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POSITION PAPER

Challenges with sirolimus experimental data to inform QSP model of post-transplantation cyclophosphamide regimens

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Abstract

Dose optimization of sirolimus may further improve outcomes in allogeneic hematopoietic cell transplant (HCT) patients receiving post-transplantation cyclophosphamide (PTCy) to prevent graft-versus-host disease (GVHD). Sirolimus exposure–response association studies in HCT patients (i.e., the association of trough concentration with clinical outcomes) have been conflicting. Sirolimus has important effects on T-cells, including conventional (Tcons) and regulatory T-cells (Tregs), both of which have been implicated in the mechanisms by which PTCy prevents GVHD, but there is an absence of validated biomarkers of sirolimus effects on these cell subsets. Considering the paucity of existing biomarkers and the complexities of the immune system, we conducted a literature review to inform a quantitative systems pharmacology (QSP) model of GVHD. The published literature presented multiple challenges. The sirolimus pharmacokinetic models insufficiently describe sirolimus distribution to relevant physiological compartments. Despite multiple publications describing sirolimus effects on Tcons and Tregs in preclinical and human ex vivo models, consistent parameters relating sirolimus concentrations to circulating Tcons and Tregs could not be found. Each aspect presents a challenge in building a QSP model of sirolimus and its temporal effects on T-cell subsets and GVHD prevention. To optimize GVHD prevention regimens, phase I studies and systematic studies of immunosuppressant concentration–effect association are needed for QSP modeling.

Ezhilpavai Mohanan and Guofang Shen have shared first authorship.

Christopher G. Kanakry, Donald E. Mager and Jeannine S. McCune have shared senior authorship.

INTRODUCTION

In allogeneic hematopoietic cell transplant (HCT), a delicate balance must be maintained between the host and the administered donor cells that are not genetically identical (i.e., allogeneic). The resulting bi-directional immunological reactions include graft-versus-host disease (GVHD), control of a malignancy (termed graft vs. tumor, GVT), the development of tolerance, and immune reconstitution. Recent phase III clinical trials established post-transplant cyclophosphamide (PTCy) with mycophenolate mofetil (MMF) and tacrolimus as a standard-of-care GVHD prophylaxis for HLA-matched and HLA partially mismatched donor HCT.[1,2] Recent preclinical data suggest that PTCy has dose-dependent effects that are associated with its efficacy in preventing GVHD, including reduced proliferation of T-conventional cells (Tcons) at Day +7, followed by the preferential expansion of regulatory T-cells (Tregs) at Day +21 after HCT in murine HLA-haploidentical HCT models.[3,4] Patients undergoing HCT with PTCy exhibited reduced proliferation of potentially alloreactive Tcons and rapid recovery of activated $CD4^{+}CD45RA^{-}Foxp3^{+hi}$ Tregs.[3,5] Tregs from patients and allogeneic mixed lymphocyte cultures (MLRs) had increased expression of aldehyde dehydrogenase (ALDH), the major in vivo detoxifying enzyme for cyclophosphamide, thus protecting Tregs from PTCy. In addition, Tregs were necessary for GVHD prevention by PTCy, suggesting Tregs' resistance to PTCy through the expression of ALDH contributed to the clinical activity of PTCy in preventing GVHD.[3,5,6] Besides Tregs that are highly enriched after PTCy, CD8+ T-cells recover more rapidly after PTCy than CD4+ T-cells, and resistance of CD8+ T-cells to PTCy occurred via increased drug efflux activity and ALDH expression in allogeneic reactions.[7]

There is a growing trend to replace tacrolimus with a mammalian target of rapamycin (mTOR) inhibitor sirolimus (i.e., PTCy and MMF plus sirolimus instead of tacrolimus). Motivations for this substitution are variable but include that tacrolimus reduces the Tregs cell pool,[8] while sirolimus supports Tregs cell proliferation ex vivo, which is beneficial for immunosuppression based on preclinical data showing greater Tregs numbers are associated with less GVHD.[3,4]

We posit that a quantitative systems pharmacology (QSP) model specific for PTCy as GVHD prevention in HCT (abbreviated QSP-PTCy) can augment therapeutic drug monitoring (TDM) and exposure–response studies of sirolimus in HCT. Developing a QSP-PTCy model based on relevant T-cell subsets can provide a framework for clinical trial simulations to design 'better' clinical trials and identify if net sirolimus exposure (i.e., the area under the concentration–time curve or AUC) or steady-state trough concentration is associated with clinical outcomes. With the long-range goal of lowering GVHD rates after PTCy-based HCT, we describe the contemporary questions (Table 1) and experimental challenges for building a QSP-PTCy model of sirolimus and various immunosuppressants and summarized temporal changes in T-cell subsets in response to sirolimus (Figure 1).

NOVEL METHODS ARE NEEDED FOR SIROLIMUS DOSE OPTIMIZATION IN HCT PATIENTS RECEIVING PTCY

HCT patients generally have their sirolimus doses personalized using TDM of trough whole blood samples. However, sirolimus trough concentrations only modestly correlate with AUC_{0-24h} (R^2 range from 0.52 to 0.84).[9,10] Thus, it is unsurprising that patients treated with sirolimus show variable pharmacokinetics, toxicity, and effectiveness.

Sirolimus blood concentrations can be influenced by medications that affect cytochrome P450 3A (CYP3A) or p-glycoprotein; notably, cyclophosphamide induces CYP3A ex vivo. Studies are needed to evaluate whether PTCy induces CYP3A activity sufficiently to increase sirolimus clearance, thereby requiring higher sirolimus loading doses, which are administered shortly after PTCy administration, to achieve the target sirolimus trough concentration.

Below are the two sirolimus-specific reasons why novel methods may augment the traditional approach of evaluating sirolimus trough concentrations (exposure) with GVHD (response) in HCT patients treated with PTCy.

Reason 1: Current exposure–response literature in HCT patients does not inform the optimal dose of sirolimus in PTCy-treated patients

Of the publications reporting sirolimus trough concentration–outcome relationships in HCT (Table S1), only Goyal et al.[11] reported that GVHD was associated with sirolimus trough concentrations. In patients receiving sirolimus with a calcineurin inhibitor and methotrexate, the trough sirolimus concentrations were lower in the 10 patients who developed grades III–IV acute GVHD compared to the 22 patients who developed grades 0–II acute GVHD (6.1 ± 2.9 vs. 9.4 ± 5.5 ng/mL; $p = 0.044$). However, the published data do not provide sufficient insight regarding the optimal sirolimus dose or exposure to achieve the desired Tregs and Tcons concentrations or GVHD rates in HCT patients treated with PTCy and sirolimus.

TABLE 1 Contemporary questions regarding sirolimus dosing when administered in the PTCy regimen. The working hypothesis is that QSP modeling can inform precision sirolimus dosing and improve non-relapse mortality by decreasing the rate and severity of acute and chronic GVHD.

Question	Challenge(s) addressing this question
For the sirolimus loading dose	
1. Are higher loading doses of sirolimus needed because sirolimus clearance may increase due to cytochrome P450 3A induction by PTCy?	Challenge 2
For daily dosing of sirolimus	
2. Does the current PK-guided dosing based on trough concentrations sufficiently reduce interpatient variability?	Challenge 2
3. Can the sirolimus AUC be estimated using popPK-limited sampling schedules and/or wearables that quantify sirolimus concentrations in the outpatient setting?	Challenge 2 and Shen et al.[12]
4. How does the between-patient variability in sirolimus blood concentrations or AUC influence Tcons and Tregs, which are particularly important to GVHD prevention in PTCy regimens?	Challenges 1, 2, 3
5. When administered with PTCy, what is the optimal whole blood AUC of sirolimus to prevent GVHD?	Challenges 1, 2, 3, 4, 5
For the GVHD prophylaxis regimen in its entirety	
6. What additional immunosuppressants are needed, and do they differ for different donor types? For example, matched siblings may need PTCy only (if using bone marrow grafts), while haploidentical donors may need additional immunosuppression (e.g., MMF with tacrolimus or sirolimus).	Challenges 4, 5
7. What is the optimal duration of sirolimus treatment?	Challenges 1, 4, 5

Reason 2: No sirolimus-specific biomarkers can be used to identify the optimal sirolimus dose with PTCy in HCT patients

The next question to be considered is—are there any sirolimus-specific biomarkers that can be used instead to identify an optimal sirolimus dose? A drug-specific pharmacodynamic biomarker for sirolimus effectiveness or toxicity has yet to be identified, but promising innovations in omics techniques exist.[12]

BUILDING A PTCY QSP PLATFORM MODEL ALIGNS WITH PROJECT OPTIMUS AND THE DEVELOPMENT OF RECENT IMMUNE-ONCOLOGY DRUGS

QSP expands the traditional focus of pharmacokinetic/pharmacodynamic (PK/PD) relationships with a mechanistic understanding of disease and pharmacological pathways.[13] This holistic approach can integrate or translate available ex vivo, preclinical (animal), and clinical (patient) data representing existing knowledge to directly inform discoveries of new and/or optimized therapies within the context of processes controlling disease progression (Figure 1).[13] By modernizing the sirolimus dose optimization in HCT patients, building a QSP-PTCy model aligns with the Food and Drug Administration's Project Optimus mission to update the paradigm for dose optimization and dose selection in the development of oncology drugs. Although HCT is a procedure, it is one that predominantly uses off-label drugs to treat patients with cancer.

PUBLISHED DATA OF SIROLIMUS AND PTCY PROVIDE MECHANISTIC REASONS TO BUILD QSP-PTCY

Recent data show that optimized timing and dosing of PTCy have specific effects on alloreactive and regulatory T-cells that correlate with maximal efficacy in preventing GVHD in murine MHC-haploidentical HCT.[3,4] Effective PTCy dosing was associated with decreased alloreactive CD4+ Tcons proliferation at Day +7 and preferential increase in Tregs at Day +21 after HCT; dosing schedules that achieved one criterion only were less effective in preventing GVHD.[3,4] Sirolimus has also been shown to

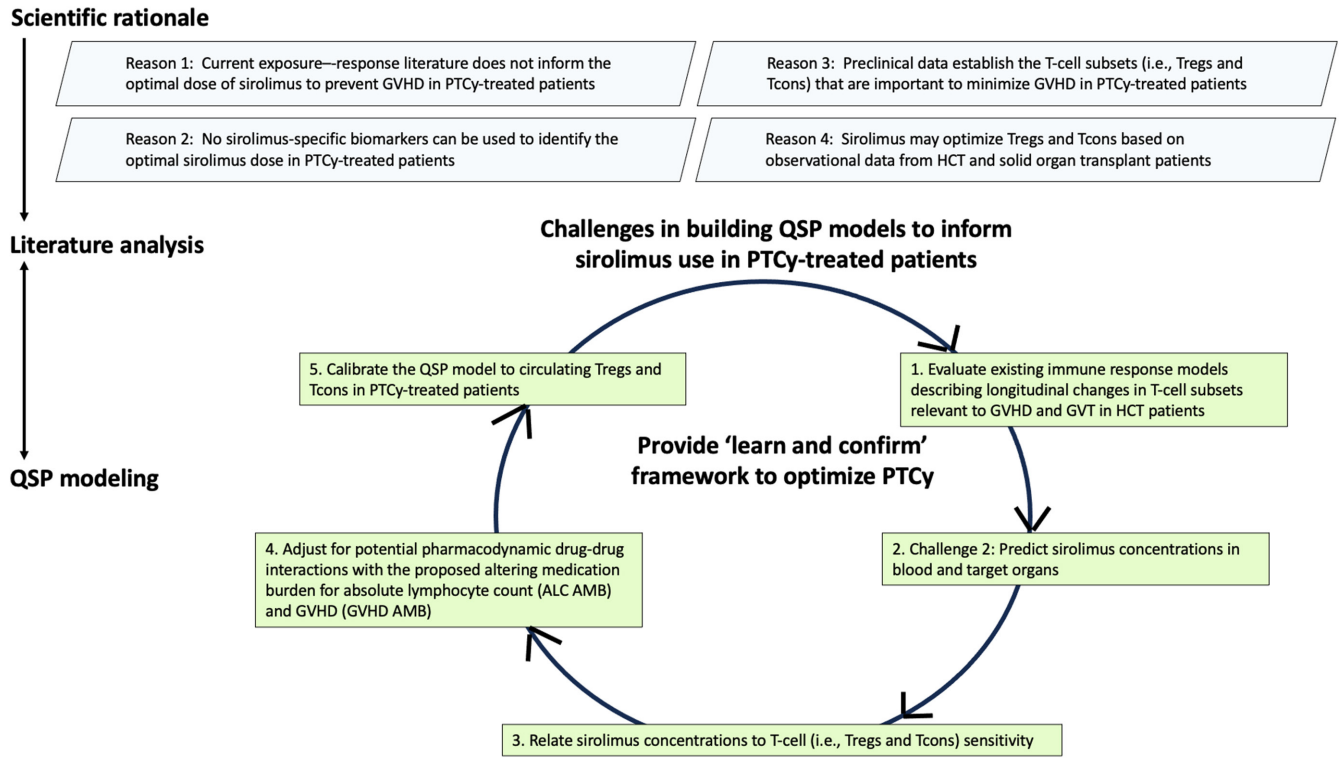

FIGURE 1 Reasons for and challenges with constructing a QSP-PTCy model.

inhibit Tcons and spare Tregs,[14] which could be beneficial for achieving targeted changes in those T-cell subsets. In addition to the two sirolimus-specific reasons for building the QSP-PTCy model, below are the mechanistic reasons to build the QSP-PTCy.

Reason 3: Preclinical data establish that T-cell subsets (i.e., Tregs and Tcons) are important for minimizing GVHD after treatment with PTCy

The Tregs population is functionally heterogeneous, and cellular selectivity depends on the antigenic stimuli and location of the immune response. Acute and chronic GVHD develop from activation of graft T-cells by alloantigen from the host and failure to develop tolerance after HCT, which is partly mediated by Tregs.[15,16] CD4 + CD25 + Foxp3+ Tregs were reduced in patients with GVHD, might increase in a compensatory manner in active acute GVHD,[5] and themselves protect against GVHD when administered to HCT patients.[17] Sirolimus was shown to selectively expand CD4 + CD25 + Foxp3+ Tregs in humans,[14,18] possibly contributing to its protective effect against GVHD. However, there are no dose–response studies of varying sirolimus doses in preclinical models of HCT to inform sirolimus dosing in HCT patients to optimize the T-cell subsets and reduce GVHD risk.

Reason 4: Sirolimus administration may optimize Tregs and Tcons based on observational data from HCT and solid organ transplant patients

To our knowledge, data describing the relationship between escalating sirolimus exposure and the T-cell subsets of interest, that is, Tcons and Tregs, in HCT patients receiving PTCy are unavailable. Preclinical data (described in Reason 3) suggest that data on sirolimus in combination with PTCy at earlier timepoints are of interest. Immune reconstitution between 1 and 24 months post-HCT has been compared between tacrolimus with methotrexate to the experimental arm of tacrolimus with sirolimus.[19]

Solid organ transplantation studies have shown higher percentages of Tregs in the peripheral blood in those receiving sirolimus rather than tacrolimus. This trend was evident even when switching the treatment from tacrolimus to sirolimus.[20] The studies by Levitsky et al.[21,22] highlighted the importance of sirolimus in HLA-matched or -mismatched MLRs from healthy volunteers or renal transplant donor-recipient pairs over

tacrolimus. These studies showed that sirolimus-treated MLR culture generated higher CD4 + CD25 + Foxp3+ cells than tacrolimus. In addition, these sirolimus-induced Tregs allospecifically inhibited the proliferation of MLR and helped in the regeneration of responder cells to show Tregs phenotype in MLR compared with tacrolimus.

CHALLENGES WITH BUILDING A QSP MODEL TO BRIDGE PRECLINICAL DATA TO HCT PATIENTS

For allogeneic HCT patients diagnosed with cancer, the goal is to maximize GVT while minimizing GVHD. Focusing upon the optimization of sirolimus in combination with PTCy and MMF, a series of challenges should be addressed to build (a) useful QSP model(s) of the allogeneic HCT process. As shown in Figure 1, we seek to connect immune response models (Challenge 1) to sirolimus pharmacokinetics (Challenge 2) using concentration–effect relationships (e.g., EC_{50} and E_{max}) from the literature (Challenge 3) while accounting for pharmacodynamic interactions of concomitant medications affecting Tcons and Tregs cells (Challenge 4), thereby creating QSP models in HCT patients (Challenge 5). These challenges are not rank ordered but are described sequentially based on our experience.

Optimal dosing of sirolimus that inhibits Tcons without compromising Tregs has yet to be systematically studied in HCT patients receiving PTCy. Therefore, optimizing the effectiveness of sirolimus in combination with PTCy is essential to prevent GVHD and maximize GVT.

Challenge 1: Evaluate existing immune response models describing longitudinal changes in T-cell subsets relevant to GVHD and GVT in HCT patients

The first challenge is characterizing the immune response of both GVHD and GVT in HCT patients. Models of immune response and circulating blood, lymphoid T, and lymphoid B tissue can inform models of allogeneic HCT. However, the inherent complexity of the immune system and the difficulty of measuring many aspects of an individual patient's immune state/status in vivo makes it challenging to develop such QSP models of the immune response.

For patients with cancer, the goal of allogeneic HCT is to maximize GVT (i.e., prevent relapse). Relevant to GVT, systems of ordinary differential equations (ODEs) have been widely used to model tumor–immune interactions at the cell population level. Such models confirm clinical observations that the conditioning regimen administered before cellular therapy administration is crucial for most patients in reducing toxicity and achieving remission.[23] Despite the success of immune response and tumor–immune interaction models, there is a paucity of models available that characterize the biodisposition of donor T-cell subpopulations in major target organs of GVHD (e.g., gastrointestinal tract, spleen, and liver). Ganusov et al.[24] developed a mathematical model to describe the kinetics of lymphocyte recirculation in the whole organism, including blood, liver, spleen, intestine, lung, and lymph node tissue spaces, by analyzing experimental measurements of labeled thoracic duct lymphocyte migration in rats and mice. One strategy would be to re-calibrate this preclinical T-cell kinetic model to measurements of T-cell subpopulations in critical tissues of interest under control and transplant conditions in mice (with and without PTCy treatment).[3,4] Standard QSP modeling workflows can be used to identify key model parameters for re-calibration and sub-models that could be integrated to address any phenomenological complexities (e.g., differential cellular proliferation and temporal delays).[13] A qualified model of T-cell subpopulation kinetics could then be scaled to humans using allometry and prior knowledge of human T-cell disposition. The next challenge is to connect such immune cells and response models to sirolimus pharmacokinetics (Challenge 2).

Challenge 2: Predict sirolimus concentrations in blood and target organs

An essential component of a useful QSP model is incorporating the blood sirolimus concentrations and linking them to the number of Tcons and Tregs within the blood and the acute GVHD target organs: the gastrointestinal, liver, and skin. There is one population pharmacokinetic (popPK) in HCT patients[11] and no physiologically-based pharmacokinetic (PBPK) models of sirolimus. Thus, it is difficult to evaluate the between-patient variability and patient factors associated with aberrant pharmacokinetics, which can be identified with popPK models. Furthermore, it is difficult to characterize how whole blood concentrations of sirolimus relate to relevant tissues (PBPK models), such as the lymph nodes or the sites of acute GVHD (i.e., the gastrointestinal tract, liver, and skin). Nevertheless, it is difficult to relate the blood concentrations to changes in the immune response. Another challenge is to relate these sirolimus blood concentrations to changes in Tregs and Tcons based on the sensitivity of each cell population to sirolimus. Challenge 3 describes this aspect.

Challenge 3: Relate sirolimus concentrations to T-cell (i.e., Tregs and Tcons) sensitivity

Challenge 3 evaluates how sirolimus concentrations directly affect Tregs and Tcons populations. In vivo preclinical and human ex vivo pharmacodynamic or concentration–effect data with sirolimus on Tregs and Tcons could inform a QSP model. More detailed descriptions of and potential solutions for the challenges in extrapolating experimental data to inform the QSP model are summarized in Table 2. Preclinical studies (Tables S2 and S3) on the T-cell populations of interest suggest that sirolimus's immunosuppressive effects may be attributed to its ability to inhibit Tcons but spare Tregs while augmenting Tregs function selectively. However, these results were obtained from different labs with various detection methods, and Tregs were defined by different T-cellular markers, making it impossible to pool the data. Most studies use one (Table S4) to three sirolimus concentrations (Table S5), which may not lead to sufficient estimation of EC_{50} and/or E_{max}. Various T-cell mitogens or T-cell receptor stimulation are essential for achieving T-cell proliferation ex vivo. It is unclear if ex vivo proliferation mimics the T-cell recovery in allogeneic HCT patients. In addition, T-cells respond differently to various stimulators, potentially leading to variations in drug effects.

In ex vivo human experiments, sirolimus has variable effects on T-cell proliferation and the suppressive function of Tregs (Tables S4 and S5). There have also been various hypotheses regarding the mechanism(s) by which sirolimus selectively expands $CD4^{+}CD25^{high}$ Tregs.

Thus, the published in vivo preclinical and ex vivo human data are too heterogeneous to inform the EC_{50} and E_{max} of sirolimus within a QSP model. In addition to the challenge presented in Challenge 2, sirolimus blood concentrations are not currently able to be mechanistically linked to changes in Tregs and Tcons. Therefore, the next challenge, Challenge 4, proposes how to account for the pharmacodynamic interactions of immunosuppressants.

Challenge 4: Adjust for potential pharmacodynamic drug–drug interactions with the proposed altering medication burden for absolute lymphocyte count (ALC AMB) and GVHD (GVHD AMB)

Because of variable conditioning regimens, allografts, and GVHD prevention protocols, there are over 123,000 potential combinations in patients with acute myeloid leukemia or myelodysplastic syndrome being treated with allogeneic HCT (Figure S1). GVHD prevention regimens include at least two immunosuppressants, with the potential for pharmacodynamic drug–drug interactions (DDI). Pharmacodynamic DDIs arise when one drug changes the pharmacological effect of another drug. A framework addressing immunosuppressants' pharmacodynamic DDI

TABLE 2 More detailed description of and potential solutions for the challenges in extrapolating experimental data to inform the QSP model.

Question	A more detailed description of and potential solutions for the challenges
What is the optimal experimental model since no gold standard exists?	• Use an experimental model that is biologically closest to HCT patients for dose-finding studies • Examples of potential experimental models are detailed in Tables S2–S5
How do we isolate the T-cell subset(s) of interest?	• Identify which T-cell subsets are associated with GVHD, which is the end point of interest • Optimize flow cytometry methods for T-cell subsets • Use controls for day-to-day consistency of quantifying the HCT patients' T-cell subsets
Because of the substantive variability in the EC_{50} and E_{max} of ex vivo data, is there a misestimation of the EC_{50} and E_{max}?	• Minimize sirolimus binding to plastic or glassware • Have at least two (ideally three, if sufficient T-cells are available) replicates • Isolate the same T-cell populations evaluated in clinical trials of PTCy—that is, use the same flow cytometry panel for sirolimus—Tregs and sirolimus—Tcons concentration—effect studies as used in clinical trials of PTCy
Do the ex vivo data of the EC_{50} and E_{max} parameters inform the QSP model?	• Table S5 describes the available ex vivo data regarding the association between sirolimus concentration—Tregs and sirolimus concentration—Tcon • Study the reasons underlying the substantial variability observed in the EC_{50} and E_{max} parameters • Use the extreme values to evaluate the sensitivity of the QSP model
Does the ex vivo data reflect the in vivo data, specifically that of an HCT patient?	• Study the effect of sirolimus on other parameters of Tregs such as metabolic and other intracellular processes that may contribute to overall effect of Tregs in vivo to compliment EC50 and E_{max} parameters • Study the relationship between the ex vivo proliferation achieved with the T-cell receptor (typically, CD3 and CD28) stimulation and the T-cell recovery in allogeneic HCT patients

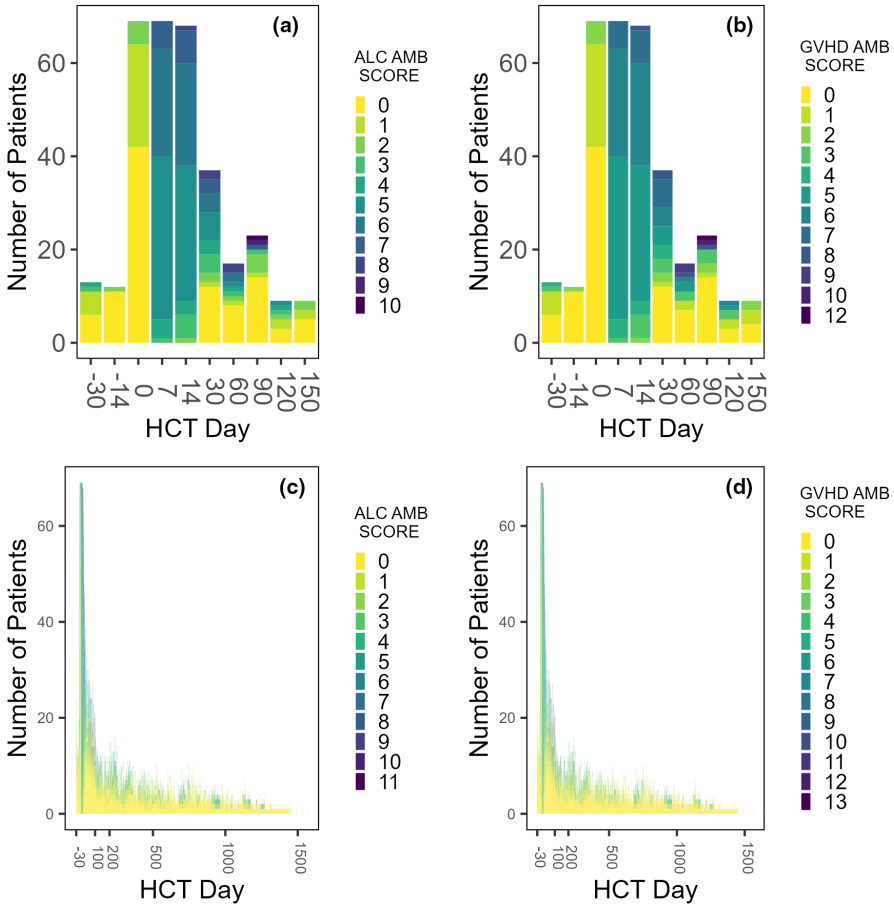

FIGURE 2 Altering medication burden for absolute lymphocyte count (ALC AMB) and graft-versus-host disease (GVHD AMB). The number of participants is shown (y-axis) by HCT Day (x-axis) for AMB that definitely (a, b) or possibly (c, d) affects the stated end points ALC (a, c) or GVHD (b, d).

could not be found. Therefore, to provide a semi-quantitative framework for pharmacodynamic DDIs, we propose the altering medication burden (AMB) method. The literature review and rationale for the AMB method are described in Method S1. Method S2 summarizes an example of how the AMB is assigned to one HCT patient on 1 day. Method S3 is a list of medications prescribed to HCT patients with their corresponding AMB for absolute lymphocyte counts (ALC AMB) and graft-versus-host disease (GVHD AMB). Figure 2 shows substantive variability in the AMB for the absolute lymphocyte count (Figure 2a,c) and GVHD (Figure 2b,d) in 70 HCT patients receiving PTCy-based GVHD prevention regimens. Also, the intensity of immunosuppressants is inconsistent between donor grafts in HCT patients (Table S1 of McCune et al.[25]). This will limit the use of a platform-based QSP model for the donor graft studied; hence, we focused only on QSP-PTCy in this manuscript.

Challenge 5: Calibrate the QSP model to circulating Tregs and Tcons in PTCy-treated patients

Relating sirolimus exposure to changes in T-cell reconstitution after HCT is essential in quantifying the effect of sirolimus on T-cell subsets relevant to GVHD. Critical aspects of mathematically characterizing the relationship of T-cell subsets with clinical outcomes are as follows: 1. characterizing the different subsets or phenotypes of Tregs and Tcons; 2. batch-to-batch variability with flow cytometry results; and 3. consistency with the flow cytometry results between participating centers. Several factors must be standardized among participating centers, including reagents, processing time, detecting instruments, and the data analysis process. Establishing a standardized protocol for cell processing and data analysis among participating centers is essential to ensure the uniformity of the data. Such factors can help overcome the limitations of the published literature described in Challenges 2, 3, and 4.

SUMMARY

The success of the potentially curative procedure of allogeneic HCT rests partly on the optimal use of repurposed drugs with or without radiation. GVHD prophylaxis could benefit from informed guidance in choosing the optimal regimen for an HCT patient and personalized dosing. A QSP-PTCy model based on T-cell response to drugs will fulfill this need. To successfully build a QSP model for

sirolimus, extensive ex vivo and in vivo data on the effect of sirolimus on T-cell subsets are needed. The currently available literature provides a partial view of the ex vivo effects. However, the variability and inconsistency in the existing data preclude its use in adequately informing the QSP-PTCy model. In addition, the existing clinical trial data are insufficient to guide subsequent clinical trials or PK-guided dosing in patient populations outside those studies. Future studies are needed to obtain informative ex vivo data and sufficient clinical data to support building a useful QSP-PTCy model for GVHD prophylaxis and sirolimus dosing.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests in this work.

DATA AVAILABILITY STATEMENT

The datasets of the altering medication burden may be available with proper approvals.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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