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Tacrolimus and Mycophenolic Acid Exposure Are Associated with Biopsy-Proven Acute Rejection: A Study to Provide Evidence for Longer-Term Target Ranges

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Evidence to define target ranges for tacrolimus (Tac) and mycophenolic acid (MPA) exposure after the first year of kidney transplantation is limited. We investigated the association of measurements at 1 year and repeated measurements of real-world Tac-trough levels (C_0) and abbreviated area under the curve from zero to 12 hours ($AUC_{0\text{-}12h}$) of Tac and MPA with biopsy-proven acute rejection (BPAR) between years 1 and 3 post-transplant in 968 kidney transplant recipients (KTRs). Thirty-five (3.6%) out of 968 KTRs experienced BPAR. Both Tac-$AUC_{0\text{-}12h}$ (hazard ratio (HR): 0.39, 95% confidence interval (CI): 0.30–0.50, $P < 0.001$), Tac-C_0 (HR: 0.46, 95% CI: 0.38–0.57, $P < 0.001$) and MPA-$AUC_{0\text{-}12h}$ at 1 year (HR: 0.80, 95% CI: 0.68–0.94, $P = 0.006$), as well as repeated measurements of Tac-C_0 (HR: 0.70, 95% credibility interval (CrI): 0.61–0.82, $P < 0.001$), and of MPA-$AUC_{0\text{-}12h}$ (HR: 0.75, 95% CrI: 0.62–0.93, $P < 0.001$) were associated with BPAR. In our population, the recommended target range for Tac-$AUC_{0\text{-}12h}$ at 1 year would be 75–95 ng*hour/mL and a Tac-C_0 5–7 ng/mL. The Tac-$AUC_{0\text{-}12h}$ predicted BPAR better than Tac-C_0 and identified KTRs with over- or underexposure despite supposedly adequate Tac-C_0. We did not find evidence to recommend another target than the consensus range of 30–60 mg*hour/L for MPA-$AUC_{0\text{-}12h}$ after the first year of transplantation. To our knowledge, this is a first study on the simultaneous exposure of Tac and MPA at year 1 and subsequent BPAR up to year 3, which may help define the therapeutic target window for the longer term.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There is scant evidence for long-term targets for maintenance immunosuppression after kidney transplantation. Therefore, the therapeutic window for tacrolimus (Tac) and mycophenolic acid (MPA) beyond the first year of transplantation is mainly extrapolated from trials limited to the first year that did not consider simultaneous exposure.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The primary and secondary objectives were to (i) investigate the relationship between simultaneous exposure to Tac/MPA and biopsy-proven acute rejection (BPAR), and (ii) explore a lower threshold of the target range.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?

☑ Using Cox regression and joint models, we showed that TAC/MPA exposure is significantly associated with BPAR beyond the first year of transplantation.

☑ A Tac-area under the curve from zero to 12 hours ($AUC_{0\text{-}12h}$) 75–95 ng*hour/mL, Tac-trough levels 5–7 ng/mL, and MPA-$AUC_{0\text{-}12h}$ 30–60 mg*hour/L might be suggested as optimal targets between the first and third years.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These results provide evidence on the long-term lower threshold of the target range. This may aid physicians in determining the longer-term therapeutic window of Tac/MPA in kidney transplantation.

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Most kidney transplant recipients (KTRs) are currently treated with triple-drug immunosuppressive maintenance therapy (TMT) consisting of tacrolimus (Tac), mycophenolic acid (MPA), and prednisolone (Pred).[1,2] The therapeutic window for Tac beyond the first year after transplantation to prevent formation of donor-specific antibodies (DSAs), acute rejection, and subsequent graft loss is extrapolated from the ELITE-Symphony study as well as smaller cohort studies investigating efficacy on end points within the first year after transplantation.[3–9] Additionally, clinically relevant interpatient variability exists in MPA drug exposure in spite of its standard-dose label.[10]

The conundrum of transplant medicine is to prolong graft survival by better defining target ranges of the lower (efficacy) and upper (toxicity) threshold for maintenance immunosuppression in the longer term.[11] Data on which to base targets after the first year post-transplant are, however, unacceptably scarce. Moreover, most studies have not considered simultaneous exposure to Tac and MPA.

The primary objective of this study was to investigate the association between simultaneous exposure measurements of both Tac area under the curve from 0 to 12 hours ($AUC_{0\text{-}12h}$), trough concentrations (C_0), and MPA-$AUC_{0\text{-}12h}$ with incidence of biopsy-proven acute rejection (BPAR) between the first and third years after kidney transplantation. A secondary objective was to find evidence for a lower threshold of the target range for both Tac and MPA to prevent BPAR.

MATERIALS AND METHODS

Study design and participants

For this real-life observational cohort study, we assessed adult KTRs transplanted between 2009 and 2018 for eligibility using our local transplant center database. We included KTRs for analysis if they received continued immunosuppressive maintenance therapy with any combination of Tac ± MPA ± Pred from year 1 after transplantation onward, regardless of the occurrence of BPAR or initial maintenance therapy within the first year post-transplant. Patients who received simultaneous organ transplants were excluded from the analysis. Our institutional review board approved analysis of impact of therapeutic drug monitoring (TDM) in our KTRs (approval number: W2020.031). All proceedings were in accordance with the Declaration of Helsinki.

Local transplant center database

The database includes adult patients who have received a kidney transplant between 1966 and 2022. It integrates deeply phenotyped transplant data from 2005 onward, including transplant characteristics, clinical outcome data, medication and drug exposure measurements, immunological data, and digital pathology originating directly from patients' electronic file records. The database has automated validation queries to optimize data completeness.

Posttransplant outpatient care

Follow-up of KTRs during the first year post-transplant occurs in declining frequency according to Kidney Disease Improving Global Outcome (KDIGO) guidelines.[12] Stable KTRs in the first year post-transplant are routinely referred back to their peripheral health care provider with (bi)-annual follow-up at our center or sooner on indication. Routine check-ups by their community nephrologist usually occurs every 3 to 4 months, limiting loss of information.

Immunosuppressive protocol

Per institutional protocol, we routinely prescribe Tac at a dose of 5 mg b.i.d. which is subsequently titrated to maintain Tac-$AUC_{0\text{-}12h}$ of 160–180 ng*hour/mL (Tac-C_0: 8–12 ng/mL) in the first 6 weeks, Tac-$AUC_{0\text{-}12h}$ of 100–120 ng*hour/L (Tac-C_0: 6–10 ng/mL) between week 6 and month 3, and Tac-$AUC_{0\text{-}12h}$ of 80–100 ng*hour/mL (Tac-C_0: 4–7 ng/mL) later on in KTRs on TMT with Tac/MPA/Pred. Notably, our program allows a Tac-C_0 target to be individualized based on patients' known AUC exposure. We maintain a target range for MPA-$AUC_{0\text{-}12h}$ 30–45 mg*hour/L during the whole period post-transplantation. Dual immunosuppressive maintenance therapy (DMT) with Tac/Pred (target Tac-$AUC_{0\text{-}12h}$: 120 ng*hour/mL or Tac-C_0: 6–8 ng/mL) is considered in case of (frequent) opportunistic infection, malignancy, or other signs of overimmunosuppression or toxicity. On exception, DMT with MPA/Pred is considered after 3 months post-transplant in selected KTRs who underwent HLA-identical sibling donation (target $AUC_{0\text{-}12h}$: 60–90 mg*hour/L). Prednisolone is tapered to a daily dose of 5 mg toward month 3 and is not routinely withdrawn in our program. Standard induction therapy consists of either an IL-2 receptor blocker (basiliximab), or a lymphocyte depleting agent (alemtuzumab (15 mg s.c. q.d. for 2 days)) for immunological high-risk transplantations (such as highly immunized patients, ABO incompatible transplantation, or presence of pretransplant DSA).

Therapeutic drug measurements

Therapeutic drug measurements are performed protocollary on weeks 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 44, and 52 for Tac-C_0; weeks 1 to 2 for Tac-$AUC_{0\text{-}12h}$; and weeks 6, 24, and 52 for both Tac- and MPA-$AUC_{0\text{-}12h}$. On indication, additional assessments are done.

We included all immunosuppressive exposure measurements (whether per protocol or for cause). Exposure at year 1 was deemed missing if no measurements between 6 and 18 months were available. We manually validated $AUC_{0\text{-}12h}$ measurements in case calculations could not be performed due to missing or incorrect blood draws. We included MPA-$AUC_{0\text{-}12h}$ measurements for both mycophenolate mofetil (MMF) and mycophenolate sodium (MPS), albeit for the latter only if maximum plasma concentration (C_{max}) was reached in the timeframe of the limited sampling strategy.

C_0 and $AUC_{0\text{-}12h}$ measurements were performed by collecting venous blood samples. The dried blood spot (DBS) technique for capillary blood collection at home was used in < 1% of all $AUC_{0\text{-}12h}$ measurements. $AUC_{0\text{-}12h}$ were calculated using a validated limited sampling strategy of MPA (0, 1, 2, and 3 hours) and Tac (0, 2, and 3 hours). For patients on once-daily formulation of Tac, $AUC_{0\text{-}12h}$ was estimated by dividing the $AUC_{0\text{-}24h}$ by 2. The quantification of MPA and Tac blood concentrations and DBS in our cohort was performed as described previously and summarized in the supplemental text (**Text S1**).[13–15]

We assessed intrapatient variability (IPV) of Tac-C_0 for each patient using the coefficient of variation $\text{IPV} = \left(\frac{\text{sd}}{\text{mean}}\right) * 100\%$ of all (non-dose-corrected) measurements performed from transplantation until year 1 post-transplant. KTRs were classified as having low or high variability depending on their IPV relative to the median-IPV. An IPV < median was classified as low, and an IPV > median as high.

BPAR assessment

We do not perform protocol biopsies as part of routine clinical care, unless performed within a clinical trial. Indication biopsies were performed when KTR suffered from a worsening in serum creatinine and/or proteinuria without evident alternative causes, and/or appearance of *de novo* DSAs on routine monitoring was observed (from 2017 onward).

The primary outcome of interest was time to first BPAR between the first and third year post-transplant. We defined BPAR as histological evidence of rejection in KTRs according to the 2019 Banff classification.[16] Both protocol and for-cause biopsies were included in this analysis. Graft failure by BPAR was classified as BPAR.

A secondary outcome of interest was treatment of rejection with or without biopsy, and thus included "blinded" rejection treatment.

Statistical analysis

All statistical analyses were performed using R-statistics (version 1.4.2), RStudio (version 1.4.1717), R-packages *ggplot* (version 3.3.6), *survminer* (version 0.4.8), *mice* (version 3.13.0), and JMbayes (version 0.8-85). Continuous data were displayed as medians and interquartile ranges (IQRs) or means and SDs with 95% confidence intervals (CIs) if applicable. Frequencies were reported as ratios.

We used two different approaches to analyze the association between Tac/MPA exposure and incidence of BPAR after the first year in the overall study cohort and in KTR on TMT with Tac/MPA/Pred. First, Cox regression analyses in which simultaneous exposure to Tac-C_0, Tac-$AUC_{0\text{-}12h}$, and MPA-$AUC_{0\text{-}12h}$ at 1 year were associated with BPAR. Second, joint models to assess the association of BPAR with all repeated measurements of Tac-C_0 and MPA-$AUC_{0\text{-}12h}$ performed since 1 year until the end of follow-up or BPAR. Additional information on joint modeling can be found in Supplementary Material (**Text S2**). We tested for the proportional-hazard assumption by looking at a non-significant relationship between residuals and time.

We adjusted hazard ratios (HRs) for HLA-antigen mismatch, virtual panel reactive antibodies (PRA), number of prior transplantations, type of donor (deceased or living), donor's and recipient's age and sex, induction therapy (interleukin 2 (IL-2)-receptor-blocker or lymphocyte depleting agent), and dialysis vintage.

We used logistic regression plots to assess lower reference values in KTR on TMT with Tac/MPA/Pred by visualizing probability of BPAR for various Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 in the overall cohort and in KTRs with low and high IPV, respectively. Moreover, we described the relation between $AUC_{0\text{-}12h}$ and C_0 using a correlation plot. At last, we stratified KTRs in low or high Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 exposure groups to assess differences in their association with BPAR. Missing data on MPA exposure at year 1 was limited (9.5%), assumed to occur at random, and imputed 10 times using chained equations with classification and regression trees. We used these imputed data sets to assess pooled HRs and CIs. KTRs were censored in case of death, graft failure, or loss-to-follow-up due to relocation.

Notably, we performed different sensitivity analyses to investigate the association between Tac/MPA exposure and BPAR in different subgroups: (i) KTRs initiated on TMT from time of transplantation, (ii) KTRs without missing MPA exposure, (iii) KTRs that did not receive alemtuzumab induction, (iv) KTRs without a previous rejection episode in the first year of transplantation, and (v) KTRs without MPS (**Tables S2–S6**). Finally, we investigated the association between Tac/MPA exposure and our secondary outcome of interest (i.e., rejection treatment; **Table S7**).

RESULTS

Study cohort

A total of 968 adult KTRs were on continued immunosuppressive maintenance therapy with Tac ± MPA ± Pred from 1 year post-transplantation onward, as depicted in **Figure S1**. Eight hundred forty-eight out of 968 KTRs were on TMT, 76 on DMT with Tac/Pred, and 44 on DMT with MPA/Pred.

Most (89%) KTRs started on Tac, MPA, and Pred at the time of transplantation (**Table 1**). A minority started on Tac/MPA with early steroid withdrawal (5%), cyclosporin/MPA/Pred (4%), Tac/Everolimus/Pred (1%), or another regimen (1%) before being on any continued combination of Tac ± MPA ± Pred from 1 year after transplantation onward. IL-2-receptor inhibition was initiated in 844 (87%), whereas only 124 (13%) KTRs received lymphocyte-depleting agents such as anti-thymocyte globulin ($n = 1$) or alemtuzumab ($n = 123$). Out of 968 KTRs, 830 (86%) received Tac b.i.d. and 138 (14%) received Tac q.d. For 892 KTRs

Table 1 Patient characteristics of the investigated population

No. of patients	968
Recipient age at transplantation—median [IQR]	55 [45–64]
Male gender recipient—*n*	585 (60%)
Dialysis prior transplantation—*n*	703 (73%)
Dialysis duration in days—median [IQR]	125 [0–326]
Donor age—median [IQR]	54 [46–63]
Male gender donor—*n*	469 (49%)
Type transplantation—*n*	
Living donor	540 (56%)
Postmortal donor	
Donation after brain death	204 (21%)
Donation after circulatory death	224 (23%)
HLA antigen mismatch—mean (SD)	
HLA-A	1.0 (0.7)
HLA-B	1.2 (0.7)
HLA-DR	1.0 (0.7)
Total -A, -B, -DR	3.2 (1.5)
Panel reactive antibodies—*n*	
<5	753 (78%)
5–84	151 (16%)
>84	64 (6%)
Pre-transplant donor-specific antibodies—*n*	42 (4.5%)
Tx rank in patient history—*n*	
#1	862 (89%)
#2	94 (10%)
#3	12 (1%)
Cold ischemia time (minutes)—median [IQR]	390 [178–779]
Delayed graft function—*n*	246 (25%)
Induction therapy—*n*	
IL-2-blocker	844 (87%)
Alemtuzumab/ATG	124 (13%)
Immunosuppression at start—*n*	
Tac/MPA/Pred	862 (89%)
Tac/MPA	52 (5%)
CsA/MPA/Pred	36 (4%)
Tac/EVL/Pred	9 (1%)
Other	9 (1%)
Immunosuppression at year 1—*n*	
Tac/MPA/Pred	848 (88%)
Tac/Pred	76 (8%)
MPA/Pred	44 (4%)
Drug exposure at year 1—median [IQR]	
Tac-$AUC_{0\text{-}12h}$—ng*hour/mL	100 [77–127]
Tac-C_0—ng/mL	6.1 [4.8–7.6]
MPA-$AUC_{0\text{-}12h}$—mg*hour/L	41 [30–53]
BPAR in the first year of transplantation—*n*	136 (14%)
eGFR at year 1 in mL/minute—median [IQR]	53 [42–66]

Patient characteristics of the investigated populations.
ATG, anti-thymocyte globulin; $AUC_{0\text{-}12h}$, area under the curve from 0 to 12 hours; BPAR, biopsy-proven acute rejection; C_0, trough level; CsA, cyclosporin A; eGFR, estimated glomerular filtration rate; EVL, everolimus; Pred, prednisolone; HLA, human leukocyte antigen; IL-2, Interleukin-2; IQR, interquartile range; MPA, mycophenolic Acid; Tac, tacrolimus; Tx, transplantation.

on MPA, 836 (94%) received MMF, whereas 56 (6%) received MPS. Mean HLA-antigen mismatch scores for HLA-A, -B, -DR, and -A/-B/-DR combined were 1.0 (0.7), 1.2 (0.7), 1.0 (0.7), and 3.2 (1.5), respectively. Of all KTR, 753 (78%) had panel reactive antibodies (PRAs) < 5%, 151 (16%) between 5% and 84%, and 64 (6%) > 84%. A total of 42 (4.5%) out of 968 KTRs had pre-transplant DSAs. Ninety percent was of White descent. Between the first and third years post-transplant, the observed rate of death-censored graft failure and mortality were 1.6% and 3.6%, respectively.

Exposure measurements

During the first 3 years of transplantation a total of 25.954 drug exposure measurements were available for analysis; 25.513 Tac-C_0, 4.098 Tac-$AUC_{0\text{-}12h}$, and 2.541 MPA-$AUC_{0\text{-}12h}$. The progression of Tac-C_0 and MPA-$AUC_{0\text{-}12h}$ over time is depicted in **Figure S2**. The median Tac-C_0 during the post-transplant period decreased from 9.2 ng/mL (IQR: 7.2–12.0) within the first 3 months to 6.1 ng/mL (IQR: 4.8–7.6) at year 1, to 5.7 ng/mL (IQR: 4.4–7.3) at year 3 post-transplantation. The median value at year 1 for Tac-$AUC_{0\text{-}12h}$ was 100 ng*hour/mL (IQR: 77–127) and MPA-$AUC_{0\text{-}12h}$ 41 mg*hour/L (IQR: 30–53). Median Tac-C_0 IPV was 40% (IQR: 33–50%).

Occurrence of primary and secondary outcomes of interest

Thirty-five out of 968 KTRs (3.6%) developed BPAR between years 1 and 3 post-transplantation. All but one were diagnosed on a for-cause biopsy. Three out of 35 KTRs (8.5%) had pre-transplant DSAs and 4 out of 35 KTR (11.4%) experienced BPAR in the first year post-transplant. Histological diagnoses of these BPAR events included chronic-active (ca) T-cell mediated rejection (TCMR), antibody mediated rejection (ABMR), and mixed (ca)TCMR/ABMR (**Table S1**).

BPAR incidence in the subgroup of Tac/MPA/Pred at 1 year was 17 of 848 (2.0%). Incidence of BPAR in KTRs on TMT with low IPV was 0.9% vs. 3.2% in KTRs with high IPV ($P = 0.028$).

Fifty out of 968 KTRs (5.2%) got rejection treatment between years 1 and 3 post-transplantation, of which 15 KTRs were treated without histological evidence.

Impact of Tac and MPA exposure on BPAR in overall study cohort

Uni- and multivariable analyses revealed that both Tac-and MPA-exposure at year 1 were associated with BPAR (**Table 2**). Every 20 ng*hour/mL increase in Tac-$AUC_{0\text{-}12h}$ gave an unadjusted HR (uHR) of 0.53 (95% CI: 0.45–0.62, $P < 0.001$) and adjusted HR (aHR) of 0.39 (95% CI: 0.30–0.50, $P < 0.001$) for BPAR. Tac-C_0 showed comparable results: every 1 ng/mL increase in Tac-C_0 gave an uHR of 0.59 (95% CI: 0.52–0.67, $P < 0.001$) and aHR of 0.46 (95% CI: 0.38–0.57, $P < 0.001$) for BPAR. MPA exposure was significantly associated with BPAR with an aHR of 0.80 (95% CI: 0.68–0.94, $P = 0.006$) for every 10 mg*hour/L increase in MPA-$AUC_{0\text{-}12h}$, irrespective of Tac exposure. No competing risk analysis was performed for the low incidence of mortality and death-censored graft failure. Proportionality of hazards' assumption was met and supported by a nonsignificant relationship between residuals and time.

Impact of Tac and MPA exposure on BPAR in subgroup of KTRs on TMT with Tac/MPA/Pred

When investigating KTRs on TMT with Tac/MPA/Pred, we found that the impact of Tac exposure did not materially change compared with the overall population (**Table 3**). However, there was no association between MPA-$AUC_{0\text{-}12h}$ at year 1 and BPAR anymore with an aHR of 0.93 ($P = 0.510$).

Impact of repeated Tac and MPA exposure on BPAR in study cohort and subgroup of KTRs on TMT with Tac/MPA/Pred

Bayesian, multivariable joint modeling revealed that repeated exposure measurements of both Tac-C_0 and MPA-$AUC_{0\text{-}12h}$ were associated with BPAR, both in the total study cohort and in the subgroup of KTRs on TMT with Tac/MPA/Pred. In the total study cohort, HRs for every 1 ng/mL increase in Tac-C_0 was 0.70 (95% credibility interval (CrI): 0.61–0.82, $P < 0.001$), and 0.75 for every 10 mg*hour/L increase in MPA-$AUC_{0\text{-}12h}$ (95% CrI: 0.62–0.93, $P < 0.001$; **Table 4**). In KTR on TMT with Tac/MPA/Pred, HR for Tac-C_0 was 0.5 (95% CrI: 0.45–0.58, $P < 0.001$), and 0.92 for every 10 units increase in MPA-$AUC_{0\text{-}12h}$ (95% CrI: 0.87–0.97, $P < 0.001$).

Identifying lower reference targets for Tac and MPA for years 1–3 for KTR on TMT with Tac/MPA/Pred

To identify lower reference targets for Tac and MPA at year 1 for KTR on TMT with Tac/MPA/Pred, we used logistic regression probability plots. These plots showed an exponential increase in BPAR if Tac-$AUC_{0\text{-}12h}$ or Tac-C_0 exposure went < 75 ng*hour/mL or < 5 ng/mL, respectively (**Figure 1**). BPAR incidence was significantly higher in KTR with Tac-C_0 < 5 ng/mL compared with Tac-C_0 > 5 ng/mL (5.4% vs. 0.8%, $P < 0.001$). KTRs with

Table 2 HRs for BPAR between years 1 and 3 post-transplantation in the total population

	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]	Multivariable HR+ (95% CI)[b]
Tac-C_0 (per 1.0 ng/mL increase)	0.59 (0.52–0.67); $P < 0.001$	0.54 (0.46–0.63); $P < 0.001$	0.46 (0.38–0.57); $P < 0.001$
Tac-AUC (per 20 ng*hour/mL increase)	0.53 (0.45–0.62); $P < 0.001$	0.47 (0.39–0.58); $P < 0.001$	0.39 (0.30–0.50); $P < 0.001$
MPA-AUC (per 10 mg*hour/L increase)	1.09 (0.97–1.22); $P = 0.182$	0.85 (0.74–0.98); $P = 0.030$	0.80 (0.68–0.94); $P = 0.006$

HRs for BPAR between years one and three post-transplantation in the total investigated population of 968 kidney transplant recipients.
AUC, area under the curve; BPAR, biopsy-proven acute rejection; CI, confidence interval; C_0, trough level; HR, hazard ratio; MPA, mycophenolic acid; Tac, tacrolimus.
BPAR incidence: 35/968 (3.6%).
[a]Adjusted for MPA exposure. [b]Adjusted for MPA exposure, HLA-mismatch, number of prior transplantations, type of transplantation, age/gender of recipient and donor, days on dialysis.

Table 3 HRs for BPAR between years 1 and 3 post-transplantation in KTRs on TMT with Tac/MPA/Pred

	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]	Multivariable HR+ (95% CI)[b]
Tac-C_0 (per 1.0 ng/mL increase)	0.49 (0.36–0.67); $P<0.001$	0.49 (0.36–0.68); $P<0.001$	0.54 (0.38–0.77); $P<0.001$
Tac-AUC (per 20 ng*hour/mL increase)	0.45 (0.31–0.64); $P<0.001$	0.45 (0.31–0.65); $P<0.001$	0.43 (0.28–0.66); $P<0.001$
MPA-AUC (per 10 mg*hour/L increase)	1.08 (0.96–1.22); $P=0.182$	1.00 (0.82–1.21); $P=0.992$	0.93 (0.74–1.16); $P=0.510$

HRs for BPAR between years 1 and 3 post-transplantation in KTRs on TMT with Tac/MPA/Pred at year 1 ($n=848$).
AUC, area under the curve; BPAR, biopsy-proven acute rejection; CI, confidence interval; C_0, trough level; HR, hazard ratio; KTR, kidney transplant recipient; MPA, mycophenolic acid; Pred, prednisolone; Tac, tacrolimus; TMT, triple immunosuppressive maintenance therapy.
BPAR incidence: 17/848 (2.0%).
[a]Adjusted for MPA exposure. [b]Adjusted for MPA exposure, HLA-mismatch, number of prior transplantations, type of transplantation, age/gender of recipient and donor, days on dialysis.

Table 4 HRs for repeated measurements of Tac and MPA exposure and BPAR between years 1 and 3 post-transplantation

	Multivariable HR (95% CrI)[a]	
	Total study cohort	KTR on TMT with Tac/MPA/Pred
Tac-C_0 (per 1.0 ng/mL increase)	0.70 (0.61–0.82) $P<0.001$	0.51 (0.44–0.59) $P<0.001$
MPA-AUC (per 10 mg*hour/L increase)	0.75 (0.62–0.93) $P<0.001$	0.92 (0.87–0.97) $P<0.001$

HRs for BPAR between years 1 and 3 post-transplantation in total study cohort ($n=968$) and in KTR on TMT with Tac/MPA/Pred ($n=848$) by using joint models with Bayesian estimation to allow analysis of longitudinal exposure measurements rather than single measurements. Output generates hazard ratios and credibility intervals (which defines the 95% probability of the HR falling within this range intervals).
AUC, area under the curve; BPAR, biopsy-proven acute rejection; C_0, trough level; CrI, credibility interval; HR, hazard ratio; KTRs, kidney transplant recipients; MPA, mycophenolic acid; Pred, prednisolone; Tac, tacrolimus; TMT, triple immunosuppressive maintenance therapy.
[a]Adjusted for MPA exposure, HLA-mismatch, number of prior transplantations, type of transplantation, age/gender of recipient and donor, days on dialysis.

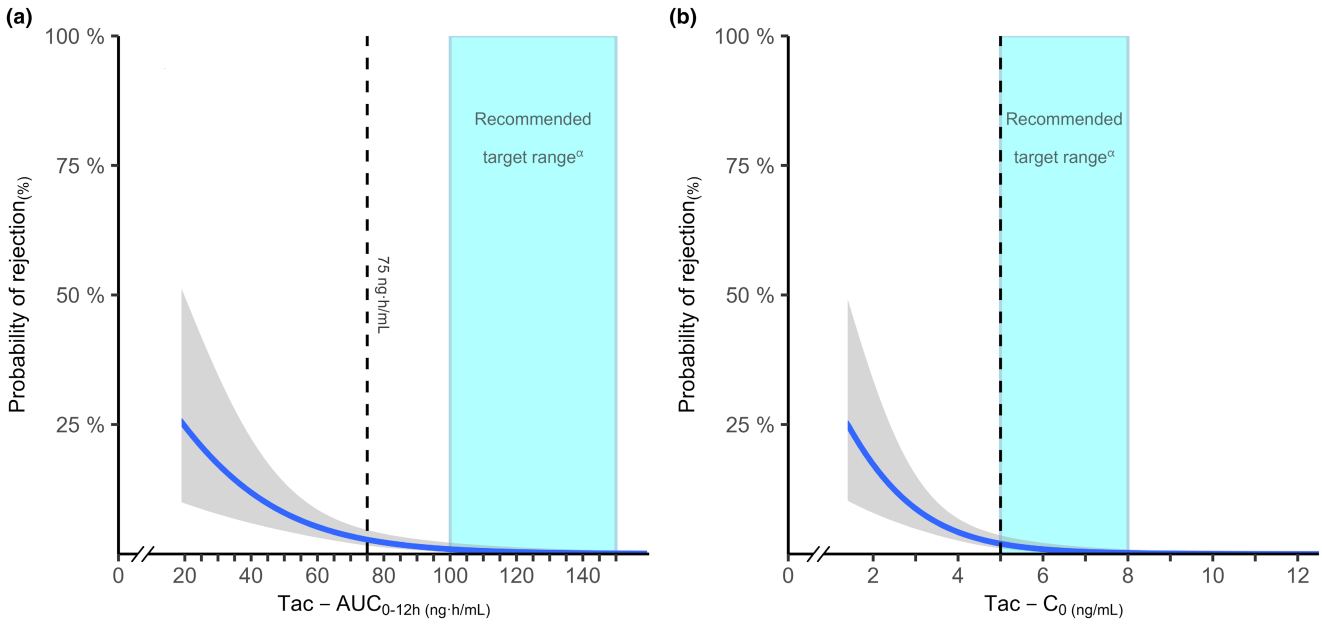

Figure 1 Tacrolimus exposure and probability of BPAR. The Y-axis depicts the probability of rejection in %. The X-axis depicts the exposure to Tac, expressed in $AUC_{0\text{-}12h}$ (**a**) and C_0 (**b**). The X-axis starts at Tac-$AUC_{0\text{-}12h}$ of 20 ng*hour/mL and Tac-C_0 of 1.5 ng/mL because these were the lowest measurements found. α: Light blue box depicts the range defined in the current consensus paper on therapeutic drug monitoring of Tac.[16] The dashed black lines illustrate the proposed lower limit for Tac-$AUC_{0\text{-}12h}$ and Tac-C_0. $AUC_{0\text{-}12h}$, area under the curve from 0 to 12 hours; BPAR, biopsy-proven acute rejection; C_0, trough level; Tac, tacrolimus. BPAR[16] Brunet *et al.*[17]

low IPV had a lower risk of BPAR than KTRs with high IPV for a similar exposure (**Figure S3**). We observed no BPAR events if Tac-$AUC_{0\text{-}12h}$ or Tac-C_0 were higher than 95 ng*hour/mL or 7 ng/mL, respectively (**Figure 1**, **Figure S3**). Therefore, a Tac-$AUC_{0\text{-}12h}$ 75–95 ng*hour/mL and Tac-C_0 5–7 ng/mL might be an optimal target based on (lack of additional) efficacy. We could not find an exposure-response relationship between MPA-$AUC_{0\text{-}12h}$ and BPAR, making it impossible to identify a long-term target for MPA other than 30–60 mg*hour/L as recommended by the consensus paper.[10]

Correlation between Tac-$AUC_{0\text{-}12h}$ and Tac-C_0

The correlation between $AUC_{0\text{-}12h}$ and Tac-C_0 was reasonably good (Pearson correlation coefficient $R=0.82$). However, the

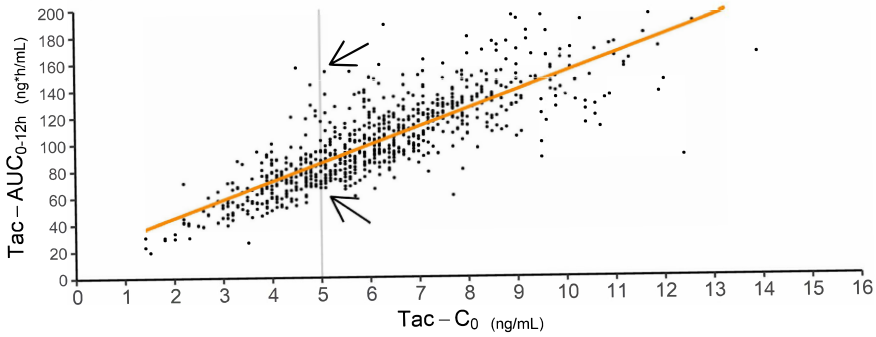

Figure 2 Correlation between Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 measured at year 1 post-transplantation. The Tac-C_0 has been projected against the Tac-$AUC_{0\text{-}12h}$. There is great interpatient variability between individuals. A Tac-$AUC_{0\text{-}12h}$ of 75 ng*hour/mL (±20%) may correspond to a C_0 between 3 and 8 ng/mL, whereas a C_0 of 4–6 ng/mL (5 ng/mL ± 20%) may correspond to an observed $AUC_{0\text{-}12h}$ between 40 and 160 ng*hour/mL. A Tac-C_0 of 5 ng/mL can result in an $AUC_{0\text{-}12h}$ of 65 ng*hour/mL or 155 ng*hour/mL. $AUC_{0\text{-}12h}$, area under the curve from 0 to 12 hours; C_0, trough level; Tac, tacrolimus.

Table 5 Incidence of BPAR for KTRs on TMT with Tac/MPA/Pred stratified in groups depending on Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 exposure

	BPAR	No BPAR
1) C_0 < 5 and $AUC_{0\text{-}12h}$ < 75	12 (8%)	148
2) C_0 < 5 and $AUC_{0\text{-}12h}$ > 75	0 (0%)	89
3) C_0 > 5 and $AUC_{0\text{-}12h}$ < 75	2 (7%)	28
4) C_0 > 5 and $AUC_{0\text{-}12h}$ > 75	3 (1%)	566

Incidence of BPAR for KTRs on TMT with Tac/MPA/Pred stratified in groups depending on Tac-$Auc_{0\text{-}12h}$ and Tac-C_0. Due to the high intrapatient variability in AUC-C_0 ratios, some KTRs might still suffer from under-exposure despite a supposedly sufficient Tac-C_0 (group 3). In contrast, some patients have a supposedly low Tac-C_0 whereas Tac-$AUC_{0\text{-}12h}$ is sufficient, not needing any increase in Tac dose (group 2).
$AUC_{0\text{-}12h}$, area under the curve from 0 to 12 hours; BPAR, biopsy-proven acute rejection; C_0, trough level; KTRs, kidney transplant recipients; MPA, mycophenolic acid; Pred, prednisolone; Tac, tacrolimus; TMT, triple immunosuppressive maintenance therapy.

relationship between Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 showed high interpatient variability (Figure 2). A Tac-$AUC_{0\text{-}12h}$ of 75 ng*hour/mL (±20%) corresponded to a C_0 between 3 and 8 ng/mL, whereas a C_0 of 4–6 ng/mL (5 ng/mL ± 20%) corresponded to an observed $AUC_{0\text{-}12h}$ between 40 and 160 ng*hour/mL. Furthermore, Figure 2 shows that a Tac-C_0 of 5 ng/mL can result in an $AUC_{0\text{-}12h}$ between 65 ng*hour/mL and 155 ng*hour/mL (illustrated with arrows).

Table 5 displays the incidence of BPAR in subgroup of KTRs on TMT with Tac/MPA/Pred stratified in the 4 suggested target groups of Tac-$AUC_{0\text{-}12h}$ and Tac-C_0. We found an extremely low incidence of BPAR in KTRs with Tac $AUC_{0\text{-}12h}$ > 75 ng*hour/mL (0.5%). However, in KTRs with Tac C_0 > 5 ng/mL and $AUC_{0\text{-}12h}$ < 75 ng*hour/mL, we found a BPAR incidence of 7%, comparable to that of KTRs with C_0 < 5 and $AUC_{0\text{-}12h}$ < 75.

Sensitivity analyses

The associations found among MPA-$AUC_{0\text{-}12h}$, Tac-$AUC_{0\text{-}12h}$, Tac-C_0, and BPAR between years 1 and 3 post-transplantation remained unchanged in multiple sensitivity analyses in different subgroups (Tables S2–S6). In our final sensitivity analysis, where we associated drug exposure with rejection therapy, we repeatedly found significant associations between Tac/MPA exposure and treatment of rejection with aHRs of 0.42 (95% CI: 0.31–0.56), 0.52 (95% CI: 0.41–0.66), and 0.78 (95% CI: 0.64–0.97), for Tac-$AUC_{0\text{-}12h}$, Tac-C_0, and MPA-$AUC_{0\text{-}12h}$, respectively (Table S7).

DISCUSSION

There is scant data on targets beyond the first year of transplantation for immunosuppressive maintenance therapy to prevent BPAR. In this study, we found that both Tac and MPA exposure were associated with BPAR between the first and third years post-transplant. We found that a Tac-$AUC_{0\text{-}12h}$: 75–95 ng*hour/mL, Tac-C_0: 5–7 ng/mL, and MPA-$AUC_{0\text{-}12h}$: 30–60 mg*hour/L might be suggested as optimal targets for KTRs on TMT at year 1 based on efficacy analysis. We deliberately investigated BPAR between years 1 and 3 post-transplant only (and not beyond) to limit the time interval between measured drug exposure at 1 year and time to BPAR.

Our findings seem in accordance with published literature, which is mainly limited to the first year post-transplant.[3,8,10,17,18] The ELITE-symphony trial reported a mean Tac-C_0 of 6.4 ng/mL at year 1 post-transplant.[3] The follow-up study reported a comparable mean Tac-C_0 of 6.5 ng/mL for the third year post-transplant with a comparable BPAR incidence of 2% to our study.[19] However, it is essential to note that, on average, KTRs in our real-world cohort have a higher immunological risk than the ELITE-Symphony trial population. First, the ELITE-Symphony trial excluded KTRs with a PRA > 20% and only 20% of those included had a PRA > 0%, whereas our cohort included all KTRs, of whom 22% had a PRA ≥ 5%. Moreover, mean HLA-antigen mismatches in our population were higher, 3.2 vs. 2.9 in the ELITE-Symphony trial. Second, the ELITE-Symphony was a randomized trial in which consenting participants were followed per study protocol. In contrast, our results are based on observational data from real-life clinical practice that may result in poorer KTRs' adherence. Another study reported that Tac-C_0 should be maintained above 7 ng/mL during the first year, without recommendations beyond this point in time.[4] In our cohort, we found little evidence to maintain this threshold for the period thereafter because we observed no BPAR

events. Additionally, maintaining a higher target may likely result in more long-term toxicity, although our study did not investigate this. Last, our study suggested lower Tac-C_0 target of 5 ng/mL for BPAR corresponds well to previous studies that investigated alternative end points, such as *de novo* DSA formation or graft survival.[20,21]

Our study builds on the existing literature by adding more advanced assessment of immunosuppressive exposure in various ways. Importantly, the ELITE-Symphony trial and previous studies on Tac exposure on BPAR did not consider exposure to all maintenance drugs simultaneously, including MPA exposure.[3,4,19,20] Rather than using only one single measurement at year 1 or a mean of multiple exposure measurements, we also used joint models to allow accurate analysis of the association between repeated Tac and MPA exposure, and subsequent BPAR. Moreover, no study before, to our knowledge, investigated the potential merit of $AUC_{0\text{-}12h}$ compared with C_0 monitoring of Tac on BPAR. At last, we validated our results by performing multiple sensitivity analyses in different subgroups reducing uncertainty of our findings.

Previous studies identified Tac-IPV as a risk factor for poor graft outcomes, such as DSA development, rejection, and impaired graft survival over the first year post-transplant.[20,22–24] This study confirms the correlation between high Tac-IPV and BPAR in the period thereafter and demonstrates that Tac-IPV modulates the lower threshold of Tac-C_0.

The correlation between Tac-C_0 and $AUC_{0\text{-}12h}$ was reasonably good (R = 0.82). However, we observed large interpatient variability in Tac $AUC_{0\text{-}12h}$, underscoring potential merit of $AUC_{0\text{-}12h}$-driven dose adjustments to prevent BPAR. It is important to note that a 20% variability in C_0 levels corresponded to a 4-fold difference in observed $AUC_{0\text{-}12h}$ exposure (between 40 and 160 ng*hour/mL). KTRs with a Tac-C_0 on target (> 5 ng/mL) could still be underexposed according to their $AUC_{0\text{-}12h}$. We showed that Tac-$AUC_{0\text{-}12h}$ obtained by a limited sampling strategy seemed to be a better exposure marker than C_0 for BPAR risk. Especially, KTRs with a low $AUC_{0\text{-}12h}/C_0$ ratio could be at increased risk of BPAR. The risk of BPAR in KTRs with Tac-C_0 < 5 ng/mL and Tac-$AUC_{0\text{-}12h}$ < 75 ng*hour/mL was 8% and comparable to the risk observed (7%) in KTRs with Tac-C_0 > 5 ng/mL & Tac-$AUC_{0\text{-}12h}$ < 75 ng*hour/mL.

Various studies found that MPA exposure was linked to graft rejection within the first year, whereas others failed to show a benefit.[8,25–29] To the best of our knowledge, evidence on the impact of MPA exposure on BPAR after the first year of transplantation is absent. However, we found that MPA exposure was associated with BPAR between years 1 and 3 post-transplantation. Moreover, we also confirmed that the association between longitudinal exposure to MPA and BPAR also exists in the long term and is not limited to the first year post-transplant.[9] As further support to these findings, the companion paper by Villeneuve *et al.* (submitted to Clinical Pharmacology and Therapeutics; November 30, 2022) also reports that MPA-$AUC_{0\text{-}12h}$ monitoring decreased the incidence of BPAR at 3 years post-transplant. The fact that we could only find an association between MPA-$AUC_{0\text{-}12h}$ and BPAR in the total population and not in the subgroup of KTRs on TMT with Tac/MPA/Pred can have several explanations. First, MPA exposure may play a more subtle role in BPAR, which is supported by the fact that there is a clear association between repeated exposure measurements of MPA and BPAR. Second, the mitigating effect of Pred on BPAR,[30] may weaken the importance of MPA-$AUC_{0\text{-}12h}$ at year 1 because corticosteroids are not routinely withdrawn in our program. Third, all KTRs on TMT were tightly titrated to an MPA-$AUC_{0\text{-}12h}$ between 30 and 45 mg*hour/L, thereby limiting variability and making it difficult to investigate the impact of other exposure levels. Consequently, we found insufficient evidence to recommend an MPA-target other than those previously published (30–60 mg*hour/L).[10]

Our real-world cohort study may falsely suggest a "one-size-fits-all" approach. In contrast, the transplant field is in desperate need of a stratified or individualized approach with regard to maintenance immunosuppression. Although we managed to formulate some degree of individualized Tac targets (depending on Tac-IPV or on Tac-$AUC_{0\text{-}12h}$) it was difficult to stratify for different immunological risk groups due to a limited number of events. Nevertheless, it seems defendable based on the immunological risk "bandwidth" as stipulated in our baseline characteristics and different sensitivity analyses that patients with a lower risk of BPAR could be more safely titrated to the lower end of the target range for Tac-$AUC_{0\text{-}12h}$: 75 ng*hour/mL or Tac-C_0: 5 ng/mL, whereas patients at a higher risk could perhaps be titrated to the higher end of the proposed range (Tac-$AUC_{0\text{-}12h}$: 95 ng*hour/mL/Tac-C_0: 7 ng/mL). Another major issue for individualization is the inability to adequately express immunological risk in an encompassing and dose-dependent way. Recently, multiple studies showed that epitope and amino-acid (rather than antigenic) mismatches of the HLA-molecule between donor and recipients could adequately classify KTR into different immunological risk groups for DSA development, acute rejection, and graft loss which could be a pivotal step toward personalized exposure targets in the future.[21,31–35] An important hurdle to overcome, however, is that assessing epitope and amino-acid mismatches requires high resolution HLA genotyping of both donors and recipient, which are not readily available in all centers.

The design of this study is also associated with some limitations. First, the limited number of BPAR events raises question on possible over-immunosuppression or under-detection. However, our incidence rate (3.5%) is more or less in line with other studies, such as the ELITE-Symphony trial (2.0%).[3,19] Moreover, our immunosuppressive exposure and protocolary targets are in line with the latest Tac and mycophenolate consensus guidelines and our 5- and 10-year patient survival (88% and 68%—unpublished data), a potential proxy of overimmunosuppression or under-detection, are completely in line with the Collaborative Transplant study (CTS-5-years survival: 87%) and the article by Hariharan *et al.* (5- and 10-years survival: ~86% and ~66%).[11,36] This supports the generalizability of our real-life data. Second, our proposed upper limit for Tac-$AUC_{0\text{-}12h}$ and Tac-C_0 is based on the lowest level above, which there is absence of additional BPAR events. This study did not focus on toxicity to better define the upper limit of the therapeutic window. One could argue that it is defendable to accept slightly increased risk for rejection in the context of observed

toxicity or comorbidity, and thus target a lower upper reference in individual cases. Yet, our proposed upper limit is lower than the currently proposed limit in the Tac consensus paper.[11,17,36] Third, the observational (nonrandomized) nature of this study is a limitation given risks of indication and selection biases. Patients may have been titrated to a certain exposure depending on various factors, such as HLA-mismatch, (drug) adherence, and toxicities which we aimed to address by adjusting for various measurable confounders, using different statistical methods, including sensitivity analyses in different subgroups, and looking at rejection therapy as secondary outcome in which our conclusions remained materially unchanged. Despite our efforts to minimize the risk of incorrect inferences, there is still an inevitable and unaccountable risk of bias that is associated with observational studies. It is, however, important to note that there is scant evidence to support longer term targets, and that it is unlikely that randomized trials will follow in the near future given their complexity and financial limitations. As a result, current evidence available beyond the first year of transplantation will largely be restricted to observational data. Importantly, both the European Medicines Agency and the US Food and Drug Administration (FDA) have published guidance documents in which the importance of observational real-world data (in addition to evidence originating from randomized clinical trials) despite its caveats has been addressed, especially in an era where more data from electronic health records are being collected.[37] To limit the bias in observational studies with real-world data, target trial emulation could be a possible elegant solution in case of sufficient events.[38] Fourth, because protocol biopsies are not part of routine clinical care, we have no information on subclinical injury and cannot rule out that subclinical cases might have been missed. However, our 5- and 10-year death-censored graft survival (as proxy of missed chronic rejection or underexposure) are 91% and 83% (unpublished data), which is also in line with other cohorts, suggesting that our real-world practice is comparable and generalizable to other cohorts.[11,35,36] Last, it is essential to note that this study included KTRs from a transplant center where TDM ($AUC_{0\text{-}12h}$ and C_0) is part of routine clinical care and where allocation is part of the Eurotransplant system. Extrapolation of our findings to other KTR populations with varying genetic backgrounds and pharmacokinetic characteristics may possibly be questioned. Possible differences in pharmacokinetics can be accounted for by measurement of exposure by Tac-$AUC_{0\text{-}12h}$ in stable KTRs.

In summary, a Tac-$AUC_{0\text{-}12h}$ range of 75–95 ng*hour/mL and Tac-C_0 5–7 ng/mL in KTRs on TMT seem reasonable targets beyond the first year of transplantation. KTRs with low IPV might benefit from even lower target ranges. Tac-$AUC_{0\text{-}12h}$ obtained by limited sampling strategy may be more informative than Tac-C_0 for BPAR risk. Although we could not formulate lower targets for MPA-$AUC_{0\text{-}12h}$ to expand on current literature, it is essential to realize that MPA is associated with BPAR after the first year post-transplant, making sufficient exposure to the drug important. Future multicenter studies, including repeated exposure measurements and information on granular immunological risk and toxicity, are necessary to aid transplant physicians in the long-term management of KTRs by further refining lower and upper limits of the therapeutic windows of Tac and MPA.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

In the last 3years T.vG. has received lecture fees and study grants from Chiesi and Astellas, in addition to consulting fees from Roche Diagnostics, Thermo Fisher, Vitaeris, CSL Behring, Astellas, and Aurinia Pharma. A.V.R. received lecture and consulting fees from Sandoz, Chiesi, Astellas, Hansa, CSL Behring, and Novartis in the past 3years, all of which went to his employer LUMC. In all cases money has been transferred to hospital accounts, and none has been paid to their personal bank accounts. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

S.M., T.vG., J.K., D.vdH., P.vdB., J.dF., D.M., and A.dV. wrote the manuscript. S.M., T.vG., D.vdH., P.vdB., J.dF., D.M., and A.dV. designed the research. S.M., T.vG., J.K., D.vdH., P.vdB., J.dF., D.M., and A.dV. performed the research. S.M., T.vG., D.M., and A.dV. analyzed the data.

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