





## ARTICLE

# Lipidomics of cyclophosphamide 4-hydroxylation in patients receiving post-transplant cyclophosphamide

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## Abstract

Biomarker-guided dosing may improve the efficacy and toxicity of cyclophosphamide (CY); however, clinical studies evaluating their association with the area under the plasma concentration–time curve (AUC) of CY and its metabolites are time- and resource-intensive. Therefore, we sought to identify lipidomic biomarkers associated with the time-varying differences in CY formation clearance to 4-hydroxycyclophosphamide (4HCY), the principal precursor to CY's cytotoxic metabolite. Hematopoietic cell transplant (HCT) patients receiving post-transplant CY (PT-CY) were enrolled, cohort 1 ( $n = 25$ ) and cohort 2 ( $n = 26$ ) donating longitudinal blood samples before they started HCT (pre-HCT), before infusion of the donor allograft (pre-graft), before the first dose of PT-CY (pre-CY) and 24 h after the first dose of PT-CY (24-h post-CY) which is also immediately before the second dose of CY. A total of 409 and 387 lipids were quantitated in the two cohorts, respectively. Associations between lipids, individually and at a class level, and the ratio of 4HCY/CY AUC (i.e., 4HCY formation clearance) were evaluated using linear regression with a false discovery rate  $<0.05$ . There were no individual lipids that passed control for false discovery at any time point. These results demonstrate the feasibility of lipidomics, but future studies in larger samples with multiple omic tools are warranted to optimize CY dosing in HCT.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The old yet commonly used drug cyclophosphamide (CY) has a complex pharmacokinetic disposition. There is a paucity of information regarding the formation clearance of 4-hydroxycyclophosphamide (4HCY), the precursor to CY's primary cytotoxic metabolite phosphoramidate mustard. To date, scientists have not been able to create a more effective or safer analog to CY or to identify a precision medicine tool consistently associated with the efficacy, toxicity, or pharmacokinetics of CY.

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### WHAT QUESTION DID THIS STUDY ADDRESS?

This study addresses the question if the plasma lipidome before post-transplant cyclophosphamide (PT-CY) administration in hematopoietic cell transplant (HCT) patients is associated with the ratio of 4HCY/CY area under the plasma concentration–time curve (AUC).

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study shows that longitudinal collection of plasma lipidomic samples is feasible in HCT patients receiving PT-CY. However, in this small patient population, we could not find an association of the plasma lipidome with the ratio of 4HCY/CY AUC. Furthermore, this study shows that the plasma lipidome changes over the ~21-day period that starts before HCT to 24-h after the first PT-CY dose. This study is also among the first to evaluate the plasma lipidome in HCT patients.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study shows how interrogating the plasma lipidome may provide insight into the pharmacokinetics of CY and how interrogating the plasma lipidome is feasible for biomarker studies.

## INTRODUCTION

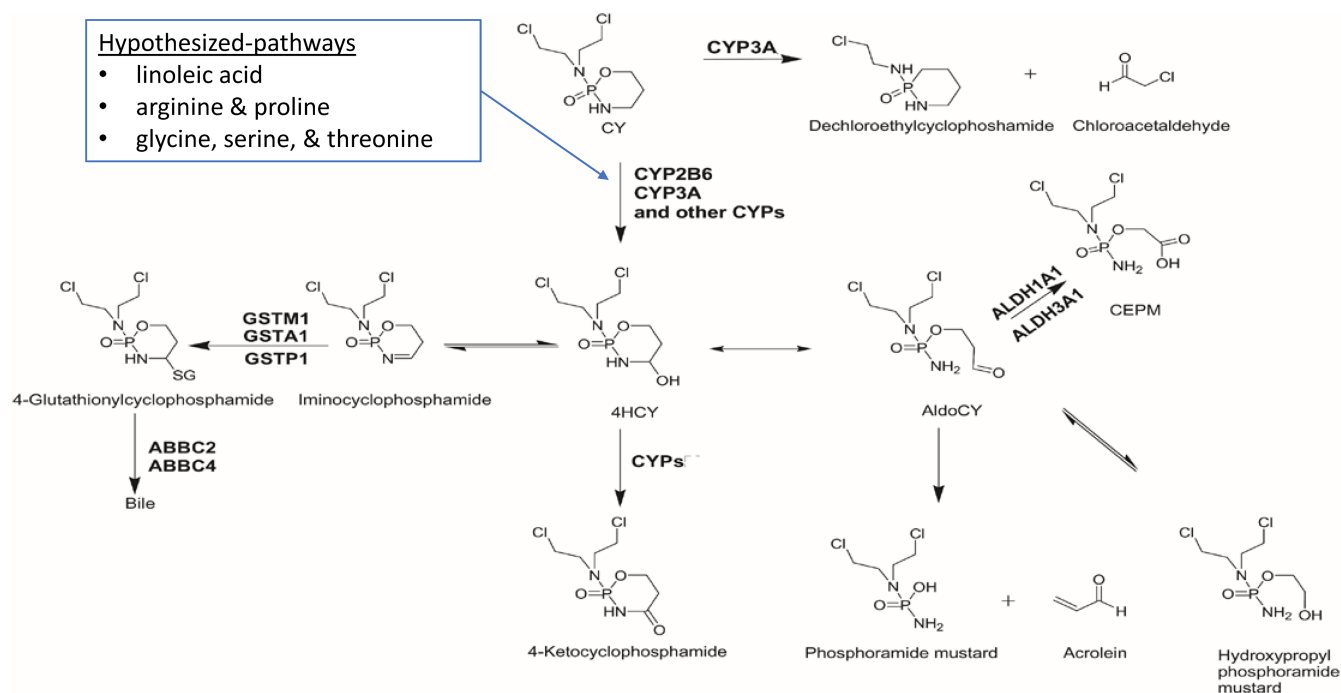
Cyclophosphamide (CY) continues to be one of the most successful and widely used antineoplastic drugs,<sup>1</sup> often in combination with other chemotherapy drugs, to treat patients with a wide range of malignancies. Given its immunosuppressive properties, patients with autoimmune and immune-mediated disorders are often treated with CY. In addition, high-dose CY is used in various parts of the hematopoietic cell transplant (HCT) procedure. Specifically, CY can be used for the mobilization of hematopoietic stem cells, conditioning regimen, and, most recently, post-graft immunosuppression, where CY is administered after allograft infusion – called post-transplant cyclophosphamide (PT-CY).<sup>1,2</sup> Preclinical studies suggest a dose-dependent effect of PT-CY when used to prevent graft-versus-host disease (GVHD), with moderate doses of PT-CY associated with the lowest GVHD rates in small animal models.<sup>2,3</sup> It is, therefore, beneficial to identify biomarkers associated with PT-CY efficacy, toxicity, and pharmacokinetics.

Over 60 years after its initial approval by the Food & Drug Administration, the efficacy, toxicity, and dosing of CY-based chemotherapy regimens have and continue to vary substantially. Identifying drugs that are better than CY and identifying biomarkers associated with the toxicity or efficacy of CY-based regimens have been elusive, potentially due to the complexity of CY metabolism. CY is a prodrug undergoing numerous metabolic activation and inactivation pathways (Figure 1).<sup>4</sup> The current methods of dosing CY lead to considerable interpatient variability in the area under the plasma concentration–time curve (AUC) to CY and its metabolites, including 4-hydroxycyclophosphamide

(4HCY), the precursor to CY's primary cytotoxic metabolite phosphoramidate mustard.<sup>4,5</sup> However, quantifying 4HCY plasma concentrations is time- and resource-intensive because it requires immediate – that is, at the bedside – processing to stabilize 4HCY, which has a short (<3 min) in vitro half-life.<sup>6</sup> Understanding factors associated with 4HCY pharmacokinetics is critical because 4HCY forms phosphoramidate mustard, which covalently cross-links DNA. In addition, because phosphoramidate mustard does not cross cell membranes appreciably, the transport of its precursor 4HCY into the cell is a crucial step for CY's cytotoxic activity.<sup>4</sup> Therefore, gaining an improved understanding of biological factors influencing the 4HCY formation clearance, defined as the 4HCY AUC<sub>0-48h</sub> divided by the CY AUC<sub>0-48h</sub> (referred to as the ratio of 4HCY/CY AUC hereafter), is desirable.

Factors associated with between-patient differences in the ratio of 4HCY/CY AUC include drug–drug interactions. In general, drugs that affect cytochrome P450 (CYP) 3A activity affect 4HCY formation, with azoles decreasing<sup>7</sup> and phenytoin increasing<sup>8</sup> the ratio of 4HCY/CY AUC. Furthermore, in patients with no apparent drug–drug interaction, the ratio of 4HCY/CY AUC changes within a patient (i.e., inpatient variability) with time in a subset of patients receiving different CY doses and regimens over time.<sup>9,10</sup> With this inpatient variability in the ratio of 4HCY/CY AUC, it is not surprising that no consistent genotype–phenotype association has been found with the pharmacokinetics, efficacy, or toxicity of CY.<sup>11,12</sup>

Within patients receiving PT-CY, we previously characterized endogenous metabolomic compounds associated with the ratio of 4HCY/CY AUC.<sup>13</sup> Given the results



**FIGURE 1** Partial metabolic schema of cyclophosphamide (CY) with the hypothesized pathways.

suggesting alterations in fatty acid metabolism before engraftment, we furthered our search for biomarkers employing lipidomics in the same cohorts of patients receiving PT-CY.<sup>13</sup> Given their signaling properties in addition to a multitude of other functions,<sup>14</sup> lipidomics may enable a deeper mechanistic understanding of the pharmacokinetic disposition, drug–target interactions, and systems physiology from the molecular (genomic, proteomic, metabolomic) to cellular to whole-body levels. These insights may provide the foundation for enhanced pharmacokinetic/dynamic modeling to comprehensive quantitative systems pharmacology models.<sup>15</sup> Thus, we wanted to gather preliminary data regarding longitudinal changes in the plasma lipidome before and during PT-CY administration. If an association is found between the plasma lipidome and the ratio of 4HCY/CY AUC, characterization of the longitudinal changes in the lipidome would inform future studies on lipidomics-guided CY dosing. The 2-week pre-HCT sample collection time was the earliest feasible time within the final HCT workup (i.e., the period in which the participant undergoes final assessment for HCT, typically up to 2 weeks). The pre-graft sample was obtained immediately before the allograft infusion after all of the conditioning regimens were administered and 72-h before the first PT-CY dose. The pre-CY sample was obtained immediately before the first PT-CY dose. The 24-h post-CY sample was obtained immediately before the second CY dose and may provide insight regarding plasma lipids associated with auto-induction of 4HCY formation.

## METHODS

### Study populations

The two datasets were cohort 1 ( $n = 25$ ) and cohort 2 ( $n = 26$ ); the samples were analyzed only within datasets from the same lipidomics run. Samples and data were prospectively collected from participants who received HCT and post-graft immunosuppression with PT-CY from June 2018 to July 2020 under the aegis of two protocols approved by the City of Hope Institutional Review Board. Participants were diagnosed with hematologic disorders and had adequate kidney and liver function. Demographic and clinical information were taken from the participants' medical charts. All participants provided written informed consent before study procedures were initiated. These participants underwent the same study procedures, but their samples were analyzed separately. Study participation did not alter the conditioning regimens and post-graft immunosuppression medications. Antibiotics, antifungals, and antiemetics were given per Institutional Standard Practice Guidelines.

### PT-CY administration and pharmacokinetic samples

The PT-CY dose was 50 mg/kg based on an adjusted ideal body weight (AIBW) IV [2-h] infusion every 24 h for two doses, administered on HCT day +3 and +4.

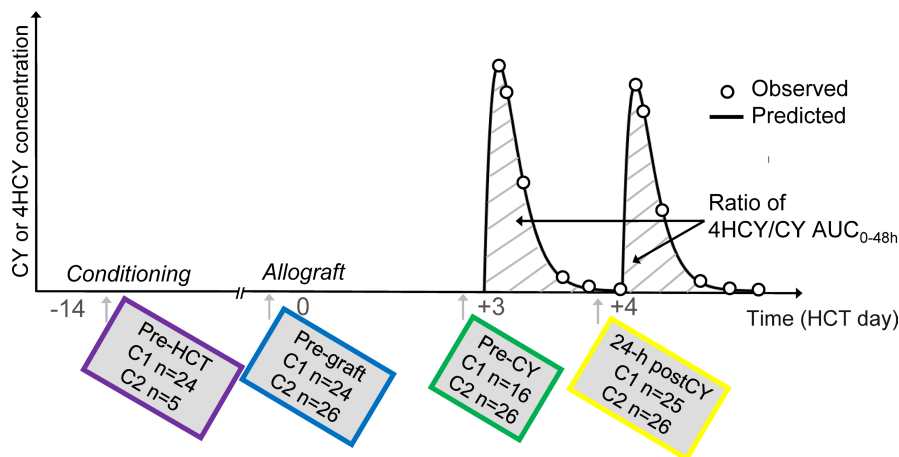
Pharmacokinetic samples were collected after each PT-CY dose: at 2 (end of infusion), 4, 8, 20, and 24 h from the start of the infusion.<sup>13</sup> Concentrations of CY, 4HCY, carboxyethylphosphoramidate mustard, deschloroethylcyclophosphamide, and 4-ketocyclophosphamide were quantitated as previously reported,<sup>13</sup> using liquid-chromatography tandem-mass-spectrometry (MS). The AUCs from time 0 h to 48 h were estimated using the trapezoidal numerical estimation function in MATLAB (v 2019a).

## Global lipidomic sample collection

Lipidomic sample collection relative to PT-CY administration and the CY pharmacokinetic sampling is shown in Figure 2. Longitudinal blood samples (3 ml/sample) were collected in EDTA tubes at four time points during PT-CY dosing: up to 2 weeks before the start of the conditioning regimen (pre-HCT sample), immediately before infusion of the allograft (pre-graft sample), immediately before administration of the first PT-CY dose (pre-CY sample) and 24 h after the first CY dose which is also immediately before the second PT-CY dose (24-h post-CY sample). In cohort 1, all 25 participants had samples for three time points, and 14 participants had samples for all four time points. In cohort 2, all 26 participants had samples for three time points, and five participants had samples for all four time points. The total samples at each time point in each cohort are shown in Figure 2.

## Untargeted lipidomics analysis

Semi-quantitative profiling of the plasma lipids was completed at the West Coast Metabolomics Center (WCMC). The samples were shipped on dry ice to WCMC and stored at  $-80^{\circ}\text{C}$  upon receipt, and underwent at most one additional freeze-thaw before lipidomic analysis (i.e., the analysis was conducted after the first or the second thaw). As previously described, samples were extracted using methanol:methyl tert-butyl ether:water.<sup>16</sup> Internal standards and chromatographic conditions were optimized by using toluene in the reconstitution solvent mixture to ensure that very lipophilic compounds are efficiently transferred column in the injection process. Data were acquired using a Waters Acquity ultra-high-performance liquid chromatography UPLC CSH C18 column (Waters). Data were collected in both positive and negative ion mode and processed using MassHunter (Agilent Technologies). Lipids were identified based on MS/MS fragmentation patterns using LipidBlast software.<sup>17</sup> Profiling of free fatty acids (FFAs) and complex lipids from several classes, including ceramides (CER), sphingomyelins (SM), cholesteryl esters (CE), lyso- and phospholipids that is, phosphatidyl ethanolamines (PE), lyso-PE (LPE), phosphatidyl cholines (PC), lyso-PC (LPC), and phosphatidyl inositols (PI), mono-, di-, and triacylglycerols (DG and TG), and acylcarnitines have been described previously.<sup>18</sup> Details on sample extraction, laboratory protocols, and data pre-processing have been described previously.<sup>19</sup> A total of 409 lipids were detected in at least 90% of samples for cohort



**FIGURE 2** Study design. Plasma lipid samples, obtained before or with the administration of the post-transplant cyclophosphamide (PT-CY) regimen, were associated with the ratio of 4-hydroxycyclophosphamide (4HCY)/CY  $\text{AUC}_{0-48\text{h}}$ . Gray boxes show the four longitudinal time points at which plasma lipid samples were obtained. The ratio of the 4HCY/CY  $\text{AUC}_{0-48\text{h}}$  was measured after both of the two PT-CY doses; the areas under the plasma concentration–time curve (AUCs) are filled with stripes. The hematopoietic cell transplant (HCT) conditioning regimen is administered before HCT day  $-1$ , the allogeneic graft infusion occurs on HCT day 0, and the PT-CY doses are administered on HCT day +3 and +4 (72 h and 96 h, respectively, after infusion of the allogeneic graft). C1 is cohort 1 and C2 is cohort 2. The time points color coding corresponds to that in Figure 3.

1 and 387 in cohort 2. Missing values were imputed with half of the lowest abundance for the lipid. Average intra-assay coefficients of variation (CVs) were 9.6 and 6.5 for cohorts 1 and 2, respectively.

## Data analysis

Given that assays were run separately and contained different sets of lipids, datasets for each cohort were analyzed separately. Lipid values were log-transformed, and each sample was centered according to its mean (i.e., centered log-ratio).<sup>20</sup> Linear regression was used to assess marginal associations for each of the lipid species individually and for each of the 12 lipid classes (FFA, CER, SM, CE, PE, LPE, PC, LPC, PI, DG, TG, and acylcarnitines), regressing on the ratio of 4HCY/CY AUC (continuous) adjusted for age, sex, ethnicity, and AIBW. This was performed for each sample time: pre-HCT, pre-graft, pre-CY, and 24-h post-CY. Only time points for which data were available were analyzed; missing time points were excluded from the analysis. Three participants received lipid-altering medications (Methods S1) after treatment commenced, two in the first cohort and one in the second; however, a sensitivity analysis showed that the results were unaffected. Benjamini–Hochberg was used to control for false discovery rate (FDR).<sup>21</sup> Individual lipids were considered for both  $p < 0.05$  and  $FDR < 0.1$ . Principal component analysis was used to visualize the variance in the lipidome across time points. All analyses were conducted using R (v 4.0.5) software.

## RESULTS

### Patient characteristics and clinical outcomes

Table 1 contains a summary of the pre-HCT characteristics of the two cohorts. The median age was 53 years (range 1.7–66.2), and slightly more participants were male (62%) and white (70%). Seventy-six (90%) participants received an HLA-identical graft, and 57 (68%) participants received a graft from an unrelated donor. Only one participant received concomitant corticosteroids, which induce CY 4-hydroxylation in vitro<sup>22</sup> and possibly in vivo. In addition, none of the participants received concomitant azoles or phenytoin, which affect plasma 4HCY concentrations in vivo.<sup>7,8</sup>

### Longitudinal changes in plasma lipidome

Principal component analysis of all samples in each run showed some separation among lipidomic profiles

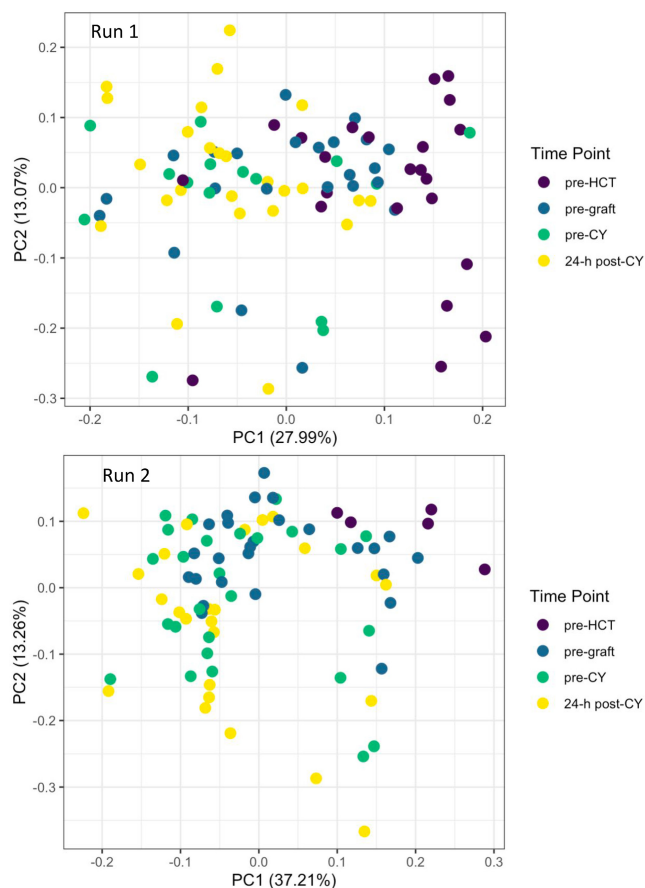
**TABLE 1** Pre-hematopoietic cell transplant characteristics of the participants<sup>a</sup>

Characteristic	Cohort 1 (n = 25)	Cohort 2 (n = 26)
Age (years)	39 (18–78)	48 (20–78)
Body mass index (kg/m <sup>2</sup> )	27 (18–43)	28 (21–53)
Sex (% male)	60%	77%
AIBW (kg)	71 (57–83)	73 (48–87)
Ratio of 4HCY/CY AUC	0.06 (0.04–0.13)	0.06 (0.02–0.13)
Self-reported race		
White	18 (72%)	18 (69%)
African American	3 (12%)	1 (4%)
American Indian or Alaska Native	1 (4%)	0 (0%)
Asian	0 (0%)	5 (19%)
Not reported	3 (12%)	2 (8%)
Self-reported ethnicity		
Hispanic or Latino	13 (52%)	6 (23%)
Non-Hispanic	12 (48%)	18 (69%)
Not reported	0 (%)	2 (8%)
Diagnosis		
Acute myeloid leukemia (AML)	10 (40%)	9 (35%)
Acute lymphoblastic leukemia (ALL)	6 (24%)	6 (23%)
Hodgkin's lymphoma	4 (16%)	4 (15%)
Chronic myeloid neoplasms	2 (8%)	4 (15%)
Non-Hodgkin's lymphoma	1 (4%)	1 (4%)
Sickle cell disease	2 (8%)	0
Other <sup>b</sup>	0	2 (8%)
Donor		
Related (haploidentical)	17 (68%)	12 (46%)
Unrelated	8 (32%)	14 (54%)
Relevant medications		
Ursodiol	25 (100%)	26 (100%)
CY as part of conditioning regimen	4 (16%)	4 (15%)
Corticosteroids with PT-CY	1 (4%)	0 (0%)
Lipid-altering drugs before or during lipidomic sampling	2 (8%)	1 (4%)
Azoles within 7 days of PT-CY	0 (0%)	0 (0%)

Abbreviations: AIBW, adjusted ideal body weight; 4HCY/CY AUC, the ratio of 4-hydroxycyclophosphamide/cyclophosphamide area under the plasma concentration–time curve; PT-CY, post-transplant cyclophosphamide.

<sup>a</sup>Data presented as: number (%) or median (range). Cohorts differed because their lipidomics analyses occurred in two separate runs.

<sup>b</sup>Other diagnoses included: myeloid sarcoma ( $n = 1$ ) and multiple myeloma ( $n = 1$ ).



**FIGURE 3** Change in plasma lipids from before post-transplant cyclophosphamide (PT-CY) administration (purple, blue, and teal dots) to after administration of the first PT-CY dose (yellow dots). Principal component analysis showing differences across time points within cohorts. The time points color coding corresponds to that in Figure 2.

between time points, particularly pre-HCT and pre-graft (Figure 3). Given that participants received a conditioning regimen during this interval, differences in the lipidome are not unexpected.

### Lipidomics: univariate and class-level analyses by cohort and longitudinal time point

We hypothesized that the sample obtained at the pre-CY time point, drawn immediately before the first CY dose, would have lipids associated with the ratio of the 4HCY/CY AUC. At the pre-CY time point, cohorts 1 and 2 had 7 and 9 lipids, respectively, which had a raw value of  $p < 0.05$ ; however, there were no significant lipids at FDR  $< 0.1$  (lipids at  $p < 0.05$  given in Tables 2 and 3; full list given in Table S1). We also evaluated the association of lipids with the ratio of the 4HCY/CY AUC with the other time points. For the pre-HCT, pre-graft, and 24-h post-CY time points,

**TABLE 2** Pre-cyclophosphamide (CY) plasma lipids associated ( $p < 0.05$ ) with the ratio of 4-hydroxycyclophosphamide/cyclophosphamide area under the plasma concentration–time curve (4HCY/CY AUC) in cohort 1 ( $n = 25$  samples)

Lipid	Coefficient (SE) <sup>a</sup>	<i>p</i> -value <sup>b</sup>	<i>q</i> -value
Phosphatidyl choline (38:5)	−8.41 (2.11)	0.003	0.97
Phosphatidyl choline (36:5)	−13.81 (4.37)	0.01	0.97
Sphingomyelin (d43:2)	−29.52 (11.41)	0.03	0.97
Phosphatidyl choline (40:6)	−6.52 (2.59)	0.03	0.97
Phosphatidyl choline (39:6)	−5.97 (2.40)	0.03	0.97
Phosphatidyl choline (38:4)	−7.78 (3.25)	0.04	0.97
Phosphatidyl ethanolamine (36:2)	9.10 (3.96)	0.04	0.97

<sup>a</sup>Beta coefficients and standard errors (SE) obtained from linear models evaluating the association between center-log ratio lipid concentrations and the ratio of 4-hydroxycyclophosphamide/cyclophosphamide area under the plasma concentration–time curve (4HCY/CY AUC) adjusted for age, sex, ethnicity, and adjusted ideal body weight (AIBW).

<sup>b</sup>The *p*-values are listed in descending order. There were no lipids significant at false discovery rate (FDR)  $< 0.1$ .

cohort 1 had 5, 7, and 4 lipids which had a raw *p*-value of 0.05, respectively. Cohort 2 had insufficient samples for analysis at the pre-HCT time point. At the pre-graft and 24-h post-CY time points, cohort 2 had 9 and 16 lipids, respectively, with raw *p*-values  $< 0.05$ . As with the pre-CY time points, no lipids were significant after controlling for FDR (Table S2). No lipids were significant at a class level at any time point in either cohort (data not shown).

## DISCUSSION

We previously evaluated an untargeted metabolomics panel and found 12 and 32 metabolites associated with pre-CY administration for cohorts 1 and 2, respectively.<sup>13</sup> In pathway analyses, fatty acid metabolism was the only pathway identified prior to engraftment. That led us to build on those results by employing a lipidomics assay in the same samples. However, no lipids were significantly associated with the ratio of the 4HCY/CY AUC.

CY use will likely expand with the increasing use of PT-CY in alternative donor HCT. Dose de-escalating studies of PT-CY doses are ongoing, with the goal of lowering the rates of GVHD. Furthermore, the precise mechanism(s) regarding how CY and its active metabolites transport into

Lipid	Coefficient (SE) <sup>a</sup>	p-value <sup>b</sup>	q-value
Phosphatidyl choline (30:0)	-8.68 (3.54)	0.02	0.99
Phosphatidyl choline (31:0)	-5.44 (2.22)	0.02	0.99
Phosphatidyl ethanolamine (36:2)	-7.50 (3.18)	0.03	0.99
Phosphatidyl ethanolamine (36:3)	-8.01 (3.47)	0.03	0.99
Phosphatidyl ethanolamine (P-36:2) or Phosphatidyl ethanolamine (O-36:3)	-29.59 (12.90)	0.03	0.99
Phosphatidyl choline (33:0)	-5.13 (2.27)	0.04	0.99
Phosphatidyl ethanolamine (34:2)	-7.78 (3.54)	0.04	0.99
Phosphatidyl choline (34:3)	-7.01 (3.21)	0.04	0.99
Phosphatidyl ethanolamine (P-38:4) or Phosphatidyl ethanolamine (O-38:5)	7.68 (3.54)	0.04	0.99

<sup>a</sup>Beta coefficients and standard errors (SE) obtained from linear models evaluating the association between the center-log ratio lipid abundances and the ratio of 4-hydroxycyclophosphamide/cyclophosphamide area under the plasma concentration–time curve (4HCY/CY AUC) adjusted for age, sex, ethnicity, and adjusted ideal body weight (AIBW).

<sup>b</sup>The p-values are listed in descending order. There were no lipids significant at false discovery rate (FDR) < 0.1.

**TABLE 3** Pre-cyclophosphamide (CY) plasma lipids associated ( $p < 0.05$ ) with the ratio of 4-hydroxycyclophosphamide/cyclophosphamide area under the plasma concentration–time curve (4HCY/CY AUC) in cohort 2 ( $n = 26$  samples)

the T-cells of interest, T regulatory and T conventional,<sup>2,3</sup> are unknown. A mechanistic understanding of factors associated with the ratio of 4HCY/CY AUC is needed to achieve the PT-CY dose associated with the optimal GVHD rates. Because of the short half-life of 4HCY in blood (~3 min), rapid processing at the patient's bedside is required to stabilize 4HCY to characterize its pharmacokinetics sufficiently. Conducting pharmacokinetic/pharmacodynamic (PK/PD) studies to test the hypothesis that 4HCY AUC is associated with clinical outcomes would be time- and resource-intensive.<sup>10</sup> Furthermore, with the increase in alternative donor HCT, there are more choices in the type of donor and, thus, a single HCT center will have increasing heterogeneity in their allografts. Thus, it is increasingly difficult for one HCT center to conduct sufficiently powered PK/PD studies to evaluate the association of the AUC of CY and its metabolites with clinical outcomes in patients receiving PT-CY.<sup>10</sup> Thus, we sought to evaluate if plasma lipidomics – which do not require bedside processing – were associated with the ratio of 4HCY/CY AUC.

We previously evaluated the endogenous metabolome with 4HCY/CY AUC in a similar fashion.<sup>13</sup> Similar to our current findings, several individual endogenous metabolites were significantly associated with 4HCY/CY metabolism, but none remained significant after controlling for multiple testing. Interestingly, in pathway analysis, fatty acid biosynthesis was associated with pre-CY at  $p = 0.04$  for cohort 2. Unfortunately, a large-scale evaluation of the lipidome in the present analysis did not yield more robust results. However, the plasma lipidome did change substantially over time (Figure 3) through an unknown

mechanism as there were very few lipid-altering medications being administered over the time of lipidomic sample collection.

Busse et al. ( $n = 12$ )<sup>9</sup> and Bemer et al. ( $n = 11$ )<sup>10</sup> each demonstrated that approximately half of the patients have a significant change in the ratio of 4HCY/CY AUC over a couple of weeks' time span. However, there was no apparent difference between patients with a consistent ratio and those with an inconsistent ratio of 4HCY/CY AUC. Therefore, we hypothesized that plasma lipids might provide insight into factors associated with the ratio of 4HCY/CY AUC. In multi-omics studies, metabolomics and its subdiscipline lipidomics are receiving increasing attention.<sup>23</sup> These endogenous metabolites are the downstream output of biological processes and have imprints of genomic, epigenomic, and environmental effects.<sup>23</sup> They are often referred to as “the link between genotype and phenotype.”<sup>24</sup> Considering the within-patient variation in the ratio of 4HCY/CY AUC, metabolomics and lipidomics are unique tools to better understand the pharmacokinetics, toxicity, and efficacy of PT-CY. Discoveries made using lipidomics are yielding new insights into how lipids influence organ function, immune function, and gut physiology.<sup>25</sup> Collectively, this work could lead to a system-wide perspective of the allogeneic HCT biology wherein lipids, proteins, and genes are understood to interact synergistically to modify the functions within the allogeneic HCT recipient.

Our dataset was limited in its sample size and thus power. However, it does represent a contemporary patient population receiving PT-CY, and the global lipidomic panel provided high accuracy of lipid identification

and semi-quantitative abundances. Our results show the feasibility of conducting lipidomics studies in allogeneic HCT patients and collecting longitudinal samples. Future studies with larger sample sizes (and, thus, more relapse and GVHD events) are needed, as they would allow for the inclusion of risk factors for relapse (e.g., cytogenetics) or GVHD (e.g., human leucocyte antigen [HLA]). In addition, integrating multi-omic platforms may yield greater mechanistic insight into the pathophysiology of relapse or GVHD while improving clinical outcomes.

### AUTHOR CONTRIBUTIONS

S.L.N., Z.Z., T.W.R., R.N., B.M.S., D.H., and J.S.M. wrote the manuscript. T.W.R. and J.S.M. designed the research. S.L.N., T.W.R., R. N., and J.S.M. performed the research. S.L.N., Z.Z., T.W.R., and J.S.M. analyzed the data.

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### CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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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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