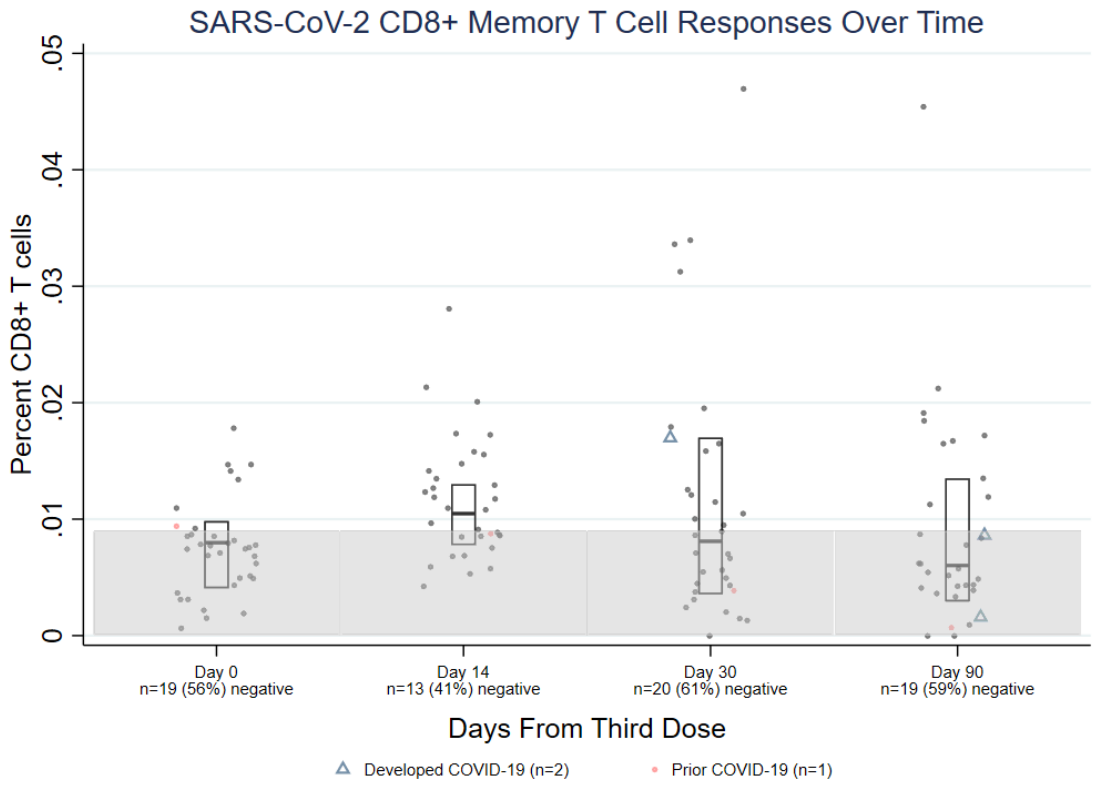
The Y axis represents percent ACE2 inhibition, ranging 0-100% with ≥20% consistent with neutralizing inhibition. Triangles denote participants with incident COVID-19 (n=4) and open circles denote participants receiving mAb (n=1). Red dots indicate participants with a prior history of COVID-19.

Figure 3: Area under the curve (AUC) of live Omicron BA.1 neutralizing antibody following a third mRNA vaccine dose, over time.



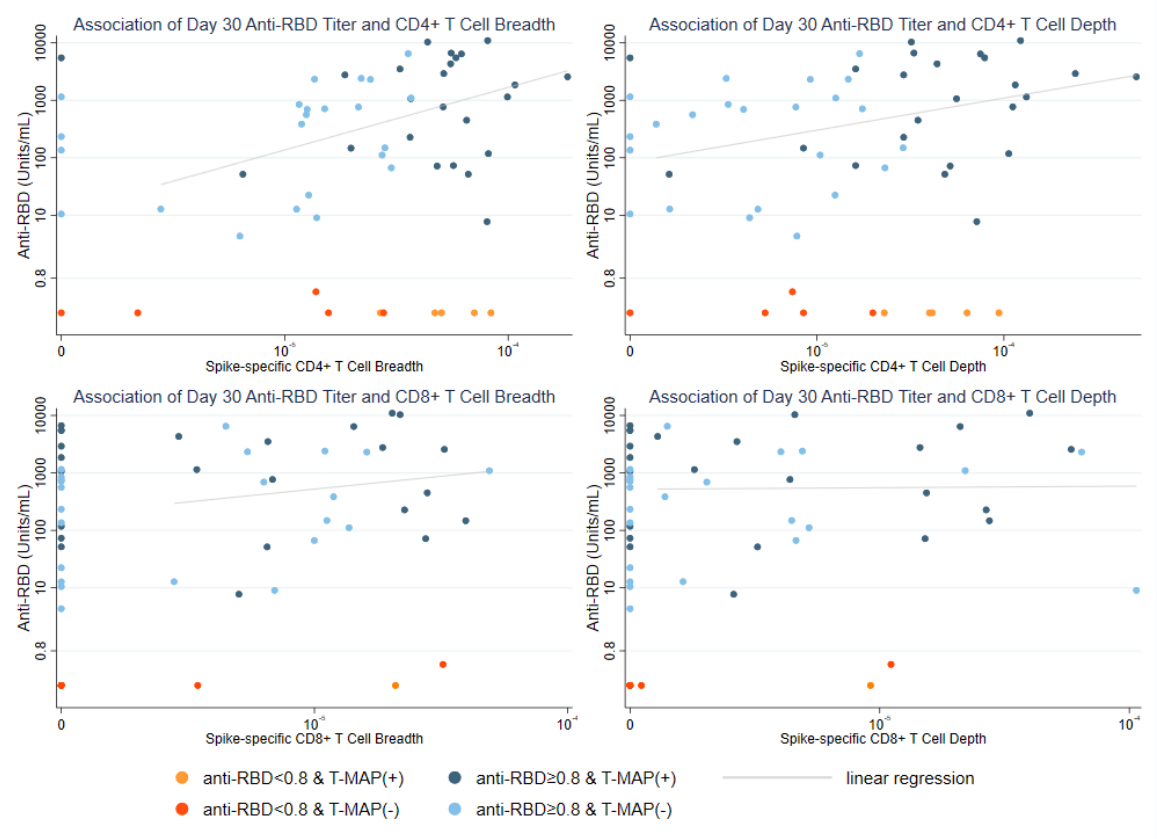
AUC is presented on the logarithmic scale. Triangles denote participants with incident COVID-19 (n=4) and open circles denote receipt of mAb (n=1). Red circles indicate participants with a prior history of COVID-19.

Figure 4: SARS-CoV-2-specific CD8+ memory T cell responses following a third mRNA vaccine dose, over time.



Flow cytometric data (epitope staining) are presented for HLA-A*02 participants. The grey shading denotes negative T cell responses (<0.009% background threshold), with the proportion of negative responses presented in the X axis labels. Triangles denote participants who developed COVID-19 (n=2).

Figure 5: Correlation of SARS-CoV-2 antibody and T cell responses after a third mRNA vaccine dose, by immune phenotype.



Scatterplot of anti-RBD level and dimensions of SARS-CoV-2 T cell receptor expansion (spike-specific CD4+ and CD8+ breadth and depth) on the logarithmic scale at day 30 post vaccination. Data points are colorized by immune phenotype, based on binary anti-RBD and TMAP™ response (SARS-CoV-2-reactive T cell repertoire). The trend lines represent correlation between anti-RBD response and TCR expansion among participants with non-negative signatures, i.e., (+)anti-RBD and non-zero SARS-CoV-2-reactive TCRs.

Chapter 5. Conclusion

The works outlined in this dissertation utilize observational and clinical trials data to study two key groups of SOT recipients who, based on distinctive immunological substrate, require personalized approaches to reducing risk for serious post-transplant infections. This included two studies of HIV+ SOT recipients and one clinical trial of SOT recipients with poor SARS-CoV-2 vaccine response. The first study assessed the landscape of infectious risk associated with utilization of organs from donors with HIV, which revealed high rates of bacterial and viral coinfection, yet reassured against risks of HIV breakthrough due to multidrug resistant virus. The second study, a national registry analysis of HIV+ KT recipients quantified the increased risk of acute organ rejection associated with the common practice of early steroid withdrawal, revealing a contributing factor to the deleterious rejection-infection cycle. Finally, in addressing the threat of the COVID-19 pandemic to SOT recipients, a clinical trial was conducted to evaluate the targeted intervention of an additional SARS-CoV-2 vaccine dose in high-risk poor antibody responders; the analysis revealed variable augmentation of immunoprotection and suggested ongoing vulnerability to the Omicron variant. These findings demonstrate the importance of dedicated study of unique SOT subpopulations, and have both specific and overarching implications regarding approaches to mitigate serious infection burden in these individuals.

Implications for Infection Screening and Prophylaxis in HIV+ SOT

The HOPE in Action HIV+ donor study (Chapter 2) captured 99% of all HIV D+/R+ SOTs in the US and detailed several interesting demographic and immunological donor characteristics. Namely, two-thirds of donors were prescribed ART and half showed HIV VL suppression on donation, yet 20% demonstrated a severely immunocompromised phenotype of high-level HIV viremia and AIDS-defining CD4%. This represents a potential higher-risk donor profile for OI transmission and suggests targeted monitoring and counseling of recipients of these organs may be indicated. Apart from less common ART use, there were no other clear distinguishing donor factors for this group, though if historical CD4% were accessible there was high correlation with CD4% on donation ($r=0.72$).

In keeping with data from the general population of PWH, donors with HIV demonstrated higher rates of certain coinfections, namely HBV, CMV, and syphilis, versus contemporaneous donors without HIV, highlighting a role for tailored antimicrobial prophylaxis in HIV D+/R+ SOT. Specifically, these findings would support the use of HBV-active ART in recipients as well as and donor screening for sexually transmitted infections as modifiable risk factors for post-transplant infections. Importantly, although donor HIV DRMs were quite common (42%), multidrug resistance that might compromise standard INSTI-based post-transplant ART regimens was very rare (2%). This supports a mechanism for the minimal observed rates of HIV breakthrough following HIV D+/R+ SOT in the US and should reassure waitlist candidates and providers against this theoretical risk.

Although this study did not directly link donor HIV status to recipient infections, other published studies of HIV+ SOT have demonstrated conflicting data regarding differential risks of infection, potentially varying by transplanted organ. For example, the HOPE in Action HIV+ KT trial did not demonstrate statistically significant difference in serious infections or HIV breakthrough incidence by donor HIV status.[128] There were numerically higher CMV reactivations with HIV D+ versus HIV D- transplant (12% vs 6%, p=0.39), yet only two episodes of atypical OIs (bartonellosis [HIV D+] and Kaposi Sarcoma [HIV D-]; weighted IRR for OI $_{0.80}2.44_{7.42}$). In contrast, the HIV+ LT study demonstrated significantly higher rates of OI (33% vs 14%, p=0.049; weighted IRR $_{1.32}5.37_{21.83}$) and infection-associated hospitalizations (weighted IRR $_{1.43}4.25_{12.62}$).[129] There was also numerically higher incidence of cancer (25% vs 10%, p=0.25) among HIV D+ versus HIV D- transplants. These differences were largely driven by herpesvirus-related complications, namely CMV as well as human herpesvirus 8 (HHV8) reactivation (causing Kaposi's sarcoma and lymphoma). Human papilloma virus-associated malignancies were also seen in 2 HIV D+ vs 0 HIV D- transplants.

Overall, these emerging findings support the work in this dissertation indicating infection-associated complications are relatively common after HIV+ SOT and may represent a different spectrum than those experienced by HIV- SOT recipients, particularly in the setting of HIV-to-HIV transplants. The notable contribution of viral infections, likely owing to a combination of higher rates of coinfection as well as complex immunodeficiency of donor and recipient, represents an opportunity to target preventative measures. For example, stemming from these findings, epidemiological and

translational work regarding the contribution of donor and recipient HHV8 serostatus to post-transplant morbidity in HIV+ SOT is currently underway.

Optimizing Immunosuppression in HIV+ SOT: Curbing the Infection-Rejection Cycle

In Chapter 3, the comprehensive nature of the SRTR was leveraged to analyze outcomes among the largest cohort of HIV+ KT recipients to date and test the hypothesis that steroid maintenance strategy may affect major transplant outcomes in this group. Indeed, ESW was associated with 39% higher hazard of AR during follow up versus SC, with a crude absolute risk increase of 6.1% by 1 year; nearly a quarter of HIV+ KT recipients undergoing ESW experienced AR by 3 years post-transplant and, unlike in the SC group, there was no evidence of a positive "period effect" to reflect improvements related to antiviral advances. Strikingly, there was major variation in ESW among transplant centers (0-90% utilization), without clear association to demographic or transplant characteristics, indicating an opportunity to harmonize immunosuppressive practices in this group. Although there was no signal for worsened graft failure or death associated with ESW, the SRTR is insensitive for capturing incident infections or associated morbidity such as hospitalizations, which is a needed area of future study and would require linkage to complementary datasets.

Notably, the study population in this work was >99% HIV D-/R+, limiting inferences for HIV D+/R+ SOT, though given rates of rejection may be even higher in this latter group, a cautious approach to immunosuppressive reduction in advisable.[128] In particular, the clear association between rejection and avoidance of potent lymphodepleting induction in the

HOPE in Action trials indicate that measures such as ESW may have further deleterious impact on rejection rates in HIV D+/R+ SOT, particularly at transplant centers that avoid lymphodepletion.

An interesting feature of this analysis includes the first application of interval censoring survival modeling to SRTR data, which represents an additional means to longitudinally analyze immunosuppressive data for HIV+ SOT recipients and test for associations with adverse outcomes. This includes opportunities to use pharmacoepidemiologic analysis to test whether certain immunosuppressive regimens reduce or exacerbate the burden of viral infections seen in HIV+ SOT. For example, use of the common antimetabolite mycophenolate is associated with lymphocyte impairments and predisposition to viral infections such as CMV the general SOT population.[130] Whereas, in contrast, mammalian target of rapamycin (mTOR) inhibitors show antiviral and antitumirogenic effects including versus HHV-8-related diseases.[131] Taken together, these findings suggest that there is considerable opportunity to optimize immunosuppressive regimens in HIV+ SOT to not only reduce rejection incidence and downstream infectious complications, but to potentially tailor regimens to mitigate the unique risks of viral infections in this group.

Improving Immunoprotection versus COVID-19 for SOT Recipients

The final scientific work in this dissertation, a Phase II clinical trial of third SARS-CoV-2 mRNA vaccines in KT recipients (Chapter 4), reflects the imperative to creatively and rapidly enroll studies that may improve protection amid the ongoing COVID-19 pandemic. This trial built upon observational data to identify highest-risk KT recipients with poor

anti-RBD response and then applied a translational approach to clarify several aspects of vaccine response, while generating hypotheses regarding underlying mechanisms. Specifically, the trial confirmed that preceding anti-RBD serostatus was a powerful clinical biomarker to predict anti-RBD response to subsequent vaccinations; nearly half of anti-RBD^{NEG} KT recipients did not achieve simple seroconversion to a third mRNA dose and none demonstrated neutralization of the Omicron variant. Certain accessible clinical and transplant features were associated with persistently poor vaccine response, such as younger transplant vintage and in particular the modifiable factor of of high-dose MMF ($aRR=_{0.02}0.06_{0.20,}$ $p<0.001$ for higher anti-RBD response).

An additional goal of this trial was to crystallize complex immunological data into digestible phenotypes of vaccine response for the transplant clinician, which revealed that even among this homogenous high-risk group of KT recipients with poor baseline anti-RBD response, there was substantial variability in dimensions of humoral and cellular immunogenicity. For example, expansions in the SARS-CoV-2-specific CD4+ T cell repertoire were associated with higher anti-RBD response in many participants, yet a subset of participants with broad CD4+ expansion were unable to generate any anti-RBD response and thus remained at high risk for COVID-19. Indeed, participants with persistent low anti-RBD represented the majority of those who experienced breakthrough infections during follow up. Indirect clinical and immunological data suggested that B cell deficiencies may be playing a role, potentially related to high-dose MMF and its effects on immunometabolism, though this hypothesis is currently undergoing intensive investigation.

This work complements a large volume of observational data indicating that the anti-RBD biomarker, specifically seronegativity, is a strong risk factor for vulnerability to SARS-CoV-2 infection.[132] For example, early case series indicated that 47/57 (82%) of SOT recipients with COVID-19 breakthrough following vaccination were found to be anti-spike or anti-RBD negative.[107,108,133] Furthermore, similar to findings in the CPAT trial, several later series studying third and fourth vaccine doses noted that only 20-50% of SOT recipients with preceding negative anti-spike or anti-RBD antibody seroconverted to a subsequent dose, typically to lower levels.[29,105,134,135] Coupled with lack of robust CD8+ T cell response (i.e., antibody-independent antiviral responses) to third mRNA vaccine doses in the CPAT trial, efforts to develop alternative vaccination strategies to improve immunoprotection in SOT recipients are vital. This includes a role for targeted modulation of immunosuppression, particularly holding or decreased MMF peri-vaccination,[124] which is subject of the active multicenter CPAT Immunosuppressive Reduction Trial (NCT05077254). Passive immunoprophylaxis for monoclonal antibody combinations[136] is another important complementary protective measure for SOT recipients with persistent poor anti-RBD response to vaccination, though these therapies must be continuously updated in order to compete with evolving immune escape of SARS-CoV-2 variants.[137]

Paths Forward: Utilizing Data to Mitigate Infectious Diseases after Transplant

A theme referenced throughout this dissertation is the symbiotic nature of observational and clinical trials data in understanding infectious risks after SOT. The history of HIV+ SOT began with a landmark trial to confirm feasibility and tolerable OI incidence, yet the analysis of observational data on a national scale was necessary to document survival

benefit, secular trends, as well as identify nuances such as immunosuppressive selection that might optimize outcomes. The sequence may soon be repeated with HIV D+/R+ SOT, as analysis and lessons from the HOPE in Action trials lay groundwork for adoption of this practice as standard of care in the US, after which observational analyses can again iterate upon management strategies such as infection prophylaxis and immunosuppressive selection as the practice expands. In similar fashion, the science of COVID-19 and transplantation was characterized by rapid accrual and dissemination of observational data, which within months identified serious outcomes and stratification schema for SOT recipients who appeared to remain at risk despite vaccination. This motivated investment in clinical trials to confirm observational findings as well as a shift from incremental data reporting to comprehensive mechanistic study of SARS-CoV-2-specific immunodeficiency, which is a much-needed model in applying translational data to infectious diseases practice in the 21st century.

Truly, the COVID-19 pandemic also demonstrated what global mobilization of academic, governmental, and industry "biomass" can accomplish in accelerating knowledge about an infectious disease in real-time. This includes unmatched research attention and output in both the epidemiological and translational spaces that have led to rapid development and assessment of diagnostics, therapeutics, and preventative measures against a ubiquitous virus that continues to threaten SOT recipients. As evidenced by the CPAT trials of SARS-CoV-2 vaccination, a high level of scientific rigor can be applied toward untangling the mechanisms of vulnerability to key pathogens. These efforts are inspiring, particularly in their creativity and collaborative nature, and it seems probable that other major infectious

complications after SOT, such as the currently incurable herpesviruses, could be surmounted if stakeholders applied similar energy, innovation, and funding.

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Appendix

SUPPLEMENT TO CHAPTER 2

Supplemental Table 1: Donor HIV screening assays.

Initial Screening Assays[a]	HIV+ Donor Reactive/Total	FP Donor Reactive/Total
Anti-HIV I/II Ab	58/58	27/34
Genetic Systems® HIV-1/HIV-2 Plus O EIA	52/52	24/31
Abbott® Prism HIV O Plus	5/5	3/3
Unspecified	1/1	..
Qualitative NAT	40/58	5/34
Cobas® Taqscreen MPX[b]	16/19	3/14
Procleix® Ultrio	15/23	2/18
Procleix® Ultrio Elite	3/10	0/1
Procleix® Ultrio Plus	5/5	0/1
Unspecified	1/1	..
Ab/Ag		
Bio-Rad BioPlex 2200	..	1/1
Unspecified	0/1	..

[a] Per manufacturer data: Sensitivity for utilized Ab assays was 100%, specificity range 99.89-99.94%. Sensitivity for utilized NAT assays ranged 99.6-100%, specificity range 99.6-100%. See Durand et al., Am J. Transplant 2018, Oct; 18(10): 2579-2586.

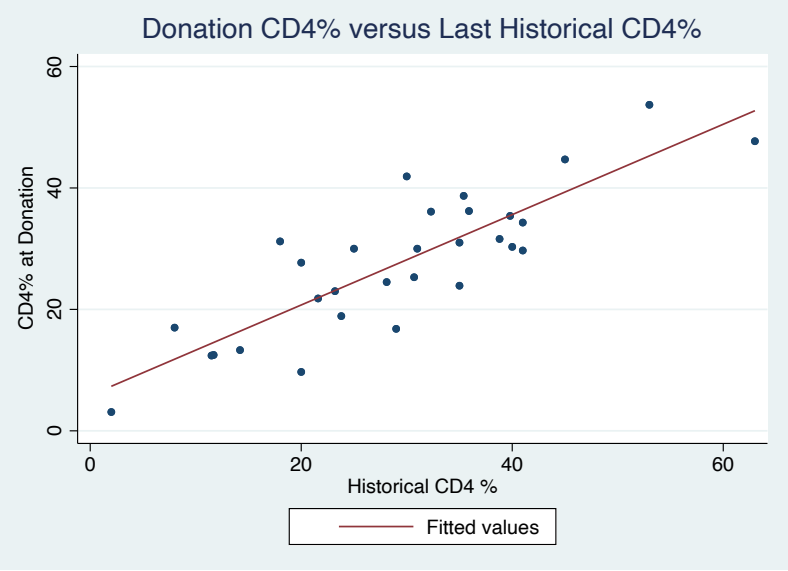
[b] Some donor laboratory reports did not explicitly identify the version or generation of Cobas® MPX assay.

Confirmatory screening assays: Among FP donors with reactive Ab, confirmatory negative assays included Western Blot (74%), fourth-generation Ab/Ag testing (7%), or both (15%). All five FP donors with reactive screening NAT underwent confirmatory negative quantitative PCR testing. For the donor with reactive Ab/Ag, FP status was confirmed by negative discriminatory NAT testing and the FP donor with inaccurate historical record was confirmed by negative fourth-generation Ab/Ag testing.

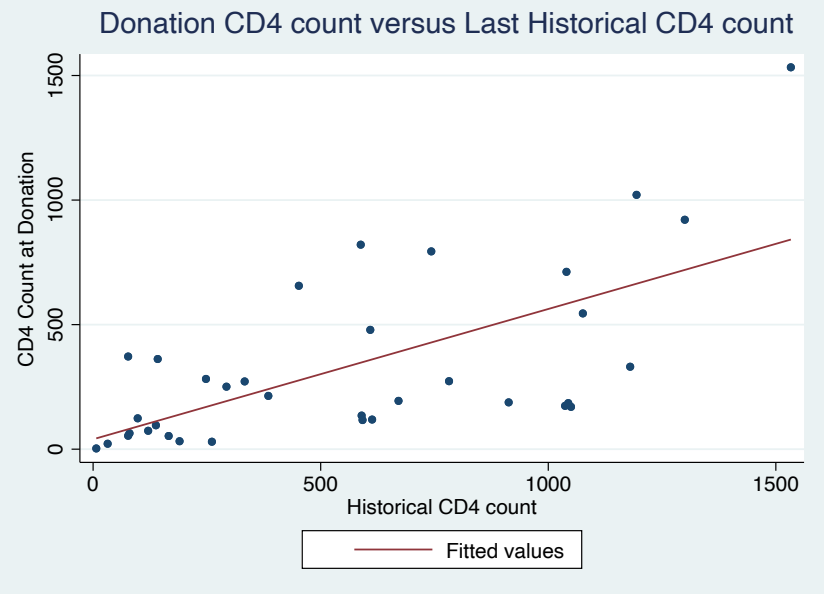
Supplemental Figure 1: Correlation between donation CD4 measures (% and counts) and last historical CD4 values.

30 HIV+ donors had both historical and donation CD4% available for analysis, which were highly, positively correlated (r=0.72) (S1a). These were predominantly donors on ART (24/30, 80%). 35 donors had both historical and donation CD4 counts, which were moderately, positively correlated (r=0.43). These were also predominantly donors on ART (29/35, 83%).

1a:

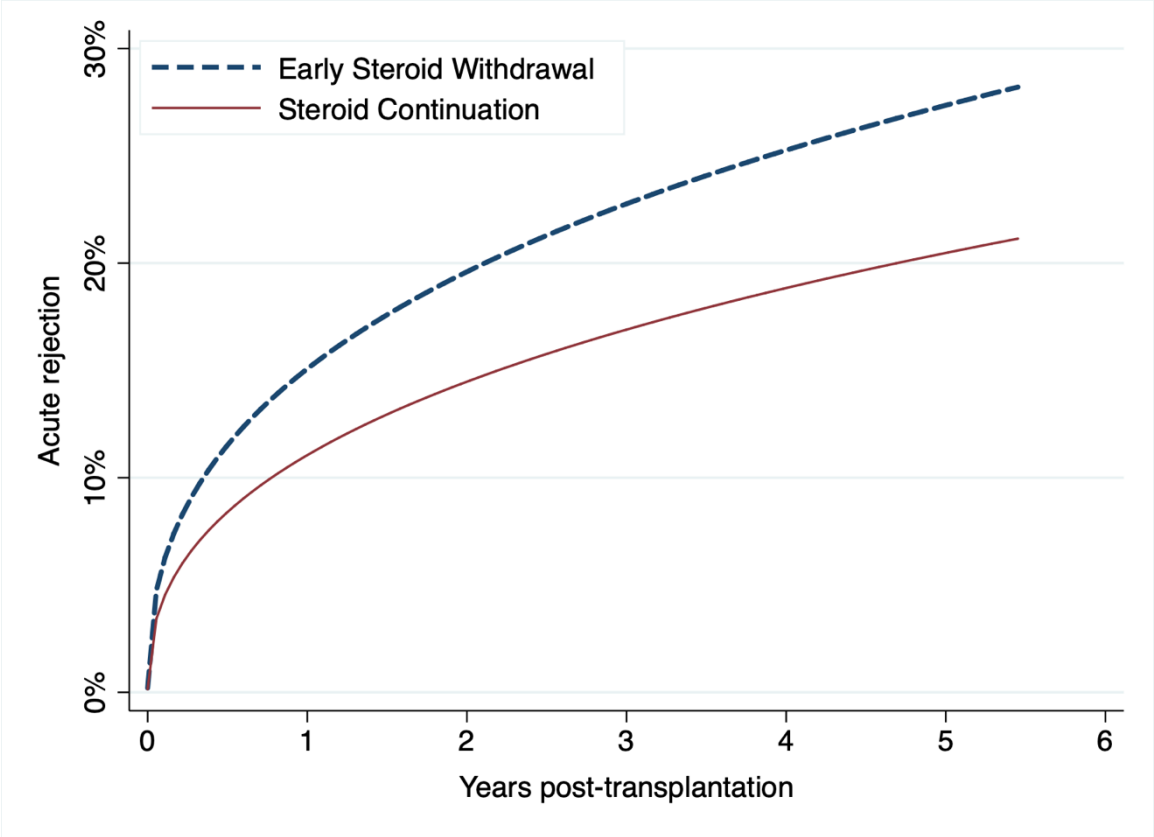


1b:



SUPPLEMENT TO CHAPTER 3

Supplemental Figure 1: Adjusted interval-censored survival analysis of time until acute rejection among HIV+ KT recipients undergoing early steroid withdrawal (ESW) versus steroid continuation (SC). The point estimate for ESW inflated further away from the null after adjustment (aHR $_{1.02}1.39_{1.90}$, p=0.03).



SUPPLEMENT TO CHAPTER 4

Methods

Study Background and Synopsis

The COVID-19 Protection After Transplant (CPAT) trials are a National Institutes of Health-funded clinical trials effort designed to investigate the safety and immunogenicity of COVID-19 vaccination strategies in solid organ transplant (SOT) recipients. The CPAT Pilot Study (ClinicalTrials.gov, NCT04969263) is a single-arm, open-label trial launched August 10, 2021 to test antibody and cellular responses to an additional (third) dose of homologous mRNA vaccination in kidney transplant recipients who failed to respond to two prior mRNA vaccine doses.

"Failure to respond" was defined anti-RBD response <50 U/mL on the Roche Elecsys anti-SARS-CoV-2 S assay. This assay was selected given in, convalescent persons and vaccinees, high correlation with neutralizing activity against SARS-CoV-2 variants. Manufacturer data suggested a cutoff of 15 U/mL concorded well with 20% ACE2 blocking (surrogate neutralization) of ancestral variants in convalescent persons, with negative and positive agreements of 90%[113]. Additionally, a cutoff of 133 U/mL maximized operating characteristics to correlate with "high level" (NT≥1:160) live virus neutralization versus ancestral variants in convalescent plasma donors[114]. A modeling study of the relationship between NT and vaccine efficacy across published trials indicated a level 2-4-fold higher of vaccine-evoked NT than that seen in convalescents using the same assays best correlates with vaccine efficacy[138]. Thus, a level of 50 U/mL was deemed a reasonable screening cutoff, i.e., a conservative lower bound under which neutralizing antibody versus ancestral variants in mRNA vaccinees was not expected.

Neutralization Assays

Meso Scale Discovery (MSD) ACE2 inhibition assay (surrogate neutralization)

Plasma from study participants was thawed and ACE2 blocking measured using the ACE2 MSD V-PLEX SARS-CoV-2 Panel 13 and 23 kits according to the manufacturer's protocol at a dilution of 1:100. Specifically, plates were pre-coated with spike proteins corresponding to variants of interest (i.e., expressing key mutations). The plates were washed and incubated with plasma for one hour followed by the addition of human ACE2 protein conjugated with a SULFO-TAG (light-emitting label) for another hour. The plates were then washed, read buffer added, and the plates were read with a MESO QuickPlex SQ 120 instrument per the manufacturer's instructions. At least four wells were left blank for calibration to 0% inhibition. Results were reported as percent ACE2 inhibition based on the equation provided by the manufacturer (1 – Average sample ECL/Average ECL signal of blank well) x100.

Live virus neutralization

VeroE6-TMPRSS2 cells19 were cultured in complete media (CM) as previously described[116]. The SARS-CoV-2/USA-WA1/2020 virus was obtained from BEI Resources. The SARS-CoV-2 Delta (hCoV19/USA/MD-HP05660/2021, EPI_ISL_2331507) and Omicron BA.1 (hCoV19/USA/MD-HP20874/2022, EPI_ISL_7160424) variants were isolated on Vero-E6-

TMPRSS2 cells plated in 6-well dishes grown to 75% confluence. CM was removed and replaced with 150 µl of infection medium (IM), which is identical to CM but contains only 2.5% fetal bovine serum, and 150 µl of the viral transport media containing a swab from a patient with SARS-CoV-2 positive. The cultures were incubated at 37°C for 2 h, the inoculum was aspirated and replaced with 0.5 mL of IM and the cells cultured at 37°C for 5 days. Cell supernatant was harvested when cytopathic effect was visible in 75% of the cells and stored at −70°C. SARS-CoV-2 was verified by extracting RNA using a viral RNA extraction kit (Qiagen), and detected using quantitative RT-PCR. The consensus sequence of the virus isolate did not differ from the sequence derived from the clinical specimen. Viral titer was determined on VeroE6-TMPRSS2 cells using a 50% tissue culture infectious dose ($TCID_{50}$) assay as previously described.[117]

Neutralizing antibody (nAb) levels were determined as described using twofold dilutions of plasma (starting at 1:20).[7] Infectious virus was added to the dilutions at a concentration of 1 × 10^4 TCID50/ml (100 TCID50 per 100 µl). Samples were incubated for 1 hour then 100 µl of each dilution was added to 1 well of a 96-well plate of VeroE6-TMPRSS2 cells in sextuplet for 6 hours at 37˚C. The inocula were removed, fresh IM was added, and the plates were incubated at 37˚C for 2 days or until complete cytopathic effect was visible in wells exposed to only virus. The cells were fixed with 4% formaldehyde, incubated for 4 hours, and then stained with Napthol Blue Black (MilliporeSigma). The nAb titer was calculated as the highest serum dilution that eliminated the cytopathic effect in 50% of the wells and area under the curve (AUC) was calculated using GraphPad Prism.

Neutralizing antibody imputation

All 81 participants had authentic live virus nAb assays performed for the ancestral and Delta variants at day 30 post third vaccine dose, while 75/81 (93%) had live virus nAb assays performed versus the Omicron BA.1 variant. The remaining 6 participants had negative assays (NT_{50} <1:20) versus the ancestral and delta variant at day 30, thus their day 30 Omicron nAb was imputed as negative given need for 10-20-fold higher titers to neutralize this variant of concern[118]. Day 90 nAb was selectively performed among n=28 participants with at least one of the following: (i) detectable nAb vs Omicron at day 30, (ii) intercurrent diagnosis of COVID-19 by positive molecular testing, clinical suspicion, or anti-nucleocapsid seroconversion, or (iii) receipt of monoclonal antibody product between day 30 and day 90. For the remaining 52 participants (1 participant did not present for day 90 testing), day 90 nAb was imputed as zero (NT_{50}<1:20) supported by the following data: 52/52 had negative Omicron nAb at day 30 and negative anti-N antibody at day 90, with a median (IQR) anti-RBD titer 45 U/mL (1-187) and median (IQR) Omicron spike ACE2 inhibition of 4.1% (1.7-6.4), consistent with negligible neutralizing capacity.

Cellular Analyses and Methodology

SARS-CoV-2 Spike-specific CD8+ Memory T cell Pentamer Staining

PBMCs from all HLA-A*02:01+ recipients were washed once in PBS and immediately stained for viability with Biolegend Live/Dead Zombie NIR Fixable Viability Dye, BD Fc Block™, and four biotinylated pentamers with immunodominant epitopes for the SARS-CoV-2 spike protein (ProImmune, sequences: FIAGLIAIV, LITGRLQSL, YLQPRTFLL, RLQSLQTYV) [119,120] for 10 min at room temperature. Cell surface staining was performed in 100uL of 20% BD Horizon™ Brilliant Stain Buffer + PBS with surface stain antibody cocktail for 20 min at 4°C. Cells were fixed and permeabilized with eBioscience™ FoxP3/Transcription Factor Staining kit 1x Fixation/Permeabilization reagent for 20 min at room temperature. Cells were washed with 1x Permeabilization/Wash buffer. Intracellular staining (ICS) was performed in 100uL 1x Permeabilization/Wash buffer with ICS antibody cocktail for 45 min at room temperature. Cells were washed once with Permeabilization/Wash buffer then resuspended in 1% Paraformaldehyde for acquisition by flow. Samples were run on a 4 laser Cytek Aurora spectral flow cytometer. FCS files were analyzed using Flowjo software (10.6.2). The frequency of spike-specific CD8 T cells was evaluated out of total memory CD8 T cells (gated on live CD3+CD4-CD8+ and excluding naïve CCR7+CD45RA+ T cells).

Surface Staining

Color	Marker	Company	Catalog Number	Clone
BV510	CD25	Biolegend	310830	24-31
BV650	CCR7	Biolegend	353234	G043H7
PE-CF594	KLRG1	BD	565393	2F1
APC	CD57			
APC Fire 750	CD28			
BUV 737	CD8			
BUV496	CD4			
BUV615	PD1	BD	612991	EH12.1
BUV661	CD3	BD	612964	UCHT1
BV605	CD95			
BV750	CX3CR1	BD		2A9-1
Spark NIR	CD27	Biolegend		
BV785	DNAM1			11A8
BV570	CD45RA	BD	612926	HI100
PECy5.5	CD69	Thermo	MHCD6918	CH/4
PE Cy7	CXCR5			
BV711	CD38			
PE Cy5	Streptavidin			

Intracellular staining

PacBlue	FOXP3	Biolegend	320116	206D
Alexa Fluor 488	CPT1a AF488	Abcam	ab171449	8F6AE9
Alexa 680	HK2 Ax680	Abcam	ab228819	EPR20839
PE	H3K27me3 PE	CST	#40724	C36B11
Alexa Fluor 532	VDAC1 AF532	Abcam	ab14734	20B12AF2
AF405	Tomm20 AF405	Abcam	ab210047	EPR15581-54
Alexa647	GLUT1 AF647	Abcam	ab195020	EPR3915

Immunosequencing of TCR Repertoires:
Sample vials from participants with sufficient viable PMBCs at day 0 and day 30 following vaccination (n=65) were processed, frozen, and sent to Adaptive Biotechnologies (Seattle, WA) for immunosequencing of the CDR3 regions of TCRβ chains using the immunoSEQ Assay®. Genomic DNA was extracted and amplified using bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered to identify and quantitate the absolute abundance of each unique TCRβ CDR3 region for further analysis as previously described[139–141].

Characteristics of prior response to SARS-CoV-2, including the breadth (proportion of unique TCR clonal rearrangements), depth (proportion of all productive TCR templates), CD4/CD8 response, and epitope-specific response were analyzed using immunoSEQ TMAP™ COVID (the same machine-learning classifier used in Adaptive Biotechnologies' Food and Drug Administration-approved T-Detect assay). Briefly, TCRs from T-cell repertoires were mapped against a set of TCRs that are known to react to SARS-CoV-2. These sequences were first identified by Multiplex Identification of T-cell Receptor Antigen Specificity (MIRA)[121]. Reactive TCRs were further screened for enrichment in COVID-19 positive repertoires collected as part of immuneCODE[122] as compared to repertoires from negative controls to remove TCRs that may be highly public or cross-reactive to common antigens. Samples were quantified by both breadth and depth of SARS-CoV-2 TCRs captured. TCRs were further analyzed at the ORF or position within ORF based on the MIRA antigens.

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Supplemental Table 1. Demographic and transplant characteristics of anti-RBD^{NEG} participants who remained anti-RBD negative versus anti-RBD positive at day 30

	Total (N=40)	Anti-RBD negative at day 30 (N=18)	Anti-RBD positive at day 30 (N=22)	p-value^{+}
Demographics				
Age (years), median (IQR)	66 (57, 73)	68 (60, 74)	64 (56, 72)	0.60
Female sex, no. (%)	16 (40%)	10 (56%)	6 (27%)	0.11
Race, no. (%)				0.94
White	22 (55%)	11 (61%)	11 (50%)	
Black/African American	13 (33%)	5 (28%)	8 (36%)	
Asian	4 (10%)	2 (11%)	2 (9%)	
BMI (kg/m^2), median (IQR)	27.4 (23.2, 31.9)	26.4 (22.3, 32.5)	27.6 (23.9, 30.2)	0.87
Medical comorbidities				
Diabetes, no. (%)	14 (35%)	6 (33%)	8 (36%)	1.00
HCV infection, no. (%)	1 (3%)	1 (6%)	0 (0%)	0.45
Lung disease, no. (%)	8 (20%)	6 (33%)	2 (9%)	0.11
Cardiovascular disease, no. (%)	34 (85%)	16 (89%)	18 (82%)	0.67
Autoimmune disease, no. (%)	5 (13%)	2 (11%)	3 (14%)	1.00
Transplant history and immunosuppression				
Years since transplant, median (IQR)	5.2 (2.0, 9.3)	6.7 (2.3, 13.0)	4.2 (2.0, 8.4)	0.29
Indication for most recent kidney transplantation, no. (%)				
Diabetes	6 (15%)	3 (17%)	3 (14%)	1.00
Hypertension	17 (43%)	6 (33%)	11 (50%)	0.35
FSGS	5 (13%)	4 (22%)	1 (5%)	0.16
Glomerulonephritis	1 (3%)	1 (6%)	0 (0%)	0.45
Cystic kidney disease	3 (8%)	1 (6%)	2 (9%)	1.00
Living donor, no. (%)	14 (35%)	7 (39%)	7 (32%)	0.74
DSA Positive at baseline, no. (%)[*]	8 (20%)	4 (22%)	4 (18%)	>0.99
Baseline Immunosuppressant, no. (%)[b]				
Mycophenolate Mofetil	27 (68%)	12 (67%)	15 (68%)	>0.99
Total daily dose (mg), median (IQR)	1000 (500, 1000)	1000 (875, 1250)	1000 (500, 1000)	0.30
Mycophenolic Acid	6 (15%)	3 (17%)	3 (14%)	>0.99
Total daily dose (mg), median (range)	810 (540, 1440)	1440 (720, 1440)	540 (500, 900)	0.12
High-dose mycophenolate	9 (23%)	5 (28%)	4 (18%)	0.71
Prednisone	37 (93%)	17 (94%)	20 (91%)	>0.99
Tacrolimus	38 (95%)	18 (100%)	20 (91%)	0.49
Cyclosporine	1 (3%)	0 (0%)	1 (5%)	>0.99
Triple IS, no. (%)	29 (73%)	14 (78%)	15 (68%)	0.72
COVID-19 and vaccination history				
Prior SARS-CoV-2 infection[^], no. (%)	3 (8%)	1 (6%)	2 (9%)	>0.99
Days between 2nd and 3rd dose, median (IQR)	159 (140, 174)	163 (145, 174)	156 (138, 175)	0.45
Vaccine manufacturer, no. (%)				>0.99
Pfizer-BioNTech (BNT162b2)	31 (78%)	14 (78%)	17 (77%)	
Moderna (mRNA-1273)	9 (23%)	4 (22%)	5 (23%)	
Laboratory results				
Creatinine (mg/dL), median (IQR)				
Day 0 (Baseline)	1.15 (1, 1.5)	1.1 (0.8, 1.4)	1.25 (1.1, 1.6)	0.25
Estimated GFR (ml/min/1.73m^2), median (IQR)				
Day 0 (Baseline)	58 (50, 75)	61 (49, 78)	57 (50, 70)	0.70
Baseline ALC (K/cu mm), median (IQR)	1.03 (0.68, 1.52)	0.77 (0.59, 1.36)	1.16 (0.93, 1.57)	0.052
Baseline Total IgG (mg/dL), median (IQR)	**872 (760, 1033)**	**779 (684, 881)**	**978 (849, 1128)**	**0.002**
Baseline CD4+ T cell count, median (IQR)[++]	172 (119, 225)	156 (102, 172)	183 (145, 258)	0.21

[*] One participant had unavailable HLA donor type.
[**] Any combination of three immunosuppressants at Day 0.
[^] By positive prior molecular testing or reactive anti-nucleocapsid antibody at enrollment.
[+] Continuous outcomes compared by Wilcoxon rank sum testing and categorical variables were compared by Fisher's exact testing.
[++] T cell subtyping performed on n=17 participants (5 negative, 12 low-titer)
BMI, body mass index; IQR, interquartile range; FSGS, focal segmental glomerulosclerosis; DSA, donor-specific antibody; IS, immunosuppressant; GFR, glomerular filtration rate; ALC, absolute lymphocyte count; IgG, Immunoglobulin G; HCV, hepatitis C virus

Supplemental Table 2: Changes in surrogate neutralization (ACE2 Inhibition) versus SARS-CoV-2 variants, by baseline anti-RBD status

(A) Day 30 surrogate neutralization (ACE2 Inhibition) versus SARS-CoV-2 variants, by Day 0 Anti-RBD status

Variant	Overall	anti-RBD^{NEG}	anti-RBD^{LO}	p-value
Ancestral	13.1 (6.3, 30.3)	6.7 (4.0, 11.3)	24.6 (13.4, 42)	<0.001
Delta	9.9 (5.2, 18.0)	5.8 (3.6, 8.6)	16.2 (11.8, 27.3)	<0.001
Omicron	5.4 (2.8, 10.4)	5.5 (3.1, 10.5)	5.3 (2.1, 10.1)	0.88

(B) Absolute and fold-change in Day 30 surrogate neutralization (ACE2 Inhibition) versus SARS-CoV-2 variants, by Day 0 Anti-RBD status

Variant	Absolute change (day 30-day 0)		p-value	Fold-change (day 30/day0)		p-value
	anti-RBD^{NEG}	anti-RBD^{LO}		anti-RBD^{NEG}	anti-RBD^{LO}	
Ancestral	3.6 (-0.8, 7.5)	21.3 (8.8, 38.7)	<0.001	2.1 (0.8, 3.6)	5.9 (2.7, 10.2)	<0.001
Delta	1.9 (0.3, 6.2)	14.1 (8.7, 25)	<0.001	1.5 (1.1, 3.2)	5.4 (3.1, 11)	<0.001
Omicron	3.6 (-0.1, 6)	2.6 (0, 8.4)	0.88	1.7 (0.7, 2.1)	1.7 (0.7, 3.5)	0.50

Values compared by Wilcoxon rank sum testing. Excludes n=1 participant with incident COVID-19 and n=1 participant who received monoclonal antibody.

Supplemental Table 3. Associations between baseline clinical factors and two measures of T cell response: spike-specific CD8+ T cell% (MHC-pentamer staining) and T-MAP^{TM} positivity (SARS-CoV-2-reactive TCR repertoire).

	Spike-specific CD8+ T cell% (day 14)	p-value	T-MAP^{TM} (day 30)	p-value
Age (per 10 yr)	$_{0.78}$ **1.00** $_{1.28}$	0.99	$_{0.74}$ **0.88** $_{1.04}$	0.14
Female sex	$_{0.62}$ **1.11** $_{2.01}$	0.72	$_{0.75}$ **1.27** $_{2.16}$	0.37
mRNA-1273 vaccine	$_{0.65}$ **1.16** $_{2.06}$	0.62	$_{0.47}$ **0.90** $_{1.75}$	0.76
Mycophenolate	$_{0.49}$ **0.93** $_{1.78}$	0.83	$_{0.61}$ **3.86** $_{24.50}$	0.15
High dose mycophenolate	$_{0.29}$ **0.82** $_{2.33}$	0.72	$_{0.61}$ **1.10** $_{2.00}$	0.75
Triple Immunosuppression	$_{0.50}$ **0.90** $_{1.62}$	0.72	$_{0.82}$ **2.27** $_{6.32}$	0.12
Transplant vintage (per 5 yr)	$_{0.83}$ **1.02** $_{1.25}$	0.85	$_{0.93}$ **1.14** $_{1.40}$	0.19
Lymphocyte <1000 cells/uL	$_{0.75}$ **1.38** $_{2.51}$	0.30	$_{0.81}$ **1.35** $_{2.26}$	0.25
CD4 count day 0 (per 100) (n=32)	$_{0.63}$ **0.86** $_{1.19}$	0.37	$_{0.60}$ **0.93** $_{1.44}$	0.75
Day 0 positive T-MAP^{TM}	$_{0.73}$ **1.31** $_{2.35}$	0.37	$_{\mathbf{1.78}}$ **2.56** $_{\mathbf{3.68}}$	**<0.01**
anti-RBD^{NEG}	$_{0.62}$ **1.11** $_{1.99}$	0.72	$_{0.34}$ **0.60** $_{1.06}$	0.09

Associations measured using Poisson regression with robust variance estimator.
Day 14 selected for CD8+ response given a priori anticipated peak response, confirmed by exploratory data analysis.

Supplemental Table 4. Demographic and transplant characteristics by day 30 T-MAP™ result (n=56)

	Negative	Positive	p-value
Demographics	28	28	
Age (years), median (IQR)	64 (56, 74)	62 (48, 70)	0.15
Female sex, no. (%)	7 (25%)	10 (36%)	0.56
BMI (kg/m2), median (IQR)	26.7 (23.9, 31.4)	26.7 (23.2, 31.2)	0.88
Medical comorbidities			
Diabetes, no. (%)	8 (29%)	7 (25%)	>0.99
HCV infection, no. (%)	2 (7%)	0 (0%)	0.49
Lung disease, no. (%)	8 (29%)	3 (11%)	0.18
Cardiovascular disease, no. (%)	25 (89%)	24 (86%)	>0.99
Autoimmune disease, no. (%)	3 (11%)	4 (14%)	>0.99
Transplant			
Years since transplant, median (IQR)	4.9 (2.2, 8.8)	8.1 (4.9, 13.3)	**0.042**
>2 years	23 (82%)	27 (96%)	0.19
>5 years	14 (50%)	20 (71%)	0.17
Indication for most recent kidney transplantation			
Diabetes	4 (14%)	3 (11%)	>0.99
Hypertension	14 (50%)	6 (21%)	0.050
FSGS	2 (7%)	2 (7%)	>0.99
Glomerulonephritis	0 (0%)	1 (4%)	>0.99
Cystic kidney disease	2 (7%)	3 (11%)	>0.99
Living donor, no (%)	12 (43%)	13 (46%)	>0.99
DSA positive at baseline, no (%)*	7 (26%)	5 (18%)	0.53
Baseline immunosuppressant			
Mycophenolate Mofetil	20 (71%)	24 (86%)	0.33
Total daily dose (mg), median (IQR)	1000 (500, 1000)	1000 (625, 1000)	0.36
Mycophenolic Acid	2 (7%)	3 (11%)	>0.99
Total daily dose (mg), median (IQR)	720 (180, 1440)	900 (360, 1440)	0.66
High dose mycophenolate	6 (21%)	7 (25%)	>0.99
Prednisone	26 (93%)	26 (93%)	>0.99
Total daily dose (mg), median (IQR)	5 (5, 5)	5 (5, 5)	0.67
Tacrolimus	24 (86%)	28 (100%)	0.11
Total daily dose (mg), median (IQR)	4 (2, 6.25)	4 (2.75, 5.5)	0.85
Cyclosporine	2 (7%)	1 (4%)	>0.99
Triple IS, no. (%)**	19 (68%)	25 (89%)	0.10
Prior SARS-CoV-2 infection, no. (%)^	1 (4%)	1 (4%)	>0.99
Days between 2nd and 3rd dose, median (IQR)	158 (138, 174)	169 (148, 183)	0.15
Vaccine manufacturer, no. (%)			>0.99
Moderna (mRNA-1273)	7 (25%)	6 (21%)	
Pfizer-BioNTech (BNT162b2)	21 (75%)	22 (79%)	
Creatinine (mg/dL), median (IQR)			
Day 0	1.3 (1.1, 1.5)	1.2 (1.1, 1.5)	0.33
Day 30	1.3 (1.2, 1.6)	1.2 (1, 1.5)	0.28
Estimated GFR (ml/min/1.73m2), median (IQR)			
Day 0	56 (46, 65)	63 (49, 77)	0.25
Day 30	55 (48, 64)	63 (46, 77)	0.30
Baseline ALC (K/mm^3), median (IQR)	1.25 (0.91, 1.58)	1.06 (0.81, 1.39)	0.33

Absolute Lymphocyte Count >1000 cells/mm^3	20 (71%)	16 (57%)	0.40
Baseline Total IgG (mg/dL), median (IQR)	886 (768, 1083)	825 (755, 1100)	0.62
Baseline CD4+ T cell count, median (IQR)[+]	174 (102, 225)	172 (156, 231)	0.72

Excludes 8 participants with indeterminate TMAP™ at day 30 and one with incident COVID-19
Continuous outcomes compared by Wilcoxon rank sum testing and categorical variables were compared by Fisher's exact testing.

[*] One participant had unavailable HLA donor type.
[**] Any combination of three immunosuppressants at Day 0.
[^] By positive prior molecular testing or reactive anti-nucleocapsid antibody at enrollment.
[+] T cell subtyping performed on n=17 participants (5 negative, 12 low-titer)

BMI, body mass index; IQR, interquartile range; FSGS, focal segmental glomerulosclerosis; DSA, donor-specific antibody; IS, immunosuppressant; GFR, glomerular filtration rate; ALC, absolute lymphocyte count; IgG, Immunoglobulin G; HCV, hepatitis C virus

Supplemental Table 5. Change in SARS-CoV-2-reactive CD4+ and CD8+ TCR breadth and depth from day 0 to day 30 post third mRNA vaccine dose.

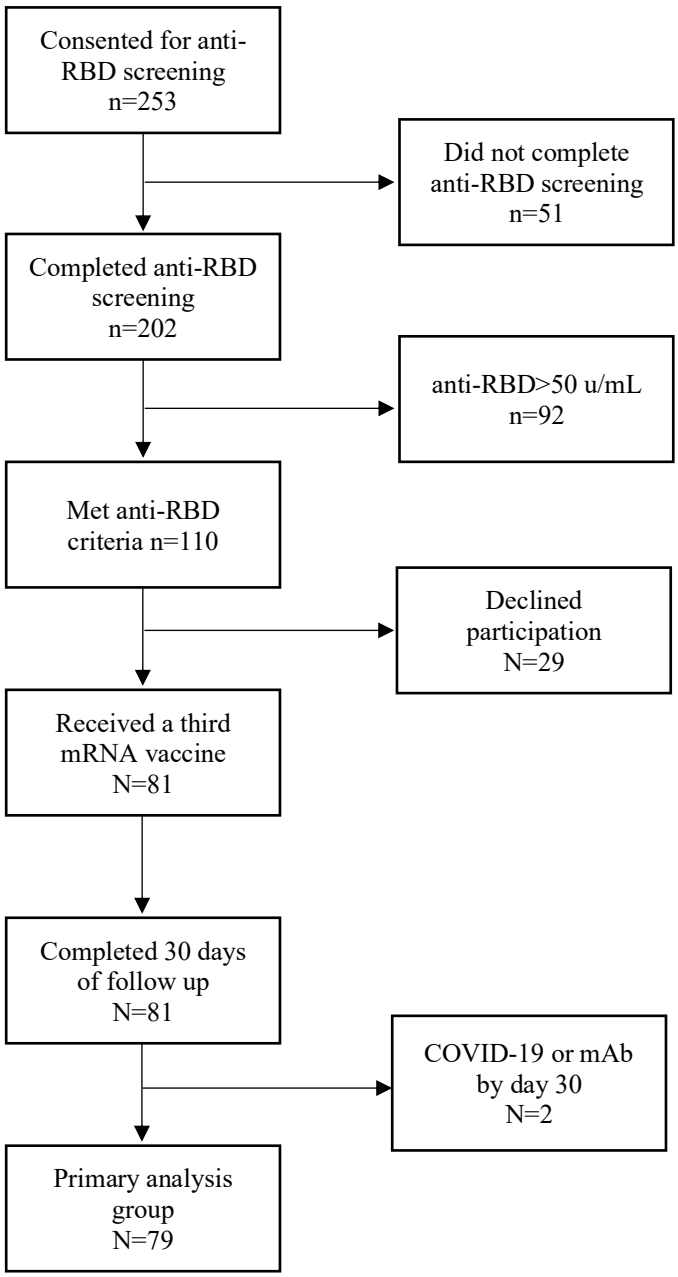
Factor	Day 0 TCR median (IQR)	Day 30 TCR median (IQR)	Wilcoxon sign-rank p
Total breadth	$1.76x10^{-5}$ ($9.70x10^{-6}$, $2.74x10^{-5}$)	$3.97x10^{-5}$ ($1.41x10^{-5}$, $6.58x10^{-5}$)	<0.001
Total depth	$9.66x10^{-6}$ ($2.16x10^{-6}$, $2.06x10^{-5}$)	$2.53x10^{-5}$ ($6.90x10^{-6}$, $6.52x10^{-5}$)	<0.001
CD4+ breadth	$1.41x10^{-5}$ ($4.64x10^{-6}$, $2.37x10^{-5}$)	$2.75x10^{-5}$ ($1.26x10^{-5}$, $5.55x10^{-5}$)	<0.001
CD4+ depth	$7.68x10^{-6}$ ($1.72x10^{-6}$, $1.65x10^{-5}$)	$1.65x10^{-5}$ ($4.60x10^{-6}$, $5.00x10^{-5}$)	<0.001
CD8+ breadth	$1x10^{-6}$ ($1x10^{-6}$, $6.62x10^{-6}$)	$2.85x10^{-6}$ ($1x10^{-6}$, $1.12x10^{-5}$)	0.010
CD8+ depth	$1x10^{-6}$ ($1x10^{-6}$, $2.06x10^{-6}$)	$1.20x10^{-6}$ ($1x10^{-6}$, $4.76x10^{-6}$)	0.005

Values of 0 TCR breadth or depth were set to $1x10^{-6}$ for analysis, as a lower bound below the distribution of values for participants with quantifiable TCR measures.

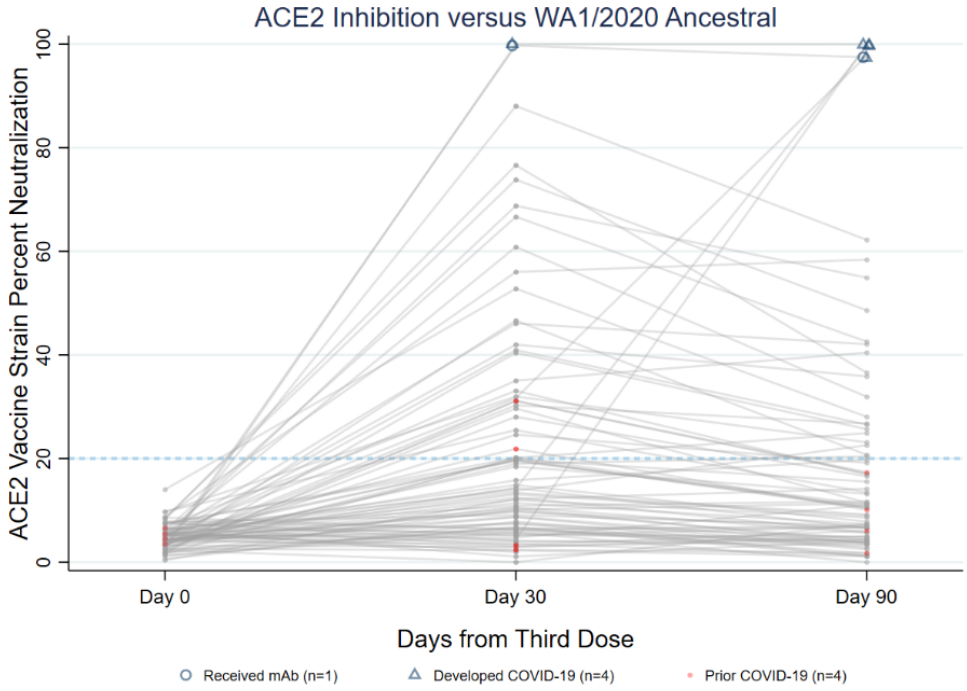
Supplemental Table 6: Clinical, transplant, and immunological characteristics according to immune phenotype (day 30 anti-RBD/TMAP^{TM}) following a third mRNA vaccine.

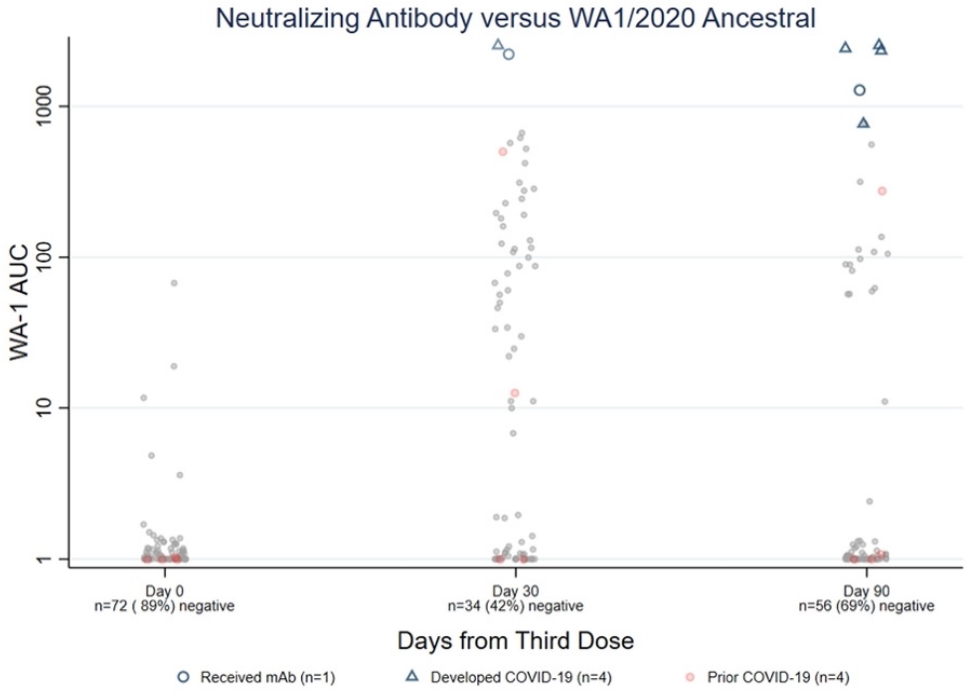
Factor	anti-RBD(-)/ TMAP(-) n=5	anti-RBD(-)/ TMAP(+) n=5	anti-RBD(+)/ TMAP(-) n=23	anti-RBD(+)/ TMAP(+) n=22	p-value
Mycophenolate	5 (100%)	5 (100%)	17 (74%)	21 (95%)	0.15
High dose mycophenolate	4 (80%)	1 (20%)	2 (9%)	6 (27%)	**0.008**
Triple IS, no. (%)	5 (100%)	5 (100%)	14 (61%)	19 (86%)	0.073
Age (per decade), median (IQR)	6.3 (6, 7.4)	6.7 (4.9, 6.9)	6.5 (5.5, 7.5)	6.2 (4.7, 7)	0.57
Years since transplant, median (IQR)	6.3 (2.3, 7.0)	13.0 (9.2, 19.2)	4.9 (2.1, 9.2)	6.3 (4.9, 12.1)	0.058
Vaccine manufacturer, no. (%)					0.64
Moderna (mRNA-1273)	0 (0%)	1 (20%)	7 (30%)	5 (23%)	
Pfizer-BioNTech (BNT162b2)	5 (100%)	4 (80%)	16 (70%)	17 (77%)	
ALC<1000	2 (40%)	1 (20%)	6 (26%)	10 (45%)	0.53
eGFR (ml/min/1.73m2) Day 0, median (IQR)	57 (55, 65)	53 (46, 91)	55 (46, 65)	66 (50, 76)	0.59
Day 0 anti-RBD					<0.001
Negative	5 (100%)	5 (100%)	12 (52%)	5 (23%)	
Positive	0 (0%)	0 (0%)	11 (48%)	17 (77%)	
Day 0 anti-RBD, median (IQR)	<0.8 (<0.8, <0.8)	<0.8 (<0.8, <0.8)	<0.8 (<0.8, 5.0)	11 (1, 29)	<0.001
Day 30 anti-RBD, median (IQR)	<0.8 (<0.8, <0.8)	<0.8 (<0.8, <0.8)	386 (22, 1096)	1499 (118, 4314)	<0.001
Day 0 TMAP					0.004
Negative	4 (80%)	2 (40%)	21 (91%)	14 (64%)	
Indeterminate	1 (20%)	0 (0%)	2 (9%)	1 (5%)	
Positive	0 (0%)	3 (60%)	0 (0%)	7 (32%)	
Overall spike-specific TCR					
Day 0 Breadth, median (IQR)	$1.1x10^{-5}$ ($1.0x10^{-6}$, $1.4x10^{-5}$)	$3.4x10^{-5}$ ($3.1x10^{-5}$, $3.7x10^{-5}$)	$1.3x10^{-5}$ ($7.7x10^{-6}$, $2.1x10^{-5}$)	$2.5x10^{-5}$ ($1.1x10^{-5}$, $4.5x10^{-5}$)	0.006
Day 30 Breadth, median (IQR)	$1.6x10^{-5}$ ($2.2x10^{-6}$, $3.1x10^{-5}$)	$6.8x10^{-5}$ ($5.0x10^{-5}$, $7.1x10^{-5}$)	$1.9x10^{-5}$ ($6.3x10^{-6}$, $3.9x10^{-5}$)	$6.2x10^{-5}$ ($5.1x10^{-5}$, $8.6x10^{-5}$)	<0.001
Day 0 Depth, median (IQR)	$3.7x10^{-6}$ ($1.0x10^{-6}$, $5.2x10^{-6}$)	$2.1x10^{-5}$ ($1.6x10^{-5}$, $2.6x10^{-5}$)	$6.2x10^{-6}$ ($1.5x10^{-6}$, $1.2x10^{-5}$)	$1.7x10^{-5}$ ($5.0x10^{-6}$, $2.5x10^{-5}$)	0.014
Day 30 Depth, median (IQR)	$8.5x10^{-6}$ ($5.3x10^{-6}$, $1.8x10^{-5}$)	$5.1x10^{-5}$ ($4.0x10^{-5}$, $6.3x10^{-5}$)	$7.8x10^{-6}$ ($2.8x10^{-6}$, $1.9x10^{-5}$)	$6.1x10^{-5}$ ($3.7x10^{-5}$, $1.1x10^{-4}$)	<0.001
CD4+ spike-specific TCR					
Day 0 Breadth, median (IQR)	$8.2x10^{-6}$ ($1.0x10^{-6}$, $8.8x10^{-6}$)	$2.8x10^{-5}$ ($1.8x10^{-5}$, $2.9x10^{-5}$)	$9.9x10^{-6}$ ($1.0x10^{-6}$, $1.7x10^{-5}$)	$2.2x10^{-5}$ ($1.0x10^{-5}$, $4.2x10^{-5}$)	0.006
Day 30 Breadth, median (IQR)	$1.4x10^{-5}$ ($2.2x10^{-6}$, $1.6x10^{-5}$)	$5.0x10^{-5}$ ($4.7x10^{-5}$, $7.1x10^{-5}$)	$1.3x10^{-5}$ ($6.3x10^{-6}$, $2.4x10^{-5}$)	$5.6x10^{-5}$ ($3.7x10^{-5}$, $8.1x10^{-5}$)	<0.001
Day 0 Depth, median (IQR)	$3.1x10^{-6}$ ($1.0x10^{-6}$, $3.7x10^{-6}$)	$1.5x10^{-5}$ ($1.4x10^{-5}$, $1.8x10^{-5}$)	$4.8x10^{-6}$ ($1.0x10^{-6}$, $1.1x10^{-5}$)	$1.4x10^{-5}$ ($3.8x10^{-6}$, $2.5x10^{-5}$)	0.012
Day 30 Depth, median (IQR)	$7.4x10^{-6}$ ($5.3x10^{-6}$, $8.5x10^{-6}$)	$4.1x10^{-5}$ ($4.0x10^{-5}$, $6.3x10^{-5}$)	$4.8x10^{-6}$ ($1.6x10^{-6}$, $1.3x10^{-5}$)	$5.4x10^{-5}$ ($2.9x10^{-5}$, $1.1x10^{-4}$)	<0.001
CD8+ spike-specific TCR					
Day 0 Breadth, median (IQR)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $2.2x10^{-6}$)	$2.3x10^{-6}$ ($1.0x10^{-6}$, $5.7x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $6.8x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $6.5x10^{-6}$)	0.90
Day 30 Breadth, median (IQR)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $3.5x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $1.0x10^{-6}$)	$2.8x10^{-6}$ ($1.0x10^{-6}$, $1.1x10^{-5}$)	$5.8x10^{-6}$ ($1.0x10^{-6}$, $2.0x10^{-5}$)	0.39
Day 0 Depth, median (IQR)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $1.7x10^{-6}$)	$1.7x10^{-6}$ ($1.0x10^{-6}$, $3.0x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $1.5x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $2.7x10^{-6}$)	0.74
Day 30 Depth, median (IQR)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $1.1x10^{-6}$)	$1.0x10^{-6}$ ($1.0x10^{-6}$, $1.0x10^{-6}$)	$1.4x10^{-6}$ ($1.0x10^{-6}$, $4.6x10^{-6}$)	$2.6x10^{-6}$ ($1.0x10^{-6}$, $1.5x10^{-5}$)	0.32

Supplemental Figure 1: Study Flow Diagram

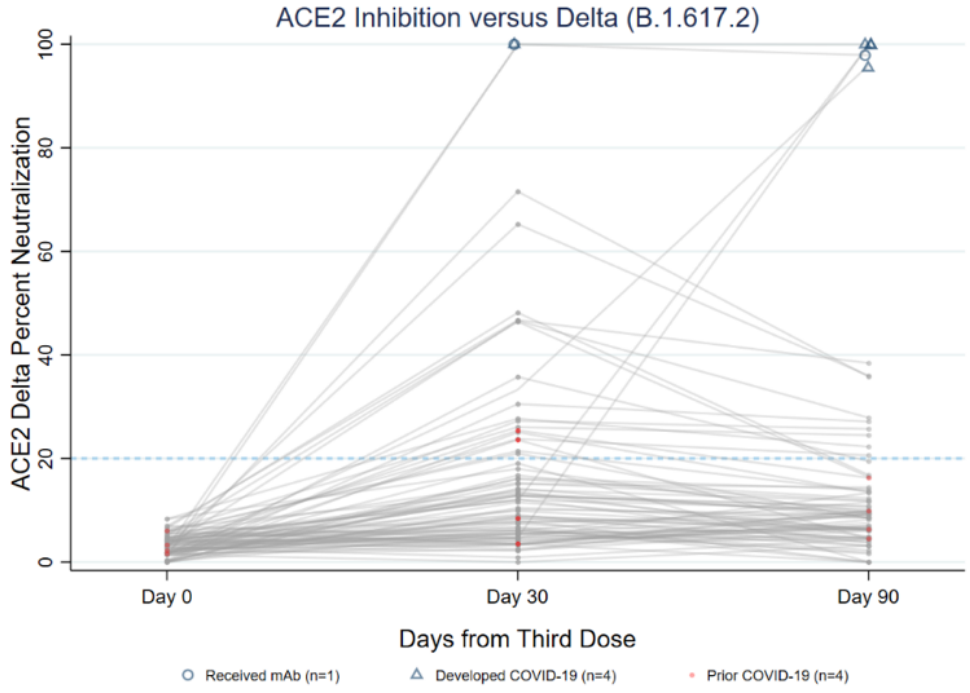


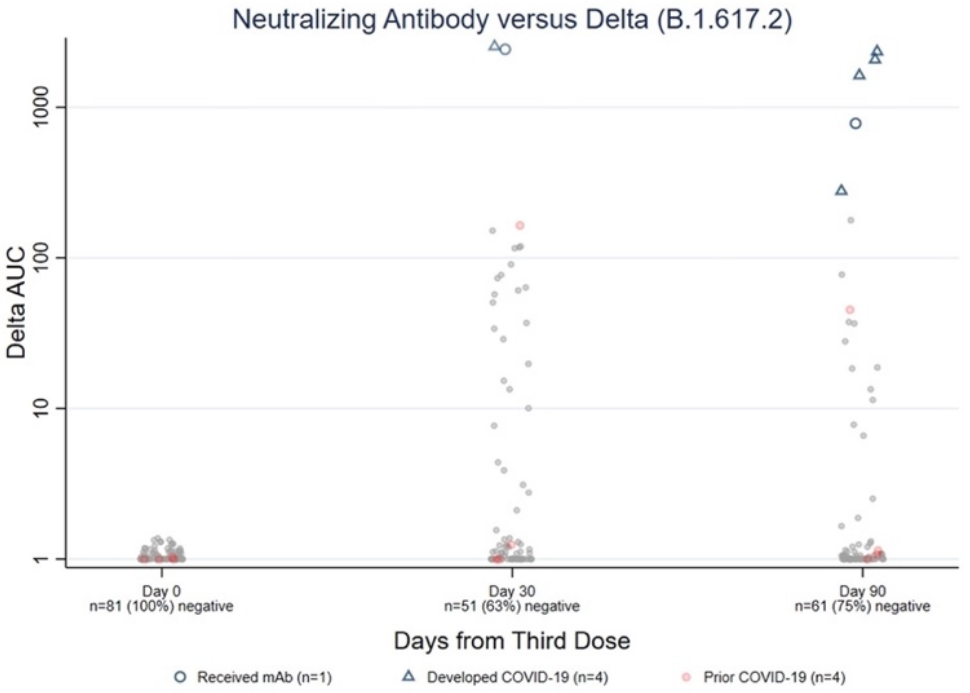
Supplemental Figure 2A: Surrogate (ACE2 inhibition) and live virus neutralization (AUC) of ancestral SARS-CoV-2 variant following a third dose of mRNA vaccine**.** Triangles represent participants with incident SARS-CoV-2 infection (n=4) and open circles represent participants receiving monoclonal antibody infusion (n=1).



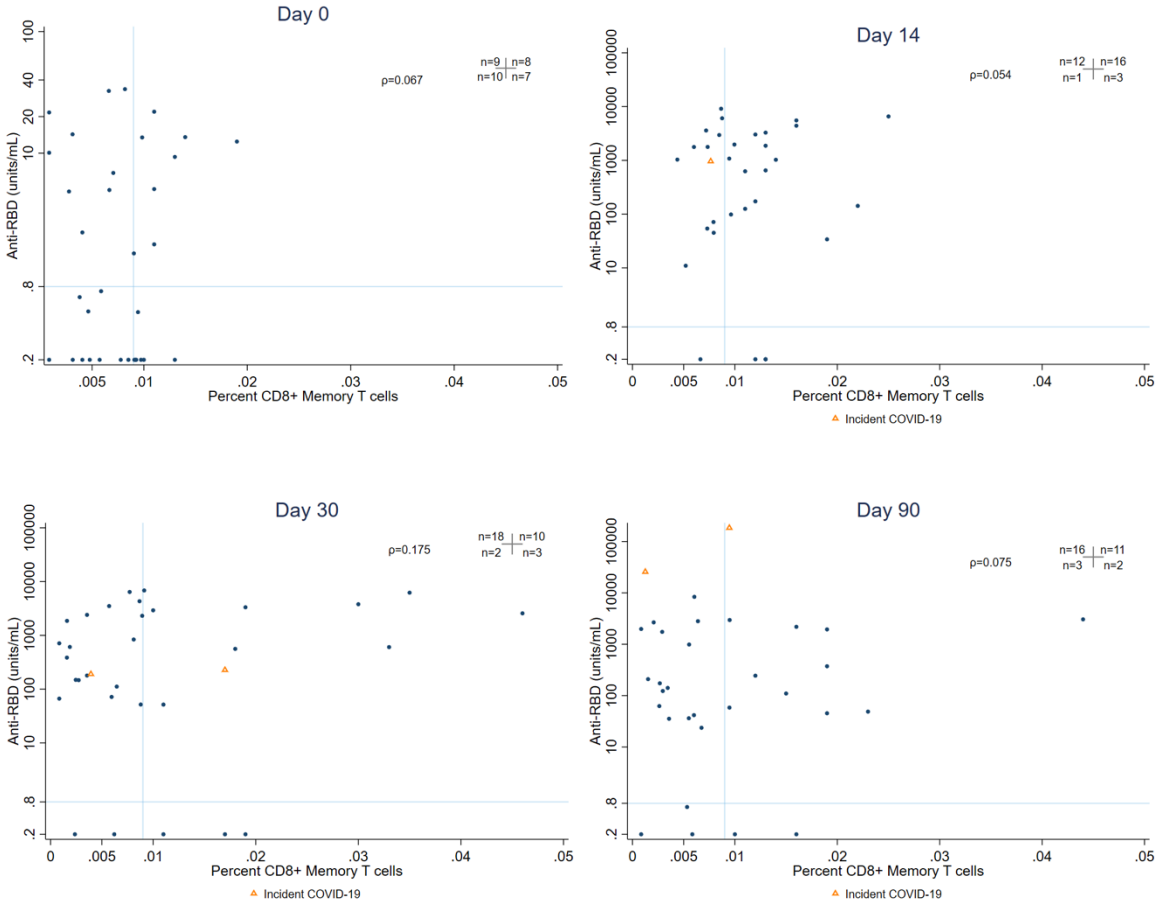


Supplemental Figure 2B: Surrogate (ACE2 inhibition) and live virus neutralization (AUC) of the delta SARS-CoV-2 variants following a third dose of mRNA vaccine. Triangles represent participants with incident SARS-CoV-2 infection (n=4) and open circles represent participants receiving monoclonal antibody infusion (n=1).



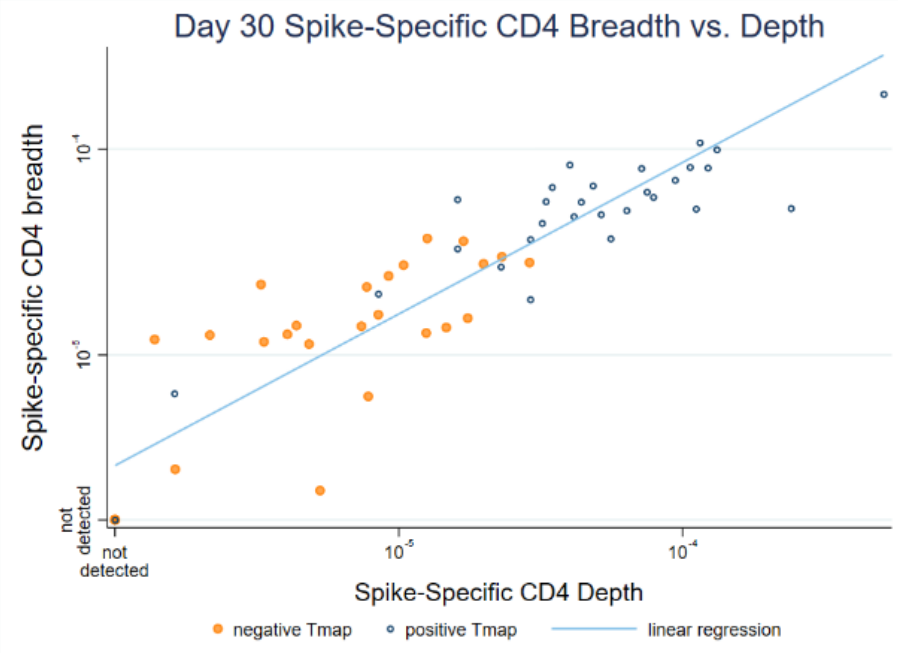


Supplemental Figure 3: Correlation of SARS-CoV-2-specific CD8+ memory T cell% with anti-RBD response following a third dose of mRNA COVID-19 vaccine, over time. Triangles indicate persons with incident SARS-CoV-2 infection (n=2). The linear correlation coefficient for each timepoint is display in within the graph body. Thresholds of CD8+ response and anti-RBD response are denoted by vertical and horizontal grey lines, respectively; the number of participants falling in each phenotype (quadrant) of antibody and cellular response is enumerated in upper right corner of each graph body.



Supplemental Figure 4: Correlation of day 30 spike-specific TCR breadth and depth, by TMAP™ result

(A) Correlation of CD4+ TCR breadth and depth at day 30 post vaccination. ρ=0.92 by Spearman correlation coefficient.



(B) Correlation of CD8+ TCR breadth and depth at day 30 post vaccination. ρ=0.96 by Spearman correlation coefficient.