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GSTT1 Null Genotype as a Pharmacogenetic Risk Marker for Infection During Cyclophosphamide Induction Therapy in Systemic Lupus Erythematosus

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Article

Keywords:

Posted Date: November 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11035298/v1

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Additional Declarations: No competing interests reported.

Abstract

Cyclophosphamide (CYC) remains an important induction therapy for severe systemic lupus erythematosus (SLE), but infection risk varies considerably among patients. This prospective study evaluated associations of pharmacokinetic and pharmacogenetic factors with infection in patients with SLE receiving intravenous CYC induction therapy. Thirty patients underwent pharmacokinetic sampling and 24-week follow-up. Infection occurred in 12 patients (40.0%). The *GSTT1* null genotype was associated with increased infection risk (RR = 3.455; BH-FDR-adjusted $p = 0.040$) and remained associated with infection after adjustment for baseline absolute lymphocyte count (ALC) (adjusted RR = 2.824; $p = 0.033$). Exploratory discrimination analysis showed the highest discrimination with combined baseline ALC and *GSTT1* genotype. The 6-hour plasma concentration after initiation of infusion (C_6) showed the strongest correlation with AUC_{0-24} ($R^2 = 0.987$). Each 1,000 ng/mL increase at C_6 or C_8 was associated with a lower risk of infection (RR = 0.908 and 0.880; BH-FDR-adjusted $p = 0.027$ and 0.018, respectively). Higher CYC clearance (CL) was associated with an early reduction in lymphocytes. These findings highlight the *GSTT1* null genotype as a potential genetic marker of infection risk in SLE. Integrating lymphocytes, CYC pharmacokinetics, and pharmacogenetics may support infection-risk stratification and could prevent the serious infection from CYC over-suppression.

Introduction

Systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disease characterized by heterogeneous clinical manifestations, can affect multiple tissues and organs. Clinical features in SLE may vary from mild disease to severe, life-threatening disease. The therapeutic approach depends on the organ system involved and the disease severity. Severe or organ-threatening manifestations, frequently require intensive immunosuppressive therapy to achieve disease control and prevent irreversible organ damage. Induction therapy is pivotal important in timely controlling severe disease flares and prevent permanent damage to vital organs. Despite advances in SLE management, treatment-related complications remain an important clinical challenge, particularly during induction therapy.

Infection, one of the leading causes of morbidity and mortality in patients with SLE, have accounted for approximately 15.0-65.8% of deaths in previous reports (1, 2, 3). Not only the increased susceptibility of SLE patients to infection but the poor clinical outcome of infection in SLE can be explained by multiple factors, including abnormalities of innate and adaptive immunity, disease-related and hematologic factors, and immunosuppressive treatment (4, 5). The risk may be particularly relevant during intensive induction therapy, when a combination of immunosuppressive drugs such as CYC, corticosteroids, and other immunosuppressive agents were given to bring about a fast remission of the disease. Infection may necessitate interruption or delay of immunosuppressive treatment, potentially compromising disease control. Therefore, identifying factors associated with infection during SLE induction therapy would be beneficial.

CYC, a potent immunosuppressive agent, remains an important induction therapy for severe and organ-threatening SLE (6, 7, 8, 9, 10). As an inactive prodrug, CYC undergoes hepatic bioactivation to 4-hydroxycyclophosphamide (4-OHCP) and downstream active metabolites, while competing metabolic pathways contribute to detoxification (11, 12). Despite conventional body surface area-based dosing, CYC exhibits substantial interindividual pharmacokinetic variability, including among patients with lupus nephritis and other autoimmune glomerular diseases, which may be influenced by renal function, patient characteristics, and genetic variation in drug-metabolizing enzymes (13, 14, 15, 16, 17, 18). This variability may be clinically relevant, as exposure to the active metabolite 4-OHCP has been associated with clinical remission in patients with lupus nephritis (19). Nevertheless, pharmacokinetic-outcome relationships remain incompletely characterized in lupus nephritis, and a validated target exposure, such as an area under the concentration-time curve (AUC) for CYC, has not been established in these populations. In particular, the relationship between parent CYC exposure and infection risk remains poorly understood. Pharmacogenetic variation in CYC-metabolizing and detoxification enzymes may further contribute to interindividual differences in drug disposition, treatment efficacy, and toxicity, with reported associations varying across populations and treatment settings (19, 20, 21, 22, 23).

Glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH) enzymes are involved in CYC detoxification (11, 15, 24). The *GSTT1* null genotype results in loss of enzyme activity and may reduce glutathione-mediated detoxification capacity, potentially contributing to interindividual susceptibility to CYC-related toxicity (25). Variants in *GSTP1* and *GSTT1* have been associated with CYC-related efficacy and toxicity (20, 21, 23) In particular, *GSTP1* rs1695 has been associated with an increased risk of CYC-related toxicity, including infection in patients with SLE or lupus nephritis (20, 26), GST polymorphisms have also been associated with treatment outcomes in CYC-treated lupus nephritis (27), while genetic variation in ALDH enzymes, including *ALDH3A1* rs2228100 and *ALDH1A1* rs6151031, has been investigated in relation to CYC disposition and adverse effects (15). The *GSTT1* null genotype is particularly common in Asian populations, with reported frequencies of approximately 50–60% (25), and has been reported at a frequency of approximately 60% in a Thai population (28). Population differences in the distribution of pharmacogenetic variants may contribute to interindividual variability in CYC disposition and clinical outcomes. Collectively, these findings provide a rationale for jointly evaluating CYC pharmacokinetics and genetic variation in detoxification pathways in Thai patients with SLE.

Lymphopenia and neutropenia, are commonly seen in SLE and may reflect immune dysfunction, disease-related factors, and immunosuppressive treatment (4, 29, 30). Lymphopenia has been associated with infection in SLE, although findings have varied across studies (5, 31). During SLE flare which required induction therapy, lymphopenia is one of the common features of active disease. At the same time, CYC is needed for the induction treatment in severe SLE and can suppress lymphocyte count profoundly. Thus, we aim to identify the pharmacokinetics and pharmacogenetics parameters that can help to predict the risk of infection in SLE patients receiving CYC induction therapy to prevent the complication of the treatment.

Results

Baseline Characteristics of the Study Population

A total of 30 patients with active SLE were included in the study. Lupus nephritis was the most common organ manifestation, affecting 22 patients (73.33%), followed by vasculitis in 12 patients (40.0%). Neuropsychiatric SLE and lupus hepatitis were observed in 4 (13.33%) and 3 (10.0%) patients, respectively, whereas lupus myocarditis was observed in 1 patient (3.33%). The mean SLEDAI-2K score was 18.23 ± 6.82, indicating high disease activity at study entry. Baseline laboratory evaluation showed the median ALC was 600 cells/μL (400-970 cells/μL). The mean C3 concentration was 0.608 ± 0.264 g/L, and low C3 (< 0.9 g/L) was present in 27 patients (90.0%). The median urine protein-to-creatinine ratio (UPCR) was 2.27 (0.43-6.90), reflecting significant proteinuria in this population. At treatment initiation, all patients received intravenous corticosteroids and hydroxychloroquine. The mean initial CYC dose was 740 ± 145.27 mg, corresponding to a mean body surface area-adjusted dose of 0.458 ± 0.073 g/m^2. The baseline demographic, clinical, laboratory, and treatment characteristics of the study population are summarized in Table 1.

Pharmacokinetic Profile of CYC

Complete 24-hour pharmacokinetic profiles were obtained for all 30 patients. The mean plasma CYC concentration-time profile following intravenous administration is presented in Figure 1A. The mean AUC_{0-24} was 163.54 ± 63.52 μg·h/mL, and the mean dose-normalized AUC was 223.68 ± 88.39 μg·h/mL/g. The mean Cmax was 23,132 ± 9,286.84 ng/mL. The mean terminal elimination rate constant (λz) was 0.1368± 0.0402h^{-1}, corresponding to a median terminal elimination half-life ($T_{1/2}$) of 5.03 h (4.13-6.32 h). The median systemic clearance (CL) was 4.29 L/h (3.62-5.79 L/h), and the median apparent volume of distribution associated with the terminal phase (Vz) was 32.52 L (23.95-51.61 L). Tmax occurred at 1 h in all patients, corresponding to the end of the 1-h CYC infusion. Pharmacokinetic parameters are summarized in Table 2.

Pharmacogenetic variants in the SLE study population

The distribution of pharmacogenetic variants is summarized in Table 3. For *GSTP1* rs1695 (c.313A>G), the AA genotype was the most prevalent, occurring in 20 patients (66.67%), followed by AG in 7 (23.33%) and GG in 3 (10.00%). The *GSTT1* present genotype was observed in 19 patients (63.33%), whereas the *GSTT1* null genotype was identified in 11 patients (36.67%). All patients carried the *GSTM1* present genotype, and no *GSTM1* null genotype was detected. For *ALDH3A1* rs2228100 (c.985C>G), the CC, CG, and GG genotypes were observed in 4 (13.33%), 15 (50.00%), and 11 (36.67%)patients, respectively.The *ALDH1A1* rs6151031 17-bp deletion variant was identified in 3 patients (10.00%), whereas the insertion genotype was observed in 27 patients (90.00%).

Association Between Pharmacogenetic Variants and CYC Pharmacokinetic Parameters

Univariable linear regression analyses were performed to evaluate associations between pharmacogenetic variants and CYC pharmacokinetic parameters. Nominal associations were observed

between the *GSTT1* null genotype and lower CYC exposure and higher CL, the data are provided in Table 4.

Compared with the *GSTT1* present genotype, the *GSTT1* null genotype was associated with lower AUC_{0-24}, lower dose-normalized AUC, and higher CL. The *ALDH3A1* rs2228100 G-allele carrier genotype (CG/GG) was nominally associated with higher Cmax compared with the CC genotype.

However, none of these associations remained statistically significant after BH-FDR correction across the 28 pharmacogenetic–pharmacokinetic comparisons (adjusted $p \geq 0.270$). No nominally significant associations were observed for *GSTP1* rs1695 G-allele carrier status or *ALDH1A1* rs6151031 deletion carrier status with the evaluated pharmacokinetic parameters (The data are provided in Table S1-S3).

Factors associated with infection and reduction of lymphocyte count during CYC induction therapy

Infectious and hematologic outcomes were prospectively monitored during CYC induction therapy. Infections requiring systemic antimicrobial treatment during hospitalization occurred in 12 of 30 patients (40.0%). Documented infections included bacterial infections (N = 10), cytomegalovirus infection (N = 7), invasive pulmonary aspergillosis (N = 4), candidiasis (N = 2), cryptococcosis (N = 1), influenza A infection (N = 1), influenza B infection (N = 1), and parainfluenza virus infection (N = 1). Some patients experienced more than one infectious event during the study period.

Reduction of lymphocyte countoccurred in 13 of 30 patients (43.3%) after CYC initiation. Given the more frequent worsening of lymphopenia, factors associated with reduction of lymphocyte countfrom weeks 2 to 4 were additionally evaluated.

Factors associated with infection and reduction of lymphocyte countwere evaluated using modified Poisson regression with robust variance (Table 5). Among baseline leukocyte parameters, higher baseline ALC was associated with a lower risk of infection, whereas baseline WBC were not significantly associated with infection after multiple-comparison correction.

Among CYC pharmacokinetic parameters, higher AUC_{0-24} and dose-normalized AUC were associated with a lower risk of infection. In contrast, higher CL, Vz and $T_{1/2}$ were associated with a higher risk of infection. Cmax and λz were not significantly associated with infection.

Among pharmacogenetic factors, the *GSTT1* null genotype was associated with a higher risk of infection compared with the *GSTT1* present genotype (RR = 3.455, 95% CI 1.345-8.870; $p = 0.010$; adjusted $p = 0.040$). No significant associations with infection were observed for *GSTP1* rs1695 G-allele carrier status, *ALDH3A1* rs2228100 G-allele carrier status, or *ALDH1A1* rs6151031 deletion carrier status.

For reduction of lymphocyte countfrom weeks 2 to 4, higher CL (RR = 1.065, 95% CI 1.026-1.105; $p = 0.001$; adjusted $p = 0.0035$) and higher Vz (RR = 1.002, 95% CI 1.001-1.003; $p < 0.001$; adjusted $p = 0.0035$) were associated with an increased risk. No significant associations with reduction of

lymphocyte countfrom weeks 2 to 4 were observed for the other baseline leukocyte, pharmacokinetic, or pharmacogenetic factors after BH-FDR correction (the data are provided in Table 5).

Exploratory multivariable analyses of factors associated with infection and reduction of lymphocyte count

Given the limited sample size and number of outcome events, parsimonious exploratory multivariable models were used to further evaluate selected factors associated with infection and reduction of lymphocyte countfrom weeks 2 to 4 (Table 6). In the infection model, the *GSTT1* null genotype remained associated with a higher risk of infection after adjustment for baseline ALC (adjusted RR = 2.824, 95% CI 1.087-7.341; $p = 0.033$), whereas higher baseline ALC remained associated with a lower risk of infection after adjustment for *GSTT1* genotype (adjusted RR = 0.873 increase in baseline ALC per 100 cells/μL, 95% CI 0.782-0.976; $p = 0.016$).

In the exploratory model for reduction of lymphocyte countfrom weeks 2 to 4, higher CL remained associated with an increased risk after adjustment for Vz (adjusted RR = 1.084 increase in CL per 1 L/h, 95% CI 1.018-1.155; $p = 0.012$), whereas Vz was no longer significantly associated with reduction of lymphocyte countfrom weeks 2 to 4 (adjusted RR = 0.999 increase in Vz per 1 L, 95% CI 0.998-1.001; $p = 0.322$). The data are provided in Table 6.

Association of Single-Time-Point Plasma CYC Concentrations With AUC_{0-24} and Infection Risk

All evaluated single-time-point plasma CYC concentrations were significantly correlated with AUC_{0-24} (all $p < 0.001$), although the strength of the correlations varied according to sampling time (Figure 1 B-C). The strongest correlation was observed at 6 h (C_6; $R^2 = 0.987$), followed by C_8 ($R^2 = 0.954$), C_4 ($R^2 = 0.936$), C_{12} ($R^2 = 0.858$), C_3 ($R^2 = 0.822$), C_2 ($R^2 = 0.764$), and C_1 ($R^2 = 0.697$). Correlations were weaker at later sampling times, with R^2 values of 0.582 at C_{18} and 0.408 at C_{24}. The strong relationship between C_6 and AUC_{0-24} is illustrated in Figure 1C

In exploratory analyses of subsequent infection risk, higher plasma CYC concentrations at C_4, C_6, C_8, and C_{12} were nominally associated with lower infection risk. After BH-FDR correction across the nine evaluated sampling time points, the associations remained significant at C_6 (RR = 0.908 increase in C_6 per 1,000-ng/mL; 95% CI, 0.848-0.973; BH-FDR-adjusted $p = 0.027$) and C_8 (RR = 0.880 per 1,000-ng/mL increase in C_8; 95% CI, 0.813-0.954; BH-FDR-adjusted $p = 0.018$) (Figure 1D). These findings indicate that C_6 showed both the strongest correlation with AUC_{0-24} and a significant inverse association with subsequent infection risk.

Exploratory discrimination analysis for subsequent infection

To further assess the potential complementary value of baseline ALC and *GSTT1* genotype for identifying patients who subsequently developed infection, an exploratory ROC analysis was performed.

Baseline ALC alone showed an AUC of 0.697 (95% CI 0.510-0.884), whereas *GSTT1* genotype alone showed an AUC of 0.750 (95% CI 0.585-0.915). The combined model incorporating baseline ALC and *GSTT1* genotype showed the highest discrimination, with an AUC of 0.843 (95% CI 0.697-0.988). Compared with baseline ALC alone, addition of *GSTT1* genotype increased the AUC from 0.697 to 0.843 (p = 0.061). These findings suggest potential complementary information from baseline ALC and *GSTT1* genotype for infection-risk discrimination. The data are provided in Table 7.

Clinical Response at Week 24

At week 24, among patients with lupus nephritis, 16 (72.7%) achieved complete response (CR), and 6 (27.3%) achieved partial response (PR) according to the KDIGO criteria. Among the eight patients without lupus nephritis, all achieved remission (100%) according to the DORIS 2021 definition.

Discussion

The baseline characteristics of our cohort reflected a population with highly active and severe SLE, characterized by high SLEDAI-2K scores, frequent major-organ involvement and active lupus nephritis, lymphopenia, and hypocomplementemia. These characteristics are clinically relevant because both SLE-related immune dysfunction and high disease activity may predispose patients to infection, in addition to the effects of immunosuppressive therapy. Previous studies have identified high disease activity, renal involvement, lymphopenia, and low complement levels as factors associated with infection in patients with SLE (5, 31, 42, 43). In particular, Wang et al. identified SLEDAI > 10 and lymphocyte count < 0.8×10^9/L as independent predictors of major infection in newly diagnosed SLE. Furthermore, SLE patients who developed serious infection were more likely to have multiple-organ disease, highlighting the contribution of underlying disease severity in this high-risk treatment setting (44). Thus, these findings suggest that the 40.0% infection rate observed in our cohort likely reflects the combined influence of severe active disease, baseline immune abnormalities, and intensive immunosuppressive therapy, providing important clinical context for interpreting the pharmacokinetic and pharmacogenetic associations with infection identified in our study.

In our cohort, CYC was initiated at the lower end of the conventional NIH dosing range (0.5 g/m^2), reflecting our institutional practice of using a cautious initial dose in patients with active SLE who may be particularly susceptible to infection. Evidence from Asian populations also supports the feasibility of CYC dosing at the lower end of the conventional range; in Korean patients with lupus nephritis, Seong et al. reported that monthly intravenous CYC at 0.5 g/m^2 achieved renal remission or response in 73.1% of patients (45). Nevertheless, despite the relatively conservative initial dosing used in our cohort, infection occurred in 40.0% of patients, suggesting that administered dose alone may not adequately capture individual susceptibility to infection.

Previous studies in SLE and lupus nephritis have suggested that CYC pharmacokinetics may be relevant for clinical outcomes. Joy et al. demonstrated interindividual variability in parent CYC and 4-OHCP

disposition in patients with lupus nephritis and other glomerular diseases, although pharmacokinetic targets for treatment were not established (18). Wang et al. reported that higher CYC exposure was significantly associated with clinical remission in patients with lupus nephritis (19). Similarly, Shu et al. demonstrated that genetically determined differences in CYC 4-hydroxylation were associated with 4-OHCP concentrations, treatment efficacy, and adverse effects in Chinese patients with SLE (46). However, the measurement of 4-OHCP, which is an unstable chemical substance, requires derivatizing agents to stabilize that we cannot obtain in Thailand. Xie et al. reported an inverse relationship between parent CYC and 4-OHCP exposure in patients with hematological malignancies, suggesting that lower parent CYP exposure may, in some patients, reflect greater metabolic conversion to 4-OHCP (47). Since the identification of parent CYC is more stable and practical, we performed the analysis of plasma CYC in SLE patients instead. However, evidence directly linking parent CYC exposure to subsequent infection risk in SLE or lupus nephritis remains limited.

In the present study, intensive pharmacokinetic sampling at 12 time points over 24 hours was performed to characterize parent CYC exposure and derive pharmacokinetic parameters. The concentration-time profiles demonstrated interindividual variability, with marked differences in plasma CYC concentrations across sampling times (Fig. 1A). Given the practical burden of intensive sampling, we further evaluated whether a single plasma concentration could provide a reliable estimate of AUC_{0-24}. Among the evaluated sampling times, the 6-h concentration (C_6) showed the strongest association with AUC_{0-24} ($R^2 = 0.987$), followed by C_8 ($R^2 = 0.954$) and C_4 ($R^2 = 0.936$). Our findings suggest that C_6 may represent a practical candidate for estimating parent CYC AUC_{0-24} while substantially reducing the sampling burden associated with full pharmacokinetic profiling.

Surprisingly, we observed that higher 6- and 8-hour parent CYC concentrations were associated with lower subsequent infection risk. Greater parent-drug clearance could potentially reflect increased metabolic conversion to active metabolites and consequently greater biologically effective exposure. Nevertheless, this interpretation remains speculative because 4-OHCP and downstream metabolites were not measured in our study. Because CYC is a prodrug, these inverse associations with parent-drug exposure should not be interpreted as indicating a protective effect of higher parent CYC exposure. Rather, the previously reported inverse relationship between parent CYC and 4-OHCP exposure provides a possible explanation whereby lower parent-drug AUC or greater clearance may reflect greater metabolic conversion to active metabolites (47), potentially contributing to greater immunosuppressive effects and susceptibility to infection. As active metabolites were not measured in our study, this proposed mechanism remains speculative and requires further investigation.

Higher CL and Vz were associated with greater risk of infection and reduction of lymphocyte count from baseline. These findings suggest that parent CYC clearance may provide additional information regarding infection susceptibility and lymphocyte count reduction beyond administered dose and baseline immune status.

Genetic variation in the CYC detoxification pathways, including *GSTT1* and *GSTM1* deletion polymorphisms, *GSTP1* rs1695, *ALDH3A1* rs2228100 (c.985C > G), and the A*LDH1A1* rs6151031 17-bp insertion/deletion, has been investigated as a potential source of variability in CYC disposition and toxicity (15, 48). Kumaraswami et al. investigated both activation and detoxification pathway genes, including *CYP2C9*, *CYP2C19*, *CYP3A4/CYP3A5*, *GSTT1*, *GSTM1*, and *GSTP1*, in patients with lupus nephritis. Their findings supported contributions of CYP-related variation to CYC treatment, while *GSTP1* contributed through gene-gene interactions associated with treatment response (49).

Glutathione S-transferase (GST) enzymes, including GSTT1, GSTM1, and GSTP1, participate in phase II detoxification by catalyzing glutathione conjugation of CYC and its reactive metabolites, phosphoramide mustard, (48, 50). The *GSTT1* and *GSTM1* null genotypes result in loss of the corresponding enzyme activity and may therefore reduce glutathione-mediated detoxification capacity (25, 50), whereas the *GSTP1* rs1695 (c.313A > G; Ile105Val) polymorphism alters GSTP1 catalytic activity and has been investigated in relation to CYC efficacy and toxicity (20, 21, 26). Notably, the *GSTT1* no-function deletion has been reported at a high frequency in the Thai population; Wankaew et al. reported a frequency of approximately 60%, although this estimate should be interpreted cautiously because of low sequencing read depth for *GSTT1* (28). In our cohort, the *GSTT1* null genotype was detected in 36.7% of patients and was associated with a substantially increased risk of infection, supporting the potential clinical relevance of this genetic background in Thai patients receiving CYC. The genes evaluated in our study were predominantly involved in downstream detoxification rather than initial CYC bioactivation. GST enzymes, including GSTT1, GSTM1, and GSTP1, participate in glutathione-mediated detoxification of CYC-related metabolites, whereas ALDH1A1 and ALDH3A1 contribute to oxidative detoxification of aldophosphamide and related metabolites (48).

In our study, the *GSTT1* null genotype showed associations with lower parent CYC AUC_{0-24} and dose-normalized AUC and higher CL; however, these associations did not remain significant after multiple-comparison correction and should therefore be interpreted as exploratory. Genetic variation in downstream detoxification pathways may have a less direct influence on parent CYC pharmacokinetics and may affect downstream metabolite disposition or toxicity instead. In addition, Ekhart et al. evaluated variants in multiple CYC activation and detoxification-related genes, including *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP3A5*, *GSTA1*, *GSTP1*, *ALDH1A1*, and *ALDH3A1*, and found that none of the investigated variants significantly explained interindividual variability in parent CYC or 4-OHCP clearance (15). Thus, our findings support the potential clinical genetic test of the *GSTT1* null genotype for infection-risk stratification during CYC therapy and warrant confirmation in independent cohorts.

Loss of GSTT1 activity may reduce overall detoxification capacity, potentially increasing susceptibility to CYC-related toxicity (48, 50). Patients with the *GSTT1* null genotype had a 3.46-fold higher risk of infection in the univariable analysis, and this association remained significant after multiple-comparison correction. The association between the *GSTT1* null genotype and infection represents an additional pharmacogenetic finding of potential clinical implementation.

While higher baseline ALC remained associated with lower infection risk (adjusted RR = 0.873 increase in baseline ALC, per 100 cells/μL, 95% CI 0.782–0.976), in the exploratory multivariable analysis, the *GSTT1* null genotype remained associated with infection after adjustment for baseline ALC (adjusted RR = 2.824, 95% CI 1.087–7.341). These findings suggest that *GSTT1* genotype could be use as a candidate genetic testing regarding infection susceptibility beyond baseline lymphocyte status.

The clinical relevance of *GST* polymorphisms may also differ across populations. Audemard-Verger et al. reported that the *GSTM1* null genotype, rather than the *GSTT1* null genotype, was independently associated with CYC-related adverse drug reactions in a predominantly Caucasian cohort of patients with proliferative lupus nephritis (27). In contrast, the *GSTM1* null genotype was not detected in our Thai SLE cohort, whereas the *GSTT1* null genotype was present in 36.7% of patients and was associated with increased infection risk. A population-based pharmacogenomic study in Thailand has also reported a high frequency of *GSTT1* deletion (28). These findings raise the possibility that population differences in the distribution of *GST* polymorphisms may contribute to variability in the pharmacogenetic determinants of CYC-related toxicity.

Higher CL and Vz were associated with reduction of lymphocyte count during weeks 2–4 after multiple-comparison correction. In the exploratory multivariable analysis, only clearance remained associated with reduction of lymphocyte count after adjustment for volume of distribution (adjusted RR = 1.084 increase in CL per 1 L/h, 95% CI 1.018–1.155). Notably, higher clearance of parent CYC was associated with both reduction of lymphocyte count and subsequent infection.

Of particular interest, the *GSTT1* null genotype remained associated with infection after adjustment for baseline ALC, suggesting that genetic information may provide complementary information beyond baseline lymphocyte status. This possibility was further supported by the exploratory discrimination analysis, in which the AUC of combination of baseline ALC and *GSTT1* genotype showed greater discrimination for subsequent infection than *GSTT1* null genotype and baseline ALC alone (0.843, 0.750, and 0.697, respectively). Nevertheless, the observed improvement in discrimination provides a rationale for evaluating whether integration of clinical and pharmacogenetic information can improve infection-risk stratification in larger independent cohorts.

Although the present cohort was modest in size, several associations remained significant after multiple-comparison correction, supporting further investigation of these signals. Future studies should determine the association between *GSTT1* genotype and infection in independent populations receiving cyclophosphamide, including patients with other autoimmune diseases, solid tumors, and those undergoing hematopoietic stem-cell transplantation.

Conclusion

Clearance of parent cyclophosphamide was associated with infection and early reduction of lymphocyte count, while C_6 correlated with infection and showed a strong association with AUC_{0-24}, supporting its potential as a simplified measure of parent cyclophosphamide exposure. Low baseline lymphocyte

count and *GSTT1* null genotype were also strongly associated with infection risk during intravenous cyclophosphamide induction therapy in Thai patients with active SLE. The initial screening of these parameters before induction therapy with IV CYC in SLE patients may provide the precision treatment and monitoring to reduce the serious complication of infection and improve the disease outcomes.

Material and method

Study Design

This prospective repeated-measures observational study was conducted at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The study was designed to characterize CYC pharmacokinetics and evaluate the associations of lymphocyte count, pharmacokinetic parameters, and pharmacogenetic factors with infection in patients with active SLE receiving intravenous CYC induction therapy. Eligible patients were enrolled between May 2020 and December 2024 and followed throughout the 24-week induction treatment period.

Participants

Patients were eligible for enrollment if they met all of the following criteria: (1) age ≥ 18 years; (2) diagnosis of SLE according to either the 1997 American College of Rheumatology (ACR) revised classification criteria (32) or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria (33); (3) severe disease flare or active disease requiring induction therapy, as determined by a rheumatologist; and (4) provision of written informed consent.

Patients were excluded if they had (1) a coexisting autoimmune disease (overlap syndrome); (2) active systemic infection at enrollment; (3) severe renal impairment, defined as creatinine clearance < 10 mL/min, or required renal replacement therapy; (4) severe hepatic dysfunction; or (5) current or previous malignancy.

All participants received monthly intravenous CYC for six treatment cycles as induction therapy for active SLE. The intended CYC dose was 0.5-1.0 g/m^2 per cycle, consistent with established high-dose intravenous CYC regimens (34, 35); however, the administered dose was individualized according to clinical judgment and could be reduced in patients with impaired renal function (36, 37).

Clinical Data Collection and Outcomes

Demographic, clinical, treatment, and laboratory data were collected prospectively from medical records and the electronic hospital database of the Faculty of Medicine Ramathibodi Hospital. Data were recorded at baseline (week 0) and during follow-up at weeks 2, 4, 8, 12, 16, 20, and 24.

Demographic and clinical variables included age, sex, body weight, height, disease duration, organ involvement, comorbidities, and disease activity assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (38). Treatment-related variables included CYC dose, concomitant immunosuppressive therapy, corticosteroid therapy, and concomitant medications with the potential to influence CYC pharmacokinetics through drug–drug interactions.

Infectious events and hematologic abnormalities were prospectively monitored throughout the 24-week study period. Infection was defined as a clinically diagnosed or microbiologically confirmed infectious event occurring after initiation of CYC treatment within 1 month and requiring more than 3 days of systemic antimicrobial therapy (antibacterial, antiviral, or antifungal) during hospitalization, based on compatible clinical manifestations supported by microbiological, laboratory, and/or imaging findings, as appropriate.

Hematologic abnormalities were assessed using absolute lymphocyte count (ALC). Lymphopenia was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (39), as follows: Grade 1, ALC 800 to 1,000 cells/mm^3; Grade 2, 500 to < 800 cells/mm^3; Grade 3, 200 to < 500 cells/mm^3; and Grade 4, < 200 cells/mm^3. Early lymphocyte dynamics were evaluated between weeks 2 and 4, with reduction of lymphocyte count defined as an increase in CTCAE lymphopenia grade from baseline to week 2 or 4.

Clinical response was assessed at week 24. For patients with LN, complete response (CR) and partial response (PR) were defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2024 Clinical Practice Guideline (6). For patients without LN, remission was assessed according to the Definitions of Remission in SLE (DORIS) 2021 framework (40). Disease activity was assessed using SLEDAI-2K at baseline and weeks 2, 4, 8, 12, 16, 20, and 24.

Pharmacokinetic Sampling and Bioanalytical Quantification of CYC

For pharmacokinetic analysis, venous blood samples were drawn before CYC administration (0 h) and at 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after initiation of the first dose of intravenous infusion. Blood samples were collected into EDTA tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was separated and stored at -80°C until analysis. Plasma CYC concentrations were quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Patient plasma samples were routinely diluted four-fold before extraction to bring CYC concentrations within the validated calibration range. Details of sample preparation, chromatographic and mass spectrometric conditions, calibration, quality control, and assay validation are provided in the Supplementary Methods.

Pharmacokinetic Analysis

CYC was administered as a 1-h intravenous infusion. Pharmacokinetic parameters were determined from plasma CYC concentration-time data. Concentrations obtained before infusion and at 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after the start of infusion were designated C_0, $C_{0.5}$, $C_{0.75}$, C_1, C_2, C_3, C_4, C_6, C_8, C_{12}, C_{18}, and C_{24}, respectively. The area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24}) was calculated using the linear logarithmic trapezoidal method, and the maximum observed plasma concentration (Cmax) was obtained directly from the concentration-time profile.

The terminal elimination rate constant (λz) was estimated by linear regression of the natural logarithm of plasma CYC concentrations versus time using the 12-, 18-, and 24-h sampling points (C_{12}, C_{18}, and C_{24}) with λz calculated as the negative slope of the regression line. Goodness of fit of the terminal log-linear regression was assessed using the coefficient of determination (R^2). The terminal elimination half-life ($T_{1/2}$) was calculated as 0.693/λz. Total systemic clearance (CL) was calculated as the administered CYC dose divided by AUC_{0-24}, and the apparent volume of distribution associated with the terminal phase (Vz) was calculated as CL/λz. Dose-normalized AUC was calculated as AUC_{0-24} divided by the administered CYC dose to account for interindividual differences in dose (41).

For the limited-sampling analysis, only concentrations obtained at or after completion of the 1-h infusion (C_1-C_{24}) were evaluated as candidate sampling points. $C_{0.5}$ and $C_{0.75}$ were excluded because they were obtained during the infusion. Associations between individual single-time-point concentrations and AUC_{0-24} were evaluated to identify potential limited-sampling markers of CYC systemic exposure.

Pharmacogenetic Analysis

Peripheral blood samples were collected at baseline (week 0) in EDTA tubes for pharmacogenetic analysis. Genomic DNA was extracted from buffy coat samples using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA integrity was evaluated by 1% agarose gel electrophoresis.

Polymerase chain reaction (PCR) amplification was performed using gene-specific primers with a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). *GSTP1* rs1695 and *ALDH3A1* rs2228100, as well as the *ALDH1A1* rs6151031 17-bp insertion/deletion variant, were determined by Sanger sequencing. *GSTT1* and *GSTM1* genotypes were determined in all 30 patients using multiplex PCR followed by agarose gel electrophoresis, with β-globin included as an internal amplification control. The presence of the corresponding gene-specific amplification product was classified as the gene-present genotype, whereas absence of the gene-specific product in the presence of successful β-globin amplification was classified as the null genotype. For additional confirmation of the multiplex PCR results, five selected patients with a *GSTT1*-present genotype underwent Sanger sequencing. Sequence chromatograms were compared with reference sequences for genotype assignment and confirmation.

Primer sequences and detailed PCR conditions are provided in Supplementary Tables 6 and 7, respectively. Representative Sanger sequencing chromatograms are provided in Supplementary Figure S1, and representative multiplex PCR gel electrophoresis for *GSTT1* and *GSTM1* genotyping is shown in Supplementary Figure S2.

Statistical Analysis

All statistical analyses were performed using Stata version 18.0 (StataCorp LLC, College Station, TX, USA). Graphs were generated using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test. Normally distributed variables are presented as mean ± standard deviation (SD), whereas non-normally distributed variables are presented as median with interquartile range (IQR). Categorical variables are presented as frequencies and percentages.

The associations of the occurrence of infections with baseline leukocyte counts (WBC, and ALC), CYC pharmacokinetic parameters (AUC_{0-24}, Dose-normalized AUC, Cmax, CL, Vz, λz, and $T_{1/2}$), and pharmacogenetic factors (*GSTT1, GSTP1, ALDH3A1*, and *ALDH1A1*) were evaluated by calculating risk ratios (RRs) with 95% CIs using modified Poisson regression with robust variance. To account for multiple comparisons, *p*-values were adjusted using the Benjamini-Hochberg false discovery rate (BH-FDR) procedure separately within each prespecified domain. Both nominal and BH-FDR-adjusted *p*-values are reported. Given the limited sample size and number of infection events, multivariable analyses were restricted to parsimonious exploratory models incorporating clinically and biologically relevant variables.

Associations between pharmacogenetic variants and continuous pharmacokinetic parameters were evaluated using linear regression and are reported as regression coefficients (β) with 95% confidence intervals (CIs). Associations between individual single-time-point plasma CYC concentrations and AUC_{0-24} were evaluated using simple linear regression, with the coefficient of determination (R^2) used to assess the proportion of variability in AUC_{0-24} explained by each concentration.

Associations between single-time-point plasma CYC concentrations and subsequent infection risk were evaluated separately at 1, 2, 3, 4, 6, 8, 12, 18, and 24 h using modified Poisson regression with robust variance. Concentrations were scaled per 1,000 ng/mL, and associations are reported as RRs with 95% CIs. To account for multiple testing across the nine sampling time points, *p* values were adjusted using the BH-FDR procedure. Multivariate analyses were performed with logistic regression. To further explore the potential complementary value of baseline ALC and *GSTT1* genotype for discrimination of subsequent infection, an exploratory receiver operating characteristic (ROC) analysis was performed. Logistic regression models were fitted using baseline ALC alone, *GSTT1* genotype alone, and the combination of baseline ALC and *GSTT1* genotype, and predicted probabilities from each model were used to construct ROC curves and estimate the area under the ROC curve (AUC) with 95% confidence

intervals. The AUC of the combined model was compared with that of the baseline ALC-only model. This analysis was considered exploratory and was not intended to develop or validate a clinical prediction model. All statistical tests were two-sided. A *p*-values of less than 0.05 were considered statistically significant.

Declarations

Acknowledgements

The authors thank all patients with SLE who participated in this study and the clinical staff of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, for their support in patient care and clinical data management. The authors gratefully acknowledge Waricha Loknimith for assistance with blood sample collection, and Wararat Chiangjong and Rawiporn Tiyasirichokchai from the Central Laboratory, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, for their technical support with LC-MS/MS analysis and pharmacogenetic testing. The authors also thank Kanokporn Ngamsawangrungrot for guidance and support in manuscript preparation.

Author contributions

W.Y. contributed to development of the research idea, study design, patient enrollment, data collection, pharmacokinetic and pharmacogenetic analysis and interpretation, statistical analysis, interpretation of results, and preparation of the initial draft of the manuscript.

S.V. contributed to study design, pharmacokinetic interpretation, statistical analysis, interpretation of results, and manuscript review and editing.

P.S. contributed to LC–MS/MS method development and validation and manuscript preparation.
P.N. contributed to patient enrollment and interpretation of SLE clinical outcomes.
T.K. contributed to patient enrollment and interpretation of SLE clinical outcomes.

P.O. contributed to patient enrollment and interpretation of SLE clinical outcomes.

C.O. contributed to patient enrollment and interpretation of SLE clinical outcomes.
S.P. contributed to LC–MS/MS method development and validation.
T.L. contributed to pharmacogenetic analysis and interpretation.
S.N. contributed to pharmacogenetic analysis, interpretation, and validation.

T.S. contributed to patient enrollment.
O.M. contributed to statistical analysis.

P.P. contributed to conceptualization and development of the research idea, study design, patient enrollment, clinical supervision, interpretation of SLE clinical outcomes, statistical analysis, interpretation of results, funding acquisition, and manuscript review and editing.

All authors reviewed and approved the submitted manuscript.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the Faculty of Medicine Ramathibodi Hospital (CF_65001), Mahidol University (Basic Research Fund: the fiscal year 2022), and Ramathibodi Foundation for Allergy, Immunology, and Rheumatology Fund (3205010007).

Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (COA. MURA2020/648). Written informed consent was obtained from all participants before enrollment.

Data Availability

The Sanger sequencing data generated in this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under project accession number **PRJEB127061** and are available through the ENA Browser (https://www.ebi.ac.uk/ena/browser/ view/PRJEB127061). The remaining datasets generated and/or analysed during the current study are not publicly available because they contain participant-level clinical and pharmacokinetic data and are subject to ethical and participant-confidentiality requirements, but de-identified data may be made available from the corresponding author upon reasonable request and subject to applicable ethical approval.

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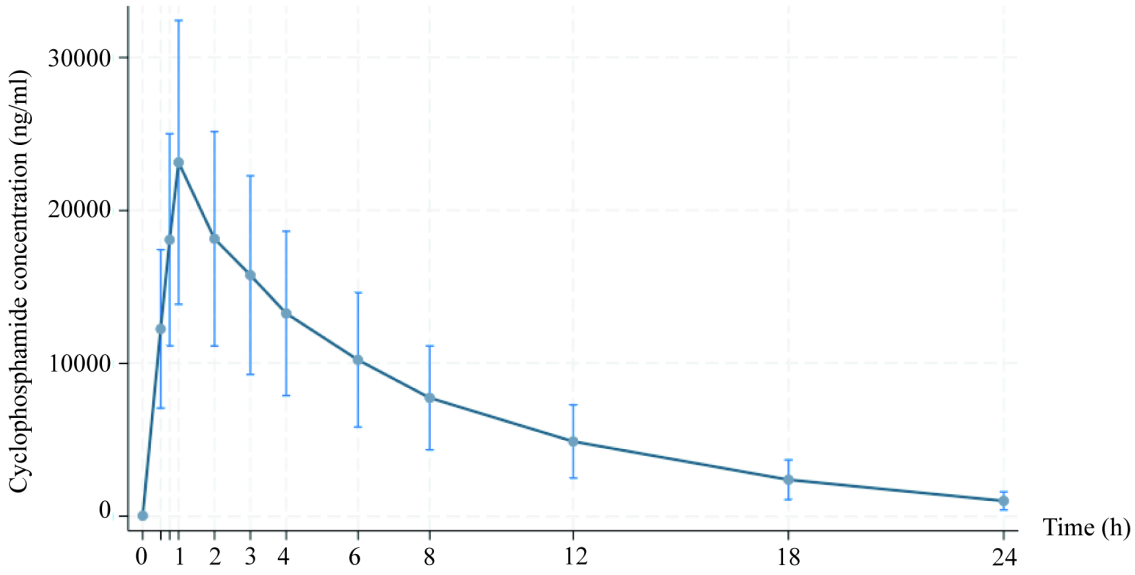
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Tables

Tables 1 to 7 are available in the Supplementary Files section.

Figures

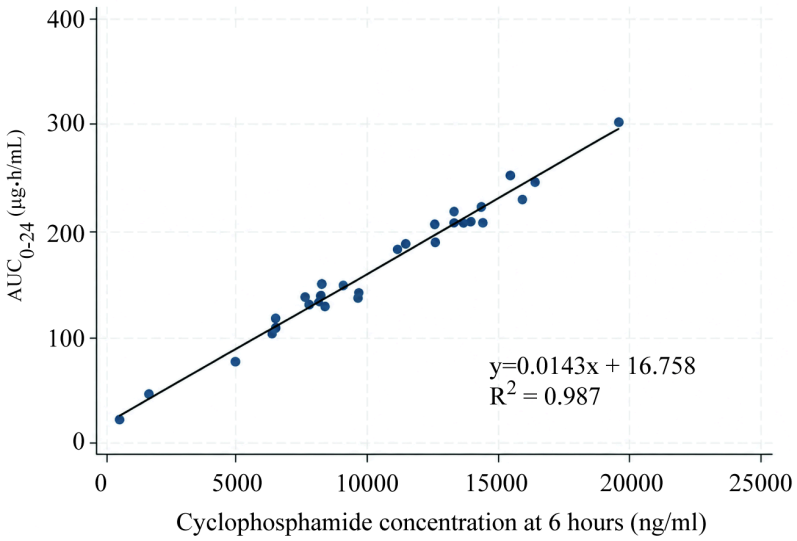
A



B

Timepoint (h)	R^2
C_1 (1 h)	0.697
C_2 (2 h)	0.764
C_3 (3 h)	0.822
C_4 (4 h)	0.936
C_6 (6 h)	0.987
C_8 (8 h)	0.954
C_{12} (12 h)	0.858
C_{18} (18 h)	0.582
C_{24} (24 h)	0.408

C



D

Concentration	RR per 1,000 ng/mL	95% CI	*p* value	BH-FDR *p* value Adj
C_6	0.908	0.848-0.973	0.006	0.027
C_8	0.880	0.813-0.954	0.002	0.018

Figure 1

(A) Mean plasma cyclophosphamide concentration–time profile over 24 h following the first intravenous cyclophosphamide dose in Thai patients with active SLE.

(B) Correlations between single-time-point plasma cyclophosphamide concentrations and AUC_{0-24} at 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after the start of cyclophosphamide infusion.

(C) Relationship between the plasma cyclophosphamide concentration measured 6 h after the start of intravenous cyclophosphamide infusion (C_6) and AUC_{0-24}. The solid line represents the fitted linear regression model ($R^2 = 0.987$, $p < 0.001$).

(D) Associations of 6- and 8-h plasma cyclophosphamide concentrations (C_6 and C_8) with subsequent infection risk. Risk ratios (RRs) are reported per 1,000-ng/mL increase in plasma cyclophosphamide concentration and were estimated using modified Poisson regression with robust variance. *p* values were adjusted using the BH-FDR procedure across the nine evaluated sampling time points.

Abbreviations: AUC_{0-24}, area under the plasma concentration-time curve from 0 to 24 h; BH-FDR, Benjamini-Hochberg false discovery rate; CI, confidence interval; C_1, C_2, C_3, C_4, C_6, C_8, C_{12}, C_{18}, and C_{24}, plasma cyclophosphamide concentrations measured 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after the start of infusion, respectively; RR, risk ratio.

Supplementary Files

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