COMPUTATIONAL MEDICINE

Individualizing liver transplant immunosuppression using a phenotypic personalized medicine platform

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Posttransplant immunosuppressive drugs such as tacrolimus have narrow therapeutic ranges. Inter- and intra-individual variability in dosing requirements conventionally use physician-guided titrated drug administration, which results in frequent deviations from the target trough ranges, particularly during the critical postoperative phase. There is a clear need for personalized management of posttransplant regimens to prevent adverse events and allow the patient to be discharged sooner. We have developed the parabolic personalized dosing (PPD) platform, which is a surface represented by a second-order algebraic equation with experimentally determined coefficients of the equation being unique to each patient. PPD uses clinical data, including blood concentrations of tacrolimus—the primary phenotypic readout for immunosuppression efficacy—to calibrate these coefficients and pinpoint the optimal doses that result in the desired patient-specific response. In this pilot randomized controlled trial, we compared four transplant patients prospectively treated with tacrolimus using PPD with four control patients treated according to the standard of care (physician guidance). Using phenotype to personalize tacrolimus dosing, PPD effectively managed patients by keeping tacrolimus blood trough levels within the target ranges. In a retrospective analysis of the control patients, PPD-optimized prednisone and tacrolimus dosing improved tacrolimus trough-level management and minimized the need to recalibrate dosing after regimen changes. PPD is independent of disease mechanism and is agnostic of indication and could therefore apply beyond transplant medicine to dosing for cancer, infectious diseases, and cardiovascular medicine, where patient response is variable and requires careful adjustments through optimized inputs.

INTRODUCTION

Posttransplant survival has greatly improved in part because of less toxic immunosuppression, improved drug monitoring protocols, and refining of target therapeutic ranges of these immunosuppressant drugs (1, 2). Transplant patients still need multiple therapeutic and prophylactic medications, with distinct pharmacologic and metabolic profiles and numerous interactions. Differences in absorption, metabolism, genotype, and comorbidities and in addition to anatomic and physiologic variations alter pharmacokinetics drastically (3–5).

In the absence of a unifying measure of immunosuppression, therapeutic drug monitoring serves as a surrogate of immunosuppression. However, even this simple measure fluctuates widely. Induction or inhibition of metabolic and transport enzymes, among other interactions, results in highly unpredictable whole-blood drug concentrations with inter- and intraindividual fluctuations that require close monitoring

and dose adjustment (6, 7). For example, tacrolimus, a mainstay of solid organ transplantation, has a narrow therapeutic window and wide pharmacokinetic variability (8). Underdosing of tacrolimus may result in underimmunosuppression and acute rejection. Overdosing puts patients at risk of considerable neuro- and nephrotoxicity (9). Tacrolimus is a substrate of cytochrome P450 and P-glycoprotein, both with genetically variable expression levels in liver and intestine. These factors combine to yield very poor inter- and intraindividual correlation between dosing and blood concentrations. In sum, dosing is clinically challenging. The lack of a consistent relationship between dose and blood concentration makes simple calculations of pharmacokinetic parameters invalid.

The standard of care is for a provider to adjust the dose of an immunosuppressant drug in response to a whole-blood trough concentration, making an educated guess about factors causing deviation from the target range and the amount by which to adjust the dose in response. These decisions are made largely on the basis of clinical experience, and patients frequently deviate from the target range, running the alternate risks of toxicity or graft rejection. Models have been developed to predict tacrolimus pharmacokinetics in solid organ recipients with multiple covariates and uncertainty regarding the importance of each covariate. These include models based on population pharmacokinetics (10), physiology-based pharmacokinetics (11, 12), genetics (13, 14), and estimative forecasting (15, 16). Attempts to increase prediction accuracy using these modeling approaches exclude complex patients to prevent too many confounding effects upon disease-causing mechanisms. These approaches have shown that drug combination performance is dose-dependent and is largely influenced by drug interactions. Although multidrug dose-modeling studies can examine

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the nonlinearity of drug-drug interactions (*17–19*), optimizing and personalizing combination therapy by correlating input stimuli (drug administration) directly with phenotype (efficacy) have not yet been realized in the clinic.

We discovered that treatment efficacy for a given patient can be related to drug dosing through a "response surface" represented by a parabola; we called this mathematical phenotype–dose relationship "parabolic personalized dosing" (PPD). PPD is a second-order algebraic equation based on coefficients specific to the application (in this case, the patient) and initially unknown as treatment commences. This approach adaptively individualizes an input on the basis of these specific coefficients. As a foundation for this clinical study, we have used the PPD platform in vitro and in vivo preclinically for applications in drug development (*20–25*). These studies showed that the parabolic response can identify optimal drug administration conditions across a broad spectrum of indications, from metabolic inhibitors to optimal combinations of antiangiogenic medicines in cancer and stem cells. We hypothesized that PPD could be extended to the clinic, specifically in the context of posttransplant regimens, where patients respond variably and a "one-size-fits-all" therapeutic paradigm is not desired.

According to most liver transplant immunosuppression protocols, only the tacrolimus dose is adjusted on the basis of a patient's daily tacrolimus whole-blood trough concentration (or trough level). Dosing of other immunosuppressants follows established protocols. Patient-specific target ranges are based on clinical context, including ethnicity, age, liver disease, disease severity, kidney function, comorbidities, concern for rejection, and use of other immunosuppressants. Despite the multiple drugs coadministered with tacrolimus, PPD optimization of the single tacrolimus input ensures that the patient's drug levels are maintained in a target range. Patient-specific coefficients that define the PPD are determined by calibrating the input doses to the phenotypic outputs, which can include trough levels of drugs or biomarkers as indicators of efficacy and safety.

In this pilot prospective randomized clinical study, PPD was used to personalize tacrolimus administration to four liver transplant patients (PPD1 to PPD4) and was compared with four control patients (C1 to C4) who received standard of care. Trough levels of tacrolimus represented the phenotype that guided the PPD process. Patients treated with PPD had significantly less variability in tacrolimus trough levels compared with control patients with physician-guided dosing. In addition, retrospective PPD for control patients indicated the possibility of better maintaining trough levels within the target range compared to the standard of care that they received. This study therefore provides a clinical proof of principle and an early demonstration of feasibility for posttransplant phenotypic personalized medicine (PPM).

RESULTS

Parabolic personalized dosing
A drug or drug combination represents an input administered to a patient. The patient then responds with phenotypic outputs, such as the efficacy and/or toxicity of therapy. We have discovered that the drug input and phenotypic output relationship is represented by a second-order polynomial equation, that is, a parabolic surface (*20–25*). Here, the trough level of tacrolimus was the "output"—which is measured routinely as part of any patient's clinical care; the input was only a one-drug dose, and three coefficients were determined using three consecutive

trough level data points (Fig. 1). Each patient response was characterized by a parabola. Barring any changes to the treatment protocol, this parabola is a robust map that identifies drug doses (inputs) that ensure that a patient will stay in a target tacrolimus range (phenotypic output). In some cases, however, introducing new drugs or procedures into the regimen, like antibiotics or hemodialysis, changes the patient's trough levels substantially. Establishing a new parabola, which we have termed recalibration, can then identify the proper tacrolimus dose to bring the trough levels back into the target range.

The PPD approach can be summarized as follows. A healthy patient is $F(S)$, and a patient with disease is $F(S')$, where S represents the patient's normal network mechanisms and S' the aberrant network mechanisms (Eq. 1). The indicator of the patient's physiological response is the phenotype of interest that can be measured clinically, such as trough level, tumor size, cell viability, or pathogen load. The diseased patient phenotype includes the parameter C—the drug dose and/or drug type (Eq. 1). Owing to the complexity of these mechanistic networks, explicit forms of these functions—$F(S)$, $F(S')$, and $F(S',C)$—are unknown. According to the Taylor expansion in mathematics, $F(S',C)$ is related to $F(S')$ by the following expression:

$$F(S',C) = F(S') + x_0 + \sum x_i c_i + \sum y_{ii} c_i^2 +$$
$$\sum z_{ij} c_i c_j + \text{high-order terms} \qquad (1)$$

where x_i is the patient response coefficient to drug i at concentration c_i, and z_{ij} is the patient response coefficient to the interaction of drug i and drug j at their respective concentrations. Because the human body responds to input in a nonlinear fashion with respect to drug i, y_{ii} represents a second-order response to the drug concentration c_i. The values of x_0, x_i, y_{ii}, and z_{ij} are experimentally determined by calibrating phenotypic outputs of a specific patient and the drug-dose inputs. Hence, the optimized drug-dose combination is personalized to this specific patient.
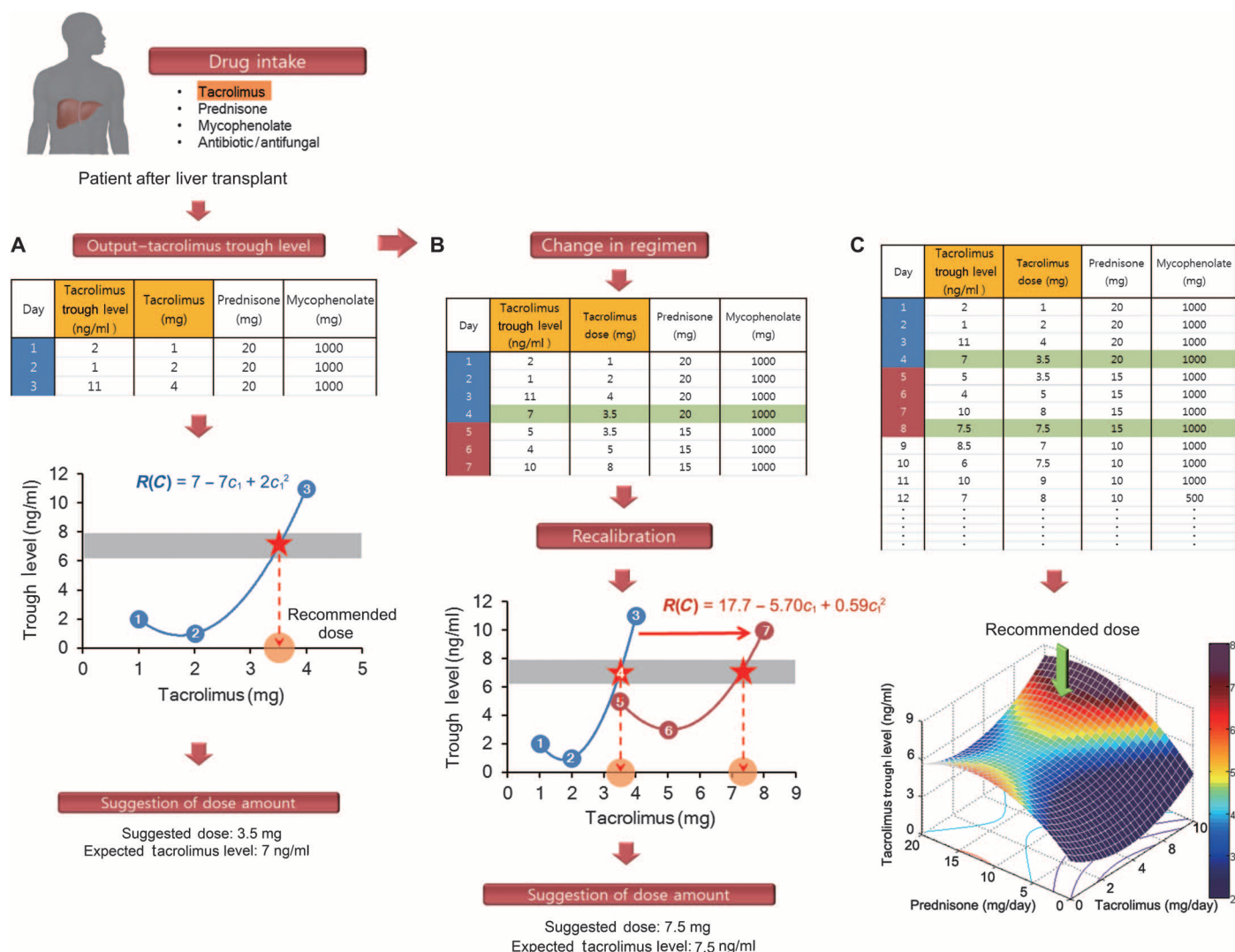
We have previously demonstrated that the high-order terms are much smaller than the first- and second-order terms (*21, 25*). Therefore, by moving $F(S')$ to the left side of the equation and removing the high-order terms, we arrive at the following Eq. 2, which is further explained in movie S1:

$$R(C) = F(S',C) - F(S') = x_0 + \sum x_i c_i + \sum y_{ii} c_i^2 + \sum z_{ij} c_i c_j \qquad (2)$$

The difference between the two unknown equations $F(S',C)$ and $F(S')$ is the overall patient phenotypic response $R(C)$ to treatment, which can be approximated by a second-order algebraic equation of drug concentrations alone, independent of the specific genomic and proteomic mechanisms. Therefore, PPD is disease mechanism–independent and disease indication–agnostic. Additionally, because experimental data are needed to construct this response surface by calibrating the coefficients, PPD is not a model-based algorithm.

A simulated case study of PPD
We first simulated PPD to demonstrate the process (Fig. 1). In this simulation, one drug, tacrolimus, was used to adjust the phenotypic output, the tacrolimus trough level. The administered drug dose, or input, is represented by c_1. Each data point, or indicator of phenotype, was represented by a daily trough level. On days 1, 2, and 3, the trough levels were 2, 1, and 11 ng/ml, respectively, and were plotted against the tacrolimus dose (mg) given. The phenotypic response (Eq. 2) was

Fig. 1. PPD process guiding tacrolimus dosing for liver transplant patients. In this simulation, a patient after transplant was prescribed tacrolimus and other medications. The patient's PPD curve (blue in select graphs) was calibrated using the trough levels from physician-guided standard-of-care dosing on days D(1) to D(3). The PPD curve was used to prospectively dose tacrolimus (red stars) to bring the patient's trough levels into the target range of 6 to 8 ng/ml (gray regions). The numbers within the circles are the dosing days with the given tacrolimus doses and the resulting trough levels. (**A**) Original PPD calibrated curve (blue) using the trough levels from D(1) to D(3), with the recommended dose for D(4) (red star). (**B**) Following regimen changes, the PPD curve was recalibrated using the trough levels from three successive days after the regimen changes. During PPD recalibration, tacrolimus doses for the patient were preemptively selected on the basis of previous correlations. The recalibrated PPD curve (red) using the trough levels from D(5) to D(7) yielded D(8)'s recommended dose (red star). (**C**) 3D PPD tacrolimus and prednisone surface calibrated using the patient's tacrolimus doses, prednisone doses, and trough levels from D(1) to D(11). Recommended tacrolimus dose is identified on the surface as noted by the green arrow.

a parabola represented by $R(C) = 7 - 7c_1 + 2c_1^2$ (Fig. 1A). Assuming no regimen changes for the subsequent day (for example, no new drugs administered, no hemodialysis, and changes in current drug dosages), according to the PPD equation above, the recommended dose for day 4 would be 3.5 mg, predicting a trough level of 7 ng/ml, within the target range (6 to 8 ng/ml) (Fig. 1A). In effect, this patient's unique set of response coefficients x_0, x_1, and y_{11} are 7, −7, and 2, respectively, based on the individual PPD quadratic equation and were determined exclusively from clinical data. The number of data points required to determine the patient's PPD equation is equal to the number of response coefficients to be calibrated (Eq. 2 and movie S1).

Assuming no regimen changes, the parabola would allow accurate dosing for several days, where the patient would continue with 3.5 mg. However, in the event of a regimen change—for instance, antibiotics administration, biliary drain capping, and prednisone dose change—the patient's response coefficients would be recalibrated by monitoring the resulting change to the trough level; the parabola is shifted and replotted to identify the new optimal drug dose (Fig. 1B). This could be accomplished using trough levels from three additional days (days 5 to 7). The newly plotted parabola is represented by $R(C) = 17.7 - 5.70c_1 + 0.59c_1^2$, with all new coefficients. If no additional changes are made to this patient's regimen for the subsequent day, the recommended

tacrolimus dose for day 8 would be 7.5 mg and would result in a trough level of 7.5 ng/ml (Fig. 1B).

If a two-drug combination is administered, we can then use three-dimensional (3D) PPD to optimize doses. In this simulated case, administering six different dose combinations of two drugs produces six coefficients (x_0, x_1, x_2, y_{11}, y_{22}, and z_{12}) (Eq. 2). The 3D PPD determines the recommended doses on day 8 as 7.5 mg of tacrolimus and 15 mg of prednisone, to achieve the tacrolimus trough level of 7.5 ng/ml, within the target range (Fig. 1C). When the doses of two different drugs, as opposed to one drug, are PPD-determined, more inputs (different dose combinations) comprehensively regulate the phenotypic output. Also, when two-drug dose inputs are modulated, the need for recalibration is minimized compared with using single-drug modulation to optimize treatment outcomes.

PPD-assisted personalized immunosuppression
In the pilot study, for all eight patients, physician-guided standard-of-care dosing was used for the first 10 days of tacrolimus dosing after liver transplantation to avoid confounding effects from the clinical protocol transitioning patients from methylprednisolone to prednisone. For the PPD-assisted patients (PPD1 to PPD4), personalized tacrolimus dosing started after these 10 days and was considered as dosing day [D(1)]. Control patients continued to receive tacrolimus via physician guidance but also reset as day 1 [D(1)] when the trial started.

PPD patient 1. At the time of transplantation, PPD1 had a model of end-stage liver disease (MELD) score of 40, which represents the highest acuity. MELD predicts patient mortality within 3 months without transplantation, and its values range from 6 to 40. Drugs administered to PPD1 included tacrolimus, prednisone, and mycophenolate mofetil (MMF) (immunosuppressants); fluconazole and cotrimoxazole (antifungal and antibiotic); and ganciclovir (antiviral). Hemodialysis was performed multiple times during the treatment period. The tacrolimus trough target range for this patient was from 6 to 8 ng/ml.

PPD was performed by plotting the trough levels 8.4, 5.3, and 4.5 ng/ml measured on D(−2) to D(0) (posttransplant tacrolimus dosing days 7 to 9) against tacrolimus doses chosen by the physician. These data resulted in a parabola corresponding to $R(C) = 48.3 − 20.4c_1 + 2.35c_1^2$ (Fig. 2A, blue). PPD recommended a dose of 7.5 mg for D(1), but this did not result in a target trough level, likely owing to two simultaneous regimen changes—ciprofloxacin (400 to 0 mg) and capping the biliary tube. PPD recalibration occurred after another regimen change (hemodialysis from continuous to single-pass) on D(2). PPD recommended the tacrolimus doses of 3.5, 3.75, 2, and 3 mg for D(2) to D(5) to recalibrate the dosing. Although only three data points are necessary for calibration, four data points [D(2) to D(5)] successfully accounted for the multiple regimen changes on D(1) and D(2). The recalibration process is represented by a shift (movie S1) from the blue to the orange [$R(C) = 30.0 − 21.4c_1 + 4.39c_1^2$] parabolas in Fig. 2A. The newly plotted orange parabola provided robust trough level control for 2 weeks at a steady tacrolimus dose of 3.25 mg. With anticipated ganciclovir dose changes on D(9) and D(12) for this patient, PPD recommended an increased tacrolimus dose of 3.5 mg to mitigate the resulting trough level deviation from the target range.

The time elapsed between the hemodialysis procedure and trough level reading had a strong effect on trough levels, shown by the clustering of D(6) to D(16) trough levels (Fig. 2A). Therefore, a correlation plot enabled preemptive tacrolimus dosing adjustments to prevent underdosing, because hemodialysis typically resulted in substantially lower trough levels

than anticipated (Fig. 2B). This analysis indicated that the trough level should be measured at least 10 hours after hemodialysis. The magnitude of the impact of hemodialysis upon trough levels was patient-specific.

For PPD1, two regimen changes—hemodialysis and cotrimoxazole dosing schedule—occurred on D(17) and D(18) (Fig. 2A). PPD recalibration using D(19) to D(21) shifted the curve from the orange to the red [$R(C) = 13.8 − 4.7c_1 + 0.6c_1^2$] parabolas in Fig. 2A. The patient's trough levels converged toward and stayed within or near the target range during the PPD treatment period until discharge from the hospital on day 33 after transplant.

PPD patient 2. PPD2 had a MELD of 25 at time of transplant, and initial target tacrolimus trough ranges were 7 to 9 ng/ml on D(−2) and 8 to 10 ng/ml for D(−1) to D(5). The target range was later changed to 9 to 11 ng/ml for D(6) to D(11) due to concerns of possible transplant rejection on the basis of elevated levels of liver enzymes in the blood, signaling hepatocyte injury in the absence of signs of other physiologic or anatomic causes. The patient's posttransplant regimen included tacrolimus, prednisone, MMF, fluconazole, ciprofloxacin, cotrimoxazole, tenofovir, and ganciclovir (table S1). PPD2 did not require hemodialysis.

PPD was calibrated by plotting the trough levels 5.4, 6.3, and 7.7 ng/ml from D(−2) to D(0) against physician-determined tacrolimus doses. These data resulted in a parabola corresponding to $R(C) = 7.92 − 1.80c_1 + 0.32c_1^2$ (Fig. 2C, blue). This parabola was used to identify tacrolimus doses for D(1) to D(3), which brought the trough levels within the target range of 8 to 10 ng/ml. Several regimen changes occurred on D(4), including a stepwise increase of MMF from 1000 to 2000 mg/day during D(4) to D(7). To compensate for the anticipated increase in the trough levels from the MMF dose increase, we preemptively decreased the tacrolimus dose—per PPD recommendations—from 5.5 mg on D(5) to 5 mg on D(6) to 4 mg on D(7); this was based on a known correlation between MMF and tacrolimus for PPD2, noted in table S4 (movie S2). Except for D(6), the trough levels from D(4) to D(7) were close to or within the target range of 8 to 10 ng/ml and 9 to 11 ng/ml (Fig. 2C). PPD recalibration as a result of regimen changes, using data from D(8) to D(10), created a new reference parabola, shifting from the blue to the red [$R(C) = 18.8 − 6c_1 + 0.8c_1^2$] (Fig. 2C). The recalibrated PPD brought the D(11) trough level into the target range. PPD2's trough levels were near or within the target range during the PPD period until the patient's discharge from the hospital on D(20).

PPD patient 3. PPD3 had a MELD of 9 at time of transplant and a target trough range of 8 to 10 ng/ml. The patient's posttransplant regimen included tacrolimus, prednisone, MMF, fluconazole, cotrimoxazole, ganciclovir, and valganciclovir (table S1). PPD3 did not require hemodialysis. For this patient, a parabola corresponding to $R(C) = 6.3 − 0.28c_1 + 0.93c_1^2$ was constructed by plotting trough levels 6.4, 7.1, 7, 8.7, and 9.3 ng/ml from D(−2) to D(2) against physician-determined tacrolimus dosages (Fig. 2D, blue). Owing to the repeated tacrolimus doses given, five data points [D(−2) to D(2)] were needed to calibrate the PPD curve. The PPD curve prospectively dosed D(3) within the target range. Despite the multiple regimen changes—cotrimoxazole (0 to 480 mg), intravenous to oral conversion of tacrolimus, fluconazole (200 to 0 mg), and ganciclovir (450 to 0 mg)—during D(4) to D(9), the PPD recommendations maintained trough levels near or within the target range during the PPD period (Fig. 2D and movie S3). PPD3 was discharged on D(10). Additionally, as an outpatient on D(12), PPD3's trough level was 9.6 ng/ml, within the target range (Fig. 2D, red).
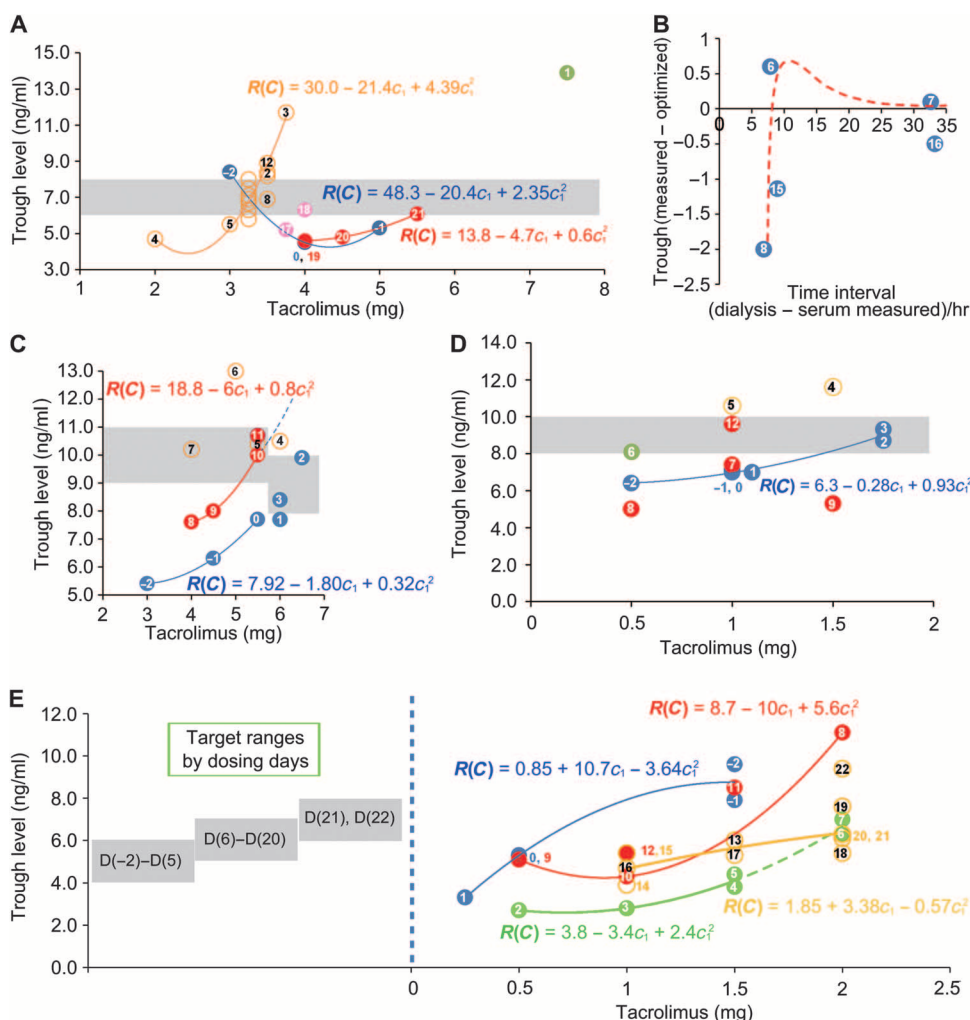
Fig. 2. PPD-assisted personalized immunosuppression for patients PPD1 to PPD4. PPD curves (blue in select graphs) were calibrated for each patient using the trough levels from physician-guided standard-of-care dosing on D(−2) to D(0). The PPD curve was used to prospectively dose tacrolimus into the target range (gray regions in select graphs). Following regimen changes, PPD curves were recalibrated using the trough levels from at least three successive days after the regimen changes. During PPD recalibration, tacrolimus doses for PPD patients were preemptively selected on the basis of previous correlations. The numbers within the circles are the dosing days with the given tacrolimus doses (mg) and the resulting trough levels (ng/ml). (**A**) PPD1: Calibrated PPD curves using the trough levels from dosing days D(−2) to D(21), with the target range of 6 to 8 ng/ml. First PPD calibrated curve: D(−2) to D(0) (blue). PPD recalibration: A regimen change (green) for D(1), the recalibrated curve (orange) for D(2) to D(5), regimen changes (pink) for D(17) and D(18), and the second recalibrated curve (red) for D(19) to D(21). (**B**) PPD1 deviations between the trough levels and PPD-projected trough levels as a function of time between hemodialysis and the measured blood trough level. The numbers within the circles (blue) are the dosing days with hemodialysis performed before the trough level reading. (**C**) PPD2: Calibrated PPD curves using the trough levels from dosing days D(−2) to D(11), with the target ranges of 7 to 9 [D(2) only, not shown in gray], 8 to 10, and 9 to 11 ng/ml. First PPD calibrated curve for D(−2) to D(0) (blue). PPD recalibration: Regimen changes (orange) for D(4) to D(7) and the recalibrated curve (red) for D(8) to D(10). (**D**) PPD3: Calibrated PPD curves using the trough levels from dosing days D(−2) to D(9), with the target range of 8 to 10 ng/ml. First PPD calibrated curve for D(−2) to D(3) (blue). PPD recalibration: Regimen changes (orange and green) for D(4) to D(6) and regimen changes (red) for D(7) to D(9). D(12) was the follow-up trough level after PPD3's discharge from the hospital. (**E**) PPD4: Calibrated PPD curves using the trough levels from dosing days D(−2) to D(22), with the target ranges of 4 to 6, 5 to 7, and 6 to 8 ng/ml. First PPD calibrated curve for D(−2) to D(1) (blue). PPD recalibration: The recalibrated curve (green) for D(2) to D(7), the second recalibrated curve (red) for D(8) to D(12), and the third recalibrated curve (orange) for D(13) to D(22).

PPD patient 4. PPD4 had a MELD of 40 at time of transplant, and a target range of 4 to 6 ng/ml for D(−2) to D(5), 5 to 7 ng/ml for D(6) to D(20), and 6 to 8 ng/ml for D(21) until discharge. The patient's posttransplant regimen included tacrolimus, prednisone, MMF, cotrimoxazole, gentamicin, linezolid, ganciclovir, and valganciclovir (table S1). PPD4 required hemodialysis. A parabola corresponding to $R(C) = 0.85 + 10.7c_1 - 3.64c_1^2$ was constructed by plotting the trough levels 7.9, 9.6, 5.3, and 3.3 ng/ml from D(−2) to D(1) against physician-determined tacrolimus dosages (Fig. 2E, blue). After a regimen change—ganciclovir (0 to 125 mg)—on D(2), PPD recalibration using D(2) to D(5) shifted the curve from the blue to the green [$R(C) = 3.8 - 3.4c_1 + 2.4c_1^2$] parabolas in Fig. 2E. This parabola was then used to prospectively identify tacrolimus dose recommendations along the green dotted line for D(6) to D(7), which brought trough levels within the target ranges. Another regimen change—fluconazole (400 to 200 mg)—occurred on D(8), and PPD recalibration using D(8) to D(10) shifted the curve from the green to the red [$R(C) = 8.7 - 10c_1 + 5.6c_1^2$] parabolas (Fig. 2E). For D(11) and D(12), single-pass hemodialysis was stopped, resulting in higher than expected PPD trough levels.

The final regimen change for PPD4—cotrimoxazole (320 to 160 mg)—occurred on D(13), and PPD recalibration using D(13) to D(21) trough levels shifted the curve from the red to the orange [$R(C) = 1.85 + 3.38c_1 - 0.57c_1^2$] parabolas (Fig. 2E). This parabola brought trough levels within the target range of 5 to 7 ng/ml. For D(19), the linezolid dose was decreased (1200 to 600 mg), resulting in a higher than expected PPD trough level, but the linezolid dose was subsequently increased to 1200 mg on D(20). On D(22), valganciclovir dose increase from 0 to 450 mg, and late tacrolimus administration to the patient resulted in a higher than expected PPD trough level. PPD4 was discharged on D(23).

Physician-guided standard of immunosuppression care: Case study C1

Patient C1 had a MELD of 36 at the time of transplantation and an initial tacrolimus target range of 8 to 10 ng/ml that was later lowered to 6.5 to 8.5 ng/ml due to

concerns for neurotoxicity and nephrotoxicity. The patient's regimen included tacrolimus, prednisone, MMF, fluconazole, cotrimoxazole, ganciclovir, and valganciclovir (table S1). A tacrolimus dose of 6 mg administered on D(−1) resulted in a trough level of 13.4 ng/ml (Fig. 3A); as a result, subsequent tacrolimus dosing dropped to 1 mg for D(1). Trough levels remained well below the target range during D(1) to D(8), and tacrolimus doses were gradually increased daily by the conventional titration process. The trough level eventually reached the target range of 6.5 to 8.5 ng/ml on D(9) (Fig. 3A). The trough levels are similarly plotted against the tacrolimus dose for control patients C2 (Fig. 3B), C3 (Fig. 3C), and C4 (Fig. 3D).

Comparing outcomes between PPD-assisted and control patients

The trough levels were plotted over time for all PPD-assisted patients ($n = 4$) and control patients ($n = 4$) to comprehensively compare the treatment outcomes (Fig. 4). C1 trough levels were out of the target range for 90% of the treatment period (Fig. 4A). The trough level management of the control patients resulted in 72.6 ± 14.3% of trough levels outside of the target range during their treatment periods, with 30.7 ± 29.2% of the trough levels ≥2 ng/ml outside of the target range (Fig. 4A). By comparison, the PPD patients' trough levels were out of range 54.2 ± 4.27% of the time, with 10.8 ± 6.54% of the trough levels ≥2 ng/ml outside of the target range. The variability for controls is notably greater, ranging from 61 to 90%; by comparison, PPD patients stayed within a tighter range of 50 to 60%.

Calculations for the areas under the curves (AUCs) and statistical analyses were performed using the data on the number of days ≥2 ng/ml outside of the target range (table S2). Shapiro-Wilk normality tests assessed the statistical significance of the variances observed between PPD and control patients. PPD did not result in extended hospital stays, because PPD subjects spent an average of 29.5 postoperative

days in the hospital compared with 48.8 for controls (Fig. 5A and table S2). PPD subjects spent fewer days with substantial deviations from the target range, defined as ≥2.0 ng/ml (median, 1.5 versus 4.0 days) (Fig. 5B). PPD subjects also had a smaller ratio of substantial deviation days to total treatment period (Fig. 5C). Ratios of AUC outside of the target range to total AUC combined the number of substantial deviation days to the magnitudes of those deviations. PPD subjects had a smaller ratio of AUC outside of the target range to total AUC compared to controls (Fig. 5D).

For the number of days in the hospital, days ≥2 ng/ml outside of the target range, and ratio of these days to total treatment days, although the averages were not statistically significant from control patients, PPD implementation resulted in significantly less interpatient variance ($P < 0.05$, one-tailed F test, two-tailed Levene's test), suggesting that PPD can better manage individual patient outcomes. Therefore, achieving significantly smaller variances in the treatment outcomes for PPD patients compared to control patients is a key benefit of personalized treatment.

We noted substantial inter- and intrapatient variability in the effect of regimen changes on tacrolimus trough levels. For example, during the administration of additional antibiotic (cotrimoxazole), antifungal (fluconazole), and/or anti-inflammatory drugs (prednisone), the trough levels changed substantially for both control and PPD patients (figs. S1 to S3 and tables S3 and S4). For PPD1, the highly convex surface indicated a synergistic tacrolimus-prednisone interaction, mediating an increasing output. Patients PPD2 and C1 demonstrated convex surfaces, which indicated a weak tacrolimus-prednisone interaction and decreasing output. However, the bulk of the surface for C1 was nearly flat, indicating an additive effect with no tacrolimus-prednisone interaction. For all patients, the 3D tacrolimus-prednisone map indicated multiple dose combinations that would bring trough levels within the target range, providing
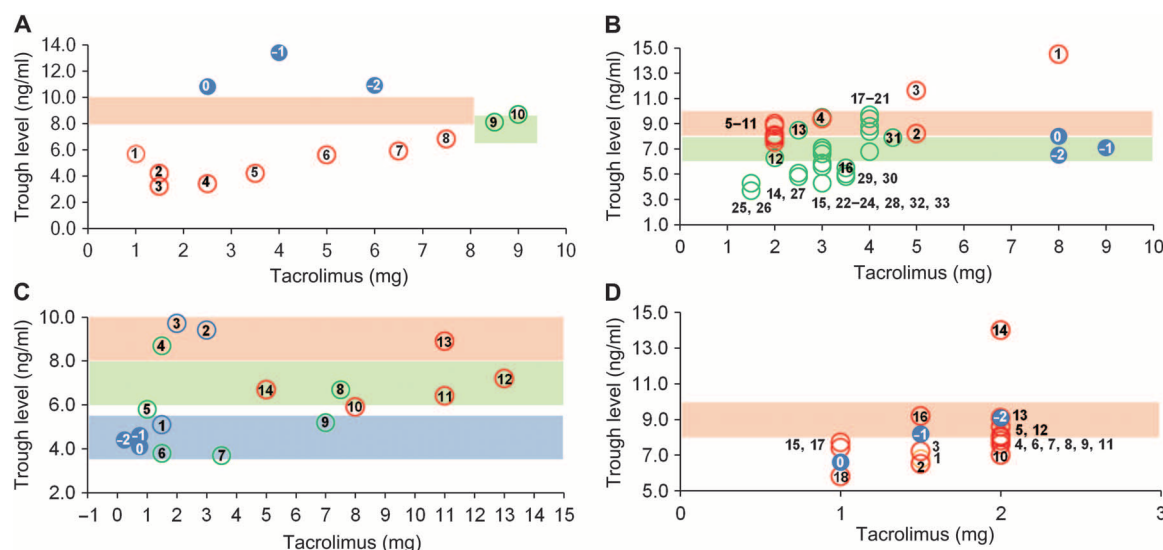

Fig. 3. Physician-guided standard-of-care immunosuppression for control patients C1 to C4. (A to D) Tacrolimus was dosed according to physician-guided standard of care. The numbers of the dosing days within the circles for the given tacrolimus doses and the resulting trough levels with the target ranges are plotted. The colors of the circles correspond to the matching colored target ranges. **(A)** C1: Trough levels from D(−2) to D(19) were plotted with the target ranges of 8 to 10 ng/ml (pink) and 6.5 to 8.5 ng/ml (green). **(B)** C2: Trough levels from D(−2) to D(33) were plotted with the target ranges of 6 to 8 ng/ml (green), 6.5 to 8.5 ng/ml (not shown), and 8 to 10 ng/ml (pink). **(C)** C3: Trough levels from D(−2) to D(14) were plotted with the target ranges of 3.5 to 5.5 ng/ml (blue), 6 to 8 ng/ml (green), and 8 to 10 ng/ml (pink). **(D)** C4: Trough levels from D(−2) to D(18) were plotted with the target range of 8 to 10 ng/ml (pink).
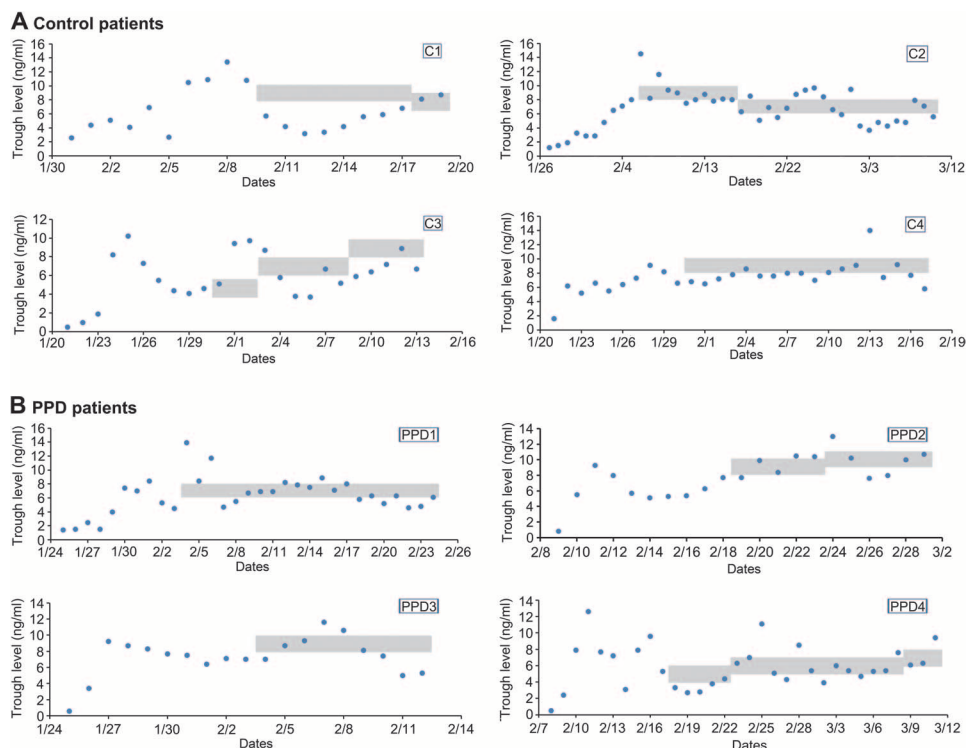
Fig. 4. PPD-treated and clinical standard control-treated patients' outcomes. (**A** and **B**) Recorded trough levels (ng/ml) were plotted against the date for each patient along with corresponding target ranges (gray regions) for control patients receiving the standard of care (A) and PPD-treated patients (B).

9.5 mg for D(2) to D(8) to maintain trough levels within the target range of 8 to 10 ng/ml (blue-shaded region) (Fig. 6, B and C). When the physician decreased the target range from 6.5 to 8.5 ng/ml for D(9) to D(10) (orange-shaded region), the PPD yielded recommended doses of 7 and 7.5 mg of tacrolimus (Fig. 6, B and C). The recalibrated PPD-identified tacrolimus doses could have therefore brought the trough levels into the target range, which is further demonstrated by comparing the retrospective ratios of [AUC inside of target range] to [total AUC] for both PPD and control patients (PPD, 0.99; control, 0.14) (Fig. 6D).

Retrospective PPD for patients C1 and C2, for combination of tacrolimus and prednisone dosing

Retrospective PPD for patients C1 and C2 demonstrated that a two-drug input (tacrolimus and prednisone) could have eliminated the need for recalibration. Specifically, by guiding multiple inputs to preemptively account for regimen changes, we could better regulate the phenotypic output. We used a modified equation, $R(C) = xc_1c_2 - yc_1^2 - zc_2^2$ [c_1, tacrolimus dose (mg); c_2, prednisone dose (mg)]. For C1, trough levels from D(−2) to D(3) (treatment days 7 to 12) were used to construct a PPD corresponding to $R(C) = 0.37c_1c_2 - 0.81c_1^2 - 0.0086c_2^2$. PPD-optimized trough levels (ng/ml) were immediately brought into the target range on D(4) and maintained within the target range for the rest of the treatment period (fig. S4A). Patient C2 frequently deviated from the target range starting on D(12) because of multiple regimen changes that were not managed using titrated dosing (fig. S4B). Trough level readings from D(−2) to D(12) were used to calibrate and construct the PPD for C2 corresponding to $R(C) = -2.82 + 8.60c_1 - 0.27c_2 - 0.68c_1c_2 - 0.23c_1^2 - 0.08c_2^2$. The PPD-optimized tacrolimus and prednisone doses brought the trough levels into the target range on D(13) and maintained the desired trough levels through the remaining treatment period (fig. S4B).

A comparison between AUC inside and AUC outside of the target range for patient C1 demonstrated a substantial improvement in treatment outcomes when using retrospective PPD optimization compared to clinically observed values (fig. S4C). Similarly, retrospective PPD optimization substantially improved the dosing outcomes of patient C2 compared to clinically observed values (fig. S4D).

DISCUSSION

Transplant patients undergo combination therapy with a substantial number of drugs and procedures. This regimen is changed constantly to account for infection, inflammation, rejection, and kidney function, among other factors, and patients respond uniquely to their constantly changing regimens. We therefore developed a clinical approach
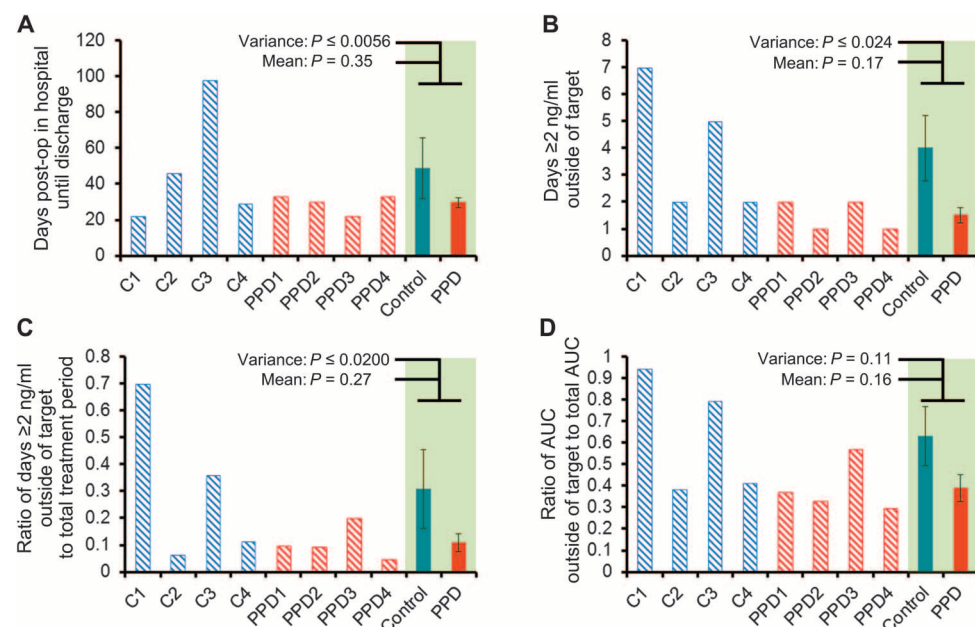
a reference for how two drugs interact under patient-specific conditions (fig. S2).

Intrapatient variability was exemplified by PPD4. The 2D tacrolimus-cotrimoxazole interaction surface transitioned between saddle-like and concave. The saddle-like surfaces constructed using treatment days 6 to 12 and 14 to 26 suggested that tacrolimus-cotrimoxazole interactions are synergistic or antagonistic, depending on the doses. However, the concave surfaces constructed using treatment days 6 to 15 and 14 to 23, for example, indicated antagonistic tacrolimus-cotrimoxazole interactions (fig. S3). Such variability in drug-drug interactions confirms the need to personalize treatment.

Retrospective PPD for control patient C1

Retrospective PPD was conducted on patient C1 to examine whether PPD could have rapidly identified tacrolimus doses to reach the target ranges. First, we identified multiple regimen changes for C1 that affected tacrolimus trough levels, such as increasing the MMF dose. Three major regimen changes occurred during this period for patient C1: no MMF for D(−2) to D(0); MMF dose increased to 500 mg for D(1); and MMF dose increased to 1000 mg for D(2) to D(10). PPD calibration used the trough levels of 10.9, 13.4, and 10.8 ng/ml from D(−2) to D(0) when no MMF was given, and a parabola corresponding to $R(C) = -2.06 + 7.27c_1 - 0.85c_1^2$ was obtained (Fig. 6A, blue). Two regimen changes—MMF dose increase (0 to 500 then to 1000 mg)—occurred on D(1) and D(2) (Fig. 6A, green). PPD recalibration using trough levels from D(2) to D(10) shifted the curve from the blue to red [$R(C) = 3.45 - 0.0046c_1 + 0.064c_1^2$] parabolas (Fig. 6A, red). The recalibrated PPD yielded recommended tacrolimus doses of 9 and

Fig. 5. Comparing PPD and control patients' outcomes. Individual PPD (red-striped) and control patients' (blue-striped) outcomes are shown. Green-shaded regions: Mean PPD (red solid) and control (blue solid) patients' outcomes are shown. Data are means ± SEM ($n = 4$). (**A**) Number of postoperative days in the hospital until discharge. P values were determined by F test and Welch t test. (**B**) Number of days with trough level ≥ 2 ng/ml outside of the target range. P values were determined by Levene's test and Wilcoxon rank sum test. (**C**) Ratio of the number of days with trough level ≥ 2 ng/ml outside of the target range to the total number of days of tacrolimus treatment. P values were determined by F test and Welch t test. (**D**) Ratio of the AUC outside of the target range to the total AUC. P values were determined by F test and Student's t test.

to personalizing drug dosing and demonstrated proof of concept in eight patients in a prospective randomized controlled study administering tacrolimus for posttransplant liver immunosuppression. Clinical data generated by standard-of-care dosing during the initial treatment period were used to calibrate patient-specific coefficients to construct a parabolic map, called PPD, to guide the immunosuppression-dosing process. Patients treated using PPD had significantly less variability in tacrolimus trough levels compared with control patients with physician-guided standard-of-care dosing. In addition, retrospective PPD for control patients indicated the possibility of better maintaining trough levels within the target range compared to the standard of care that patients received. Regardless of the dosing approach, all eight patients revealed intra- and interpatient variability as a result of drug-drug interactions and procedures, further supporting the need for such phenotypic platforms in personalized medicine.

Our PPD approach is broadly applicable, as we have demonstrated previously in vitro and in vivo in animals (20–25). This clinical study has shown that this parabolic surface represents patient phenotypic responses to monotherapy and combination therapies well, serving as a powerful foundation for expanding the PPD process toward other indications. The PPD 3D drug interaction map may provide further insight into the effects of patient-specific drug additivity, synergism, or antagonism on drug levels to assist with clinical decision-making. Notably, PPD is not a pharmacogenomic or pharmacokinetic predictive modeling approach. Instead, PPD uses phenotypic outputs such as clin-

ical efficacy and/or safety to plot the parabolic surface. The phenotype innately accounts for molecular and pharmacokinetic determinants, serving as the foundation for the disease mechanism–independent and indication-agnostic nature of PPD. This also differentiates PPD from systems biology approaches (26–28).

With PPD, we can visualize the phenotypic effects of drug-drug interactions and procedural changes on trough levels. For example, hemodialysis alters total body water and can therefore affect tacrolimus redistribution during and after dialysis (29). There is also an unpredictable correlation between MMF dose and tacrolimus levels (change in absorption levels and in intestinal transit time). Both tacrolimus and MMF absorption and metabolism are affected by cytochrome P450 and P-glycoprotein. Such interactions could be further evaluated in humans using PPD to optimize dosing regimens. This pilot study guided tacrolimus dosing only; therefore, a recalibration process addressed regimen changes to maintain trough levels within target ranges. Although the recalibration process managed trough levels, retrospective PPD modulated multidrug dosing to eliminate recalibration altogether (which would reduce the incidence of target range deviations). Specifically, when a regimen change—change in drug dose, hemodialysis, etc.—is anticipated, deviations from target ranges are typically imminent because modifying tacrolimus dosing alone is not sufficient to account for changes in dosages of other drugs or the introduction of additional drugs into the regimen.

This preliminary study was based on four patients, where mean analysis of the outcomes was not statistically significant but the reduction of interpatient variance was statistically significant. Continued PPD scale-up for widespread clinical application should focus on enhancing preemptive management of trough levels or novel immunosuppression markers with personalized multidrug dosing. Scale-up would also include integrating PPD with outpatient immunosuppression to assess long-term patient response, because high levels of calcineurin trough level variability adversely affect graft outcomes (30). For this initial validation of PPD, we chose to limit our study to the inpatient setting to facilitate data collection, to ensure that the patients are receiving the drugs that they are prescribed, and to allow for the incorporation of as broad a range of clinical data as possible.

This study also raises the question of whether a second-order polynomial is more effective in dosage guidance compared to a line. The clinical drug titration standard is based on the linear approach. This is evident for patient C1, where for most of the treatment period, the tacrolimus trough level was not in the target range; with the parabolic response surface, as shown by retrospective PPD optimization, the target trough level would have been reached much more quickly. The parabola
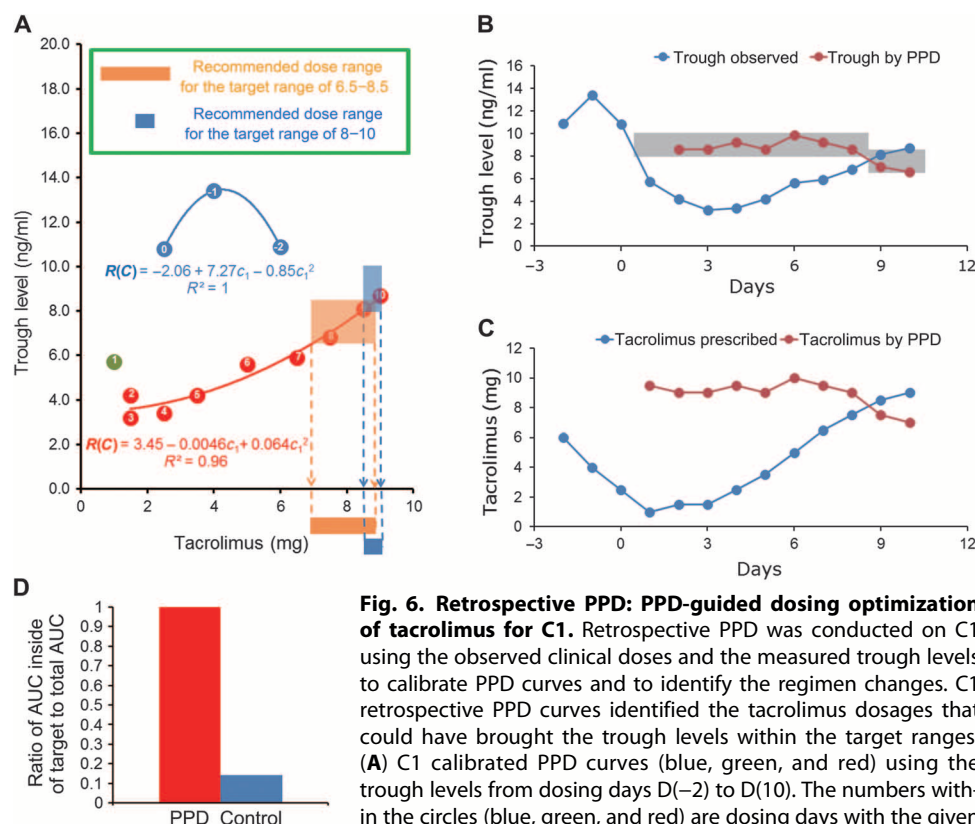
Fig. 6. Retrospective PPD: PPD-guided dosing optimization of tacrolimus for C1. Retrospective PPD was conducted on C1 using the observed clinical doses and the measured trough levels to calibrate PPD curves and to identify the regimen changes. C1 retrospective PPD curves identified the tacrolimus dosages that could have brought the trough levels within the target ranges. (**A**) C1 calibrated PPD curves (blue, green, and red) using the trough levels from dosing days D(−2) to D(10). The numbers within the circles (blue, green, and red) are dosing days with the given tacrolimus doses (mg) and the resulting trough levels (ng/ml). The shaded regions (orange and blue) are the target ranges 8 to 10 ng/ml and 6.5 to 8.5 ng/ml. (**B** and **C**) PPD-optimized (red) and clinically observed (blue) trough levels (ng/ml) (B) and tacrolimus doses (mg) (C) plotted against the dosing days. The gray-shaded region is the target range. (**D**) PPD (red) and clinically observed control (blue) ratios of the AUC inside of the target range to the total AUC.

may also intersect the tacrolimus target range at two points to identify two possible dose suggestions; a line cannot accomplish this and therefore may prevent optimized dosing. The linear approach is, at best, an approximation of one side of a parabola, whereas Eq. 2 (PPD) shows that the efficacy-dose response surface is inherently parabolic (*20–25*).

The current clinical practice is to personalize immunosuppression for each recipient by setting the tacrolimus target range according to the clinical scenario. An alternative indicator could be CD4+ T cell activation, which the Cylex ImmuKnow assay was designed to measure. This assay was never embraced clinically, because its utility was never definitively demonstrated (*31, 32*). Therefore, in the absence of a clinically useful and validated measure of immunosuppression, the tacrolimus trough level has become the standard. Factors such as kidney function, comorbidities, race, and disease severity are therefore taken into account in this determination. However, PPD could be adapted to any novel indicator of immunosuppression that may be more effective than trough levels (for instance, T cell alloreactivity or donor-specific anti–human leukocyte antigen antibodies) (*33, 34*).

The PPD platform implemented in this study is markedly different from the clinical standard of care that relies on titration or incremental dosing using educated guesses. PPD has thus far not been automated so that the clinician is given the final say in approving dosing orders, to minimize patient risk. PPD is embedded with upper and lower dosing limits to prevent over- and underdosing, so automation is possible depending on the indication. PPD implementation could be completed within minutes, allowing one person to manage many patients. PPD can also be implemented in an outpatient setting where tacrolimus levels are recorded every few days, and dosing prescriptions can be given to patients through their outpatient care provider. Therefore, PPD could maximize patient benefit and turnaround time, as well as financial considerations, such as reimbursement, associated with reducing treatment complications and duration of postoperative hospitalization. In sum, this parameter was used to demonstrate that PPD implementation did not result in apparent adverse events, complications in administrating PPD, or other barriers that required prolonged hospitalization. Our preliminary clinical study of a phenotypic medicine approach will serve as a foundation for the expansion of PPD toward other disease indications, such as cancer, infectious diseases, and cardiovascular disorders, where dosing could be better controlled and personalized.

MATERIALS AND METHODS

Study design
This study was conducted to compare the effectiveness of a PPM approach toward optimized tacrolimus dosing with conventional physician-guided dosing. To assess the broad applicability of the PPM process, no exclusion criteria were implemented. Eight patients consented and were enrolled and randomized in this study at the University of California, Los Angeles (UCLA)–Dumont Liver Transplant Center under Institutional Review Board no. 14-001682 approved by the UCLA Office of the Human Research Protection Program. Four patients were randomly assigned to the PPD-assisted immunosuppression dosing arm, and the other four received standard-of-care immunosuppression dosing.

The number of recruited patients was determined to eliminate overlap with other ongoing studies while also serving an adequate test population for a series where a substantially different multidrug regimen was administered to each patient and each regimen was optimized using the personalized medicine approach. The trough level was selected as the primary efficacy (phenotypic) result for this study. The MELD score was calculated for each patient on the basis of serum bilirubin and creatinine levels and international normalized ratio. The target range for each patient was assigned by the primary surgeon on the basis of ethnicity, age, etiology of liver disease, disease severity, kidney function, comorbidities, and the use of other immunosuppressants. Data collection was stopped at the point of patient discharge or at a physician-determined time point when discharge was imminent. Metrics to compare PPD with controls included the number of days

that trough levels were ≥2 ng/ml outside of the target range and the number of postoperative days in the hospital; from these, ratios and patient intra- and intervariability were also calculated. Postoperative days in the hospital vary substantially between treatment centers and are not typically used as outcome metrics, but the Dumont–UCLA Liver Transplant Center consistently has the highest median MELD score at transplant in the United States, and patient acuity is among the highest, if not the highest, in the country. Thus, these patients' experiences were ideal for presenting challenging situations for PPD.

Prospective and retrospective clinical PPD process

Trough levels, drug regimen dosages, and other events such as hemodialysis were obtained every morning before analysis. To project optimal dosages, a second-order polynomial fit for each patient was made from linear regression with mainly two variables, such as trough level (ng/ml) and tacrolimus dose (mg), and it was calibrated using at least three previous data points from the specific patient. Additionally, the effect and degree of drug-drug interactions on individual patients obtained during the prospective study were considered when recommending the better dosage regimen. PPD 2D and 3D drug interaction maps were plotted using MATLAB R2014a (MathWorks Inc.), with a matrix input of the drug concentration values correlated to the trough levels. AUCs were calculated using MATLAB R2014a (MathWorks Inc.), with a matrix input of the trough levels and the target ranges. Additional information pertaining to retrospective multidrug optimization can be found in the Supplementary Methods.

Statistical analysis

Normal distribution was determined by the Shapiro-Wilk normality test. In the case of parametric (normal) distributions, one-tailed F test was used to compare variances, and the appropriate two-tailed Welch t test or Student's t test was used to compare means. In the case of nonparametric (nonnormal) distributions, two-tailed Levene's test was used to compare variances, and two-tailed Wilcoxon rank sum test was used to compare medians. α was set at 0.05, and $P < 0.05$ was considered significant. We performed all statistical analyses using R version 3.1.1.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/8/333/333ra49/DC1
Subject clinical details
Methods
Fig. S1. The effect of cotrimoxazole and fluconazole dosing on patients' trough levels.
Fig. S2. PPD tacrolimus-prednisone interaction plots for patients PPD1, PPD2, and C1.
Fig. S3. PPD tacrolimus-cotrimoxazole interaction plots for patient PPD4.
Fig. S4. Retrospective PPD-guided tacrolimus and prednisone dosing optimization for patients C1 and C2.
Table S1. Clinical summaries of control patients (C1 to C4) and PPD-guided patients (PPD1 to PPD4).
Table S2. Data used for statistical comparisons in Fig. 5.
Table S3. Patient-specific antibiotic and antifungal dosing changes and corresponding tacrolimus trough levels.
Table S4. Patient-specific anti-inflammatory and immunosuppressant dosing changes and corresponding trough levels.
Movie S1. Introduction to PPD and patient recalibration.
Movie S2. Patient PPD2 recalibration tutorial.
Movie S3. Patient PPD3 recalibration tutorial.

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Individualizing liver transplant immunosuppression using a phenotypic personalized medicine platform

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Personalizing drug dosing

After organ transplant, patients are on a merry-go-round of medicines and procedures to make sure that the graft is not rejected. Currently, physicians use dosing guidelines for drugs meant to suppress the immune system, but also use educated guesses in choosing dose, to account for variability in patient response to the drugs and drug-drug interactions. Now, Zarrinpar and colleagues have come up with a mathematical approach to remove the guesswork. Their approach, called parabolic personalized dosing (PPD), relies on algebraic equations to relate phenotype (in this case, trough level of an immunosuppressant, tacrolimus) to input (tacrolimus concentration). By mapping patient response over the course of treatment, the equation produces a two-dimensional (2D) parabola that indicates the next dose that the patient should receive. The parabola shifts as drugs are added or taken away, or as the patient undergoes additional clinical procedures, such as hemodialysis, which can interfere with drug distribution within the body. The PPD approach was tested in four patients and compared to the standard of care, physician guidance. The PPD patients were out of trough range less frequently and for shorter periods of time than controls, suggesting that the equation was predicting next doses accurately. Future studies will involve more patients and will expand the PPD equation to represent a 3D parabolic surface, which will factor in drug combinations. The PPD approach will have broad applicability beyond transplant medicine, because it is independent of disease mechanism or drug of choice and could thus personalize regimens for many types of patients.

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