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Early measurement of ST2 and REG3 α has a prognostic impact in haploidentical transplantation with post-transplant cyclophosphamide

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Author contributions

M.F-S., M.G-A., L.L-C., L.A.M. and M.A. conceived and designed the study. L.L-C. obtained financial support. M.G.A., R.M. A.I.S. and I.S-V. processed the samples. M.G.A., L.A.M. and F.J.G-E. analysed and interpreted protein analysis. L.A.C.S and M.F-S. analysed and interpreted statistical data. A.G.B., R.G.M., A.A., M.L-P., M.B., A.C., M.L.M-M., O.L-V., S.M., A.A.M., E.P., M.C., F.S-G., L.V. and R.G-S. were responsible for the clinical management of the patients and provided clinical data and study materials. M.G-A., M.F-S. and L.A.C.S. wrote the first draft of the manuscript. M.A. and L.L-C. re-wrote the manuscript and gave the final approval for the publication. All authors were involved in the review and acceptance of the final version of the manuscript.

Data availability statement

The datasets used and/or analysed during the current study are available from the first and corresponding author on reasonable request.

Artificial intelligence: Artificial intelligence-based tools were used solely for checking grammar, spelling, and language. No advanced artificial intelligence

(aAI) tools were used for drafting, revising, formatting, data analysis, interpretation, or reference selection.

ABSTRACT

Research on predictive biomarkers in haploidentical SCT (haplo-HSCT) with post-transplant cyclophosphamide (PTCy) remains limited, particularly regarding the critical first week post-infusion. We prospectively evaluated serum ST2, REG3 α , and the MAGIC algorithm pre-conditioning and at days 0, +7, +14, and +21 in 81 adult patients undergoing haplo-HSCT with PTCy.

ST2 and REG3 α surged significantly from pre-conditioning to day 0 ($p=0.031$ and $p<0.001$). At day 0, elevated ST2 independently predicted inferior OS ($p=0.016$) and EFS ($p=0.002$), while REG3 α predicted inferior GRFS ($p=0.041$) in multivariate analysis. Post-infusion, ST2 independently portended higher NRM at days +7 and +14 ($p=0.009$ and $p=0.011$), inferior OS across days +7 ($p=0.001$), +14 ($p=0.005$), and +21 ($p=0.019$), EFS at days +7 ($p<0.001$), +14 ($p=0.002$), and +21 ($p=0.019$) and grade II–IV aGVHD at days +14 ($p=0.013$) and +21 ($p<0.001$). REG3 α independently predicted NRM at days +14 ($p=0.028$) and +21 ($p=0.023$), lower OS at days +7 ($p=0.045$), +14 ($p=0.007$), and +21 ($p=0.021$) and inferior GRFS at days +14 ($p=0.007$) and +21 ($p=0.006$). Longitudinal ST2 trajectory models significantly discriminated NRM ($p=0.006$), OS ($p=0.031$), EFS ($p=0.046$), and aGVHD ($p=0.014$). Multivariable models confirmed the MAGIC algorithm as an independent predictor of EFS at days 0 ($p=0.013$) and +14 ($p=0.043$) and GRFS at day +14 ($p=0.025$).

This study confirms the relevance of ST2 and REG3 α in haplo-SCT and demonstrates the prognostic impact of pre-infusion and day +7 levels across multiple endpoints, establishing the MAGIC algorithm's utility in haplo-SCT with PTCy.

INTRODUCTION

HLA-haploidentical hematopoietic stem cell transplantation (haplo-HSCT) with high-dose post-transplant cyclophosphamide (PTCy) has become an increasingly utilized strategy for patients lacking an HLA-matched sibling or unrelated donor^{1–5}. PTCy has proven effective in preventing graft-versus-host disease (GVHD), thereby enabling successful transplantation from partially matched donors^{3,6}. Despite these advances, the 2-year non-relapse mortality (NRM) after haplo-HSCT with PTCy remains approximately 25%, comparable to historical results in HLA-matched donor transplants, and acute GVHD (aGVHD) continues to be a major complication with an incidence of grades II to IV ranging from 21% to 47%^{7–11}.

Although several pre-transplant prognostic scoring systems exist to identify patients at higher risk of adverse outcomes, biomarkers offer additional potential for the early prediction of transplant-related complications. They may guide clinical decision-making, refine follow-up in high-risk patients, and offer insight into the biological mechanisms underlying allo-HSCT outcomes¹². Different serum biomarkers have been investigated in recent years to predict complications related to allo-HSCT, improve outcomes and to identify patients with poor prognosis¹³.

These biomarkers —mainly cytokines involved in inflammatory responses, tissue damage, or endothelial injury— have consistently been found to be associated with clinical outcomes after allo-HSCT^{13–19}. Among these, suppression of tumorigenicity 2 (ST2) and regenerating islet-derived 3-alpha (REG3α) are the most extensively studied. ST2, a member of the interleukin-1 receptor family whose ligand is IL-33, is released by several cell types in

response to inflammatory stimuli, promoting a Th1-mediated immune response. In the transplant setting, ST2 is associated with gastrointestinal crypt damage in GvHD and with endothelial complications such as transplantation-associated thrombotic microangiopathy (TA-TMA)^{20–24}. REG3 α , an antimicrobial peptide produced by intestinal Paneth cells, reaches the bloodstream when intestinal crypts are damaged by GVHD or other enteropathies. Both biomarkers are strong and independent predictors of aGVHD severity and NRM, especially when measured at day +14 post-HSCT or at GvHD onset^{13,16,20,25,26}.

The Mount Sinai Acute GVHD International Consortium (MAGIC) validated an algorithm based on ST2 and REG3 α levels, which, when measured on day +7 post-HSCT, stratified patients into high- and low-risk groups for severe aGVHD, NRM, and overall survival (OS), and when applied at the time of GVHD onset, the algorithm further identified patients at high risk of NRM and poor response to corticosteroid therapy²⁷. The algorithm has subsequently been used at different time points to predict outcomes including NRM, severe aGVHD, endothelial injury, and corticosteroid response^{28,29}. However, these studies were conducted mainly in HLA-matched or mismatched unrelated donor transplantation, or evaluated haploidentical recipients within heterogeneous transplant cohorts rather than evaluated as a dedicated haploidentical PTCy population^{16,17,20,25,27,30–32}.

These pathways may differ in haplo-HSCT with PTCy due to the singularities of this platform. To date, only two studies have specifically evaluated ST2 and REG3 α in dedicated haplo-HSCT cohorts receiving PTCy^{22,33}. Kanakry *et al.* evaluated seven different biomarkers in a mixed cohort of haplo-HSCT and HLA-matched HSCT with PTCy, myeloablative conditioning (MAC) and bone

marrow graft observing that higher ST2 and REG3 α levels at day +30 post-HSCT were associated with higher NRM at three months³³. Solan et al. assessed these biomarkers in patients undergoing MAC and reduced-intensity (RIC) haplo-HSCT with PTCy using peripheral blood stem cells. ST2 levels at day +30 post-HSCT were associated with higher NRM and increased aGVHD II–IV in the RIC cohort, while REG3 α levels at day +30 post-HSCT correlated with higher aGVHD II–IV rates in both cohorts²². Importantly, neither study examined biomarkers before day +15 or assessed the MAGIC algorithm in this setting.

The aim of this study was to evaluate the role of ST2 and REG3 α —individually and within the MAGIC algorithm—measured prior to conditioning and at early time points (days 0 (pre-infusion), +7, +14, and +21) after haplo-HSCT with PTCy in a homogeneous cohort. Furthermore, we utilized longitudinal linear mixed-effects modelling to characterize dynamic biomarker trajectories over time. We hypothesized that earlier detection of elevated biomarker levels could identify patients at increased risk of adverse outcomes.

METHODS

Study population

Serial serum samples were prospectively collected from 81 patients (>18 years) undergoing haplo-HSCT with PTCy at the University Hospital of Salamanca between 2012 and 2020 (follow-up cutoff: December 31, 2021). A sensitivity analysis confirmed no significant differences in baseline characteristics between patients with and without available samples during the recruiting period of time, indicating no major selection bias (Tables S1-S2).

Patients received myeloablative (MAC), reduced-Intensity (RIC) or sequential conditioning. All received mycophenolate mofetil (MMF) and calcineurin inhibitor (CNI).

MAC consisted of FluBu-MAC (busulfan 3.2 mg/kg/day, days -4 to -2; fludarabine 30 mg/m²/day, days -6 to -2; cyclophosphamide 14.5 mg/kg/day, days -6 and -5) or TBF-MAC (thiotepa 5 mg/kg/day, days -6 to -5; busulfan 3.2 mg/kg/day, days -4 to -2; fludarabine 50 mg/m²/day, days -4 to -2) following Raiola *et al*³⁴. RIC was used in patients ≥60 years or with comorbidities, prior transplantation, or specific diagnoses.

RIC regimens included FluBu-RIC (fludarabine 30 mg/m²/day, days -6 to -2; cyclophosphamide 14.5 mg/kg/day, days -6 and -5; busulfan 3.2 mg/kg/day, days -4 to -3), Flu-Cy-TBI (fludarabine 30 mg/m²/day, days -6 to -2; cyclophosphamide 14.5 mg/kg/day, days -6 and -5; TBI 2 Gy on day -1), and TBF-RIC (thiotepa 5 mg/kg/day, days -6 to -5; busulfan 3.2 mg/kg/day, days -4 to -3; fludarabine 50 mg/m²/day, days -4 to -2) a modification of Raiola *et al*.³⁴.

GVHD prophylaxis consisted of IV Cy (50 mg/kg/d) on days +3 and +4, followed by CNI (cyclosporine or tacrolimus) and MMF on day +5.

The study was approved by the ethics committee and conducted according to the Declaration of Helsinki. Written informed consent included HSCT and biomarker analyses.

Sample collection, biomarker analysis, and statistical methods

Serum samples from 81 patients were collected prior to conditioning and on days 0 (pre-infusion), +7, +14, and +21 post-HSCT, centrifuged (1,800 × g, 10 min), and stored at -80°C. ST2 and REG3α concentrations were measured in

duplicate using the Luminex X-MAP platform (R&D Systems) and converted to pg/mL using 5-parameter logistic standard curves fitted by nonlinear least squares.

ST2 and REG3 α concentrations were log2-transformed. Between-group levels were compared using Wilcoxon-Mann-Whitney tests, and paired pre-conditioning and day-0 measurements using two-sided Wilcoxon signed-rank tests with Benjamini-Hochberg correction. Longitudinal trajectories through day +21 were evaluated using linear mixed-effects models, including time point, clinical outcome, and their interaction as fixed effects and patient as a random intercept; the time-by-outcome interaction was the primary test of differential trajectories. Estimated marginal means were obtained using emmeans.

High/low thresholds for ST2 and REG3 α were determined per endpoint and time point using maximally selected rank statistics (CutoffFinder³⁵; ≥ 10 patients/group; Table S3). The threshold yielding the strongest separation according to the Cox score test was selected. Outcome-specific thresholds were considered exploratory and require external validation. For competing-risk endpoints, thresholds were derived from cause-specific analysis and subsequently applied unchanged to cumulative-incidence (Table S3). The MAGIC Algorithm Probability was classified using the previously validated 0.16 threshold described by Hartwell et al ²⁷.

For non-competing risks (OS, event-free survival (EFS), and GVHD-free, relapse-free survival (GRFS) survival was estimated using Kaplan–Meier methods, comparison between groups with log-rank tests, and univariable and multivariable analysis with Cox proportional-hazards models (associations are reported as hazard ratios (HRs) with 95% confidence intervals (CIs), using

Efron's approximation for tied event times. For aGVHD, NRM, and relapse, cumulative incidence was estimated using the Aalen–Johansen estimator and compared between groups using Gray's test, accounting for the corresponding competing events. Fine–Gray models were used, where applicable, to estimate subdistribution hazard ratios (SHRs) with 95% CIs, and cumulative-incidence plots were generated. Multivariable models included biomarkers and clinically relevant or significant variables from univariable models and all analysed endpoints and time points were reported irrespective of statistical significance. All tests were two-sided, with $P < 0.05$ considered statistically significant. Analyses were performed in R v4.5.1.

RESULTS

Patient and transplant characteristics

The clinical and transplant characteristics of the 81 patients included in the biomarker analysis are detailed in Table 1.

The median recipient and donor ages were 57 (18-70) and 36 (14-75) years, respectively. The most common indications for haplo-HSCT were acute myeloid leukaemia (43.3%), Hodgkin's lymphoma (16.0%), non-Hodgkin lymphoma (14.8%) and myelodysplastic syndrome (13.6%). A total of 22 patients (27.2%) had an HCT-CI ≥ 3 ; 13 patients (16.1%) had a high or very high Disease Risk Index (DRI); and 27 patients (33.3%) had previously undergone an autologous or allo-HSCT. The majority of patients received reduced-intensity conditioning (RIC; 70.4%), followed by myeloablative conditioning (MAC; 25.9%). Peripheral blood was the stem-cell source employed in all cases. GVHD prophylaxis was based on PTCy combined with MMF and a calcineurin inhibitor.

Overall outcomes

The median follow-up of the alive patients was 52 months (range, 14-89). The cumulative incidence of grade II-IV and III-IV aGVHD at day +180 was 65.3% (95% CI, 54.7–75.9), and 14.3% (95% CI, 6.5–22.1), respectively, with a median onset of aGVHD II-IV of 29 days (range, 14-138), and with 75% of patients developing it after day +21. The 2-year cumulative incidence of cGvHD was 39.6% (95% CI, 26.7–52.5) and moderate-severe cGvHD 14.7% (95% CI, 5.7–23.7).

The +180 OS was 76.5% (95% CI, 67.3–85.7), NRM 17.5%, (95% CI, 9.1–25.9), EFS 72.8% (95% CI, 63.2–82.4) and relapse incidence was 10.0% (95% CI, 2.9–17.1). GRFS at 2 years was 49.2% (95% CI, 38.2 – 60.2). All key clinical outcomes, including detailed confidence intervals at multiple time points, are summarized in Table S4.

A total of 35 patients died from NRM. The most relevant causes were infections (n=14) and aGVHD (n=11). Other causes included haemorrhage (n=4, three of which were associated with primary graft failure), sinusoidal obstruction syndrome (SOS) (n=4), and other less frequent events such as secondary neoplasm (n=2) and haemophagocytic syndrome (n=1).

Impact of pre-conditioning and pre-infusion ST2 and REG3 α levels in transplant outcomes

To evaluate early biomarker dynamics prior to graft infusion, we first analysed paired baseline (pre-conditioning) and day 0 (pre-infusion) serum samples. Both REG3 α and ST2 levels increased significantly from pre-conditioning to day 0 (REG3 α $p<0.001$ and ST2 $p=0.031$). This early elevation

may reflect acute tissue injury occurring during the conditioning period prior to stem cell infusion. Interestingly, when analysing whether this early biomarker increase was associated with transplant-related variables, including conditioning, it did not significantly differ according to conditioning intensity (MAC vs. RIC; $p=0.150$ for REG3 α , $p=1.000$ for ST2) or any other evaluated transplant-related clinical variable. (Figure S1 and Supplementary Table S5).

We then analysed the association between pre-infusion (day 0) levels of ST2 and REG3 α and the risk of aGVHD, NRM, EFS, OS and GRFS at day +180 in patients with available serum samples.

Higher ST2 levels were significantly associated with an increased risk of NRM ($p= 0.011$), and inferior EFS ($p= 0.001$), OS ($p= 0.004$) and GRFS ($p= 0.040$) (Figure 1). Additionally, elevated REG3 α levels were also associated with inferior EFS ($p=0.047$) and OS ($p= 0.015$). No significant association was observed between ST2 or REG3 α and either the cumulative incidence or the severity of aGVHD. (Table S6).

In the multivariate analysis, recipient age and HCT-CI ≥ 3 were independently associated with an increased risk of NRM ($p= 0.019$). HCT-CI ≥ 3 remained a strong independent prognostic factor for OS ($p= 0.028$), EFS ($p<0.001$), and GRFS ($p= 0.008$). Elevated ST2 levels independently predicted inferior OS ($p= 0.016$) and EFS ($p= 0.002$), while REG3 α levels were independently associated with inferior GRFS ($p= 0.041$). In addition, male recipient sex was independently associated with a higher risk of grade II-IV aGVHD ($p= 0.032$). (Table 2).

Impact of post-transplant ST2 and REG3 α levels in transplant outcomes

We further analysed the association between ST2 and REG3 α levels at different time-points after infusion, focusing on early assessment at days +7 and +14, and extending to day +21, using the same clinical endpoints.

Interestingly, early assessment of elevated levels of ST2 had predictive value for higher risk of NRM at day +7 ($p= 0.012$) and day +14 ($p= 0.005$). Elevated ST2 levels were also significantly associated with inferior survival outcomes, including lower OS at day +7 ($p< 0.001$) and day +14 ($p=0.004$), lower GRFS at day +7 ($p= 0.012$) and day +14 ($p= 0.005$) and lower EFS at day +7 ($p< 0.001$) and day +14 ($p= 0.002$). In addition, higher ST2 levels at day +14 were predictive of grade II-IV ($p= 0.014$). (Figures 1 and 2).

Similarly, higher levels of REG3 α at day +7 ($p= 0.033$) and day +14 ($p= 0.022$) were predictive of increased NRM. Elevated REG3 α levels were also associated with lower OS at day +7 ($p= 0.005$) and day +14 ($p= 0.007$), lower GRFS at day +7 ($p= 0.043$) and day +14 ($p= 0.008$) and lower EFS at day +7 ($p= 0.004$) and day +14 ($p= 0.028$). There was no association with aGVHD on days +7 and +14. (Figures 1 and 2).

At day +21, ST2 remained significantly associated with lower OS ($p= 0.020$) and was predictive of grade II-IV aGVHD ($p= 0.001$). At this later time point, REG3 α remained predictive of increased NRM ($p= 0.015$), lower OS ($p= 0.013$), and inferior GRFS ($p= 0.005$). There was no association of REG3 α levels at day +21 with the severity of acute GVHD. Univariate analysis for every endpoint and time is shown in Tables S7-S8).

Multivariate analyses were performed for each endpoint. As summarized in Table 3 (full multivariate models shown in tables S9-S13), both biomarkers were confirmed as independent predictors of adverse outcomes across different

post-transplant time points. ST2 was independently associated with higher NRM at days +7 ($p= 0.009$) and +14 ($p= 0.011$), lower OS at days +7 ($p= 0.001$), +14 ($p= 0.005$), and +21 ($p= 0.019$), and inferior EFS at days +7 ($p< 0.001$), +14 ($p= 0.002$), and +21 ($p= 0.019$). In addition, elevated ST2 levels independently predicted a higher risk of severe aGVHD at days +14 ($p= 0.013$) and +21 ($p< 0.001$). REG3 α was independently associated with an increased risk of NRM at day +14 ($p= 0.028$) and +21 ($p= 0.023$), lower OS at day +7 ($p= 0.045$), day +14 ($p= 0.007$), and day +21 ($p= 0.021$), inferior GRFS at day +14 ($p= 0.007$), and day +21 ($p= 0.006$), and inferior EFS at days +7 ($p= 0.035$), +14 ($p= 0.019$), and +21 ($p= 0.031$).

A high HCT-CI (≥ 3) was associated with higher NRM at day +7 ($p= 0.009$), +14 ($p= 0.021$) and +21 ($p= 0.026$), lower OS at day +7 ($p= 0.024$), inferior GRFS at days +14 ($p= 0.029$) and +21 ($p= 0.044$) and inferior EFS at days +7 ($p= 0.001$), +14 ($p= 0.006$), +21 ($p= 0.003$). Older recipient age was independently associated with lower EFS at days +14 ($p= 0.030$) and +21 ($p= 0.040$), previous allogeneic transplantation with higher aGVHD II-IV at day +7 ($p= 0.037$), and male recipient sex with a higher risk of grade II-IV aGVHD at days +14 ($p= 0.030$) and +21 ($p= 0.011$).

Impact of longitudinal ST2 and REG3 α dynamics on transplant outcomes

To evaluate the prognostic impact of biomarker changes over time, longitudinal trajectories of ST2 and REG3 α from pre-conditioning through day +21 were analysed. Significant time-by-outcome interactions were observed for ST2 regarding NRM ($p=0.006$), OS ($p=0.031$), EFS ($p=0.046$), and grade II–IV aGVHD ($p=0.014$), indicating significantly different longitudinal ST2 trajectories between patients with and without these events (NRM, OS, and EFS on day +14,

and grade II–IV aGVHD on day +21). No significant difference in ST2 trajectories was observed for GRFS. In contrast, although REG3 α concentrations changed significantly over time across the post-transplant period ($p < 0.001$), no significant time-by-outcome interactions were identified for any of the evaluated clinical endpoints, indicating that longitudinal REG3 α trajectories did not differ between patients with and without these events. (Figures S2-S6)

Impact of MAGIC algorithm in transplant outcomes

Following the individual biomarker analysis, we proceeded to evaluate the impact of the MAGIC algorithm in our cohort. In the univariate analysis, the MAGIC algorithm distinguished two statistically significant risk groups of patients at day 0 for NRM ($p = 0.041$), OS ($p = 0.020$) and EFS ($p = 0.008$). Furthermore, at day +14, the algorithm retained its predictive ability, distinguishing two risk groups for EFS ($p = 0.030$) and GRFS ($p = 0.004$). Finally, at day +21, the MAGIC algorithm demonstrated predictive capacity for grade II-IV aGVHD ($p = 0.028$). (Figure 3 and Table S14).

In multivariate analysis, the MAGIC algorithm retained independent prognostic value for GRFS at day +14 ($p = 0.025$) and for EFS at days 0 ($p = 0.014$) and +14 ($p = 0.043$). (Table 4 and Tables S15-S19).

DISCUSSION

In the last decade, serum biomarkers and the MAGIC algorithm have been increasingly studied in allo-HSCT setting due to their potential predictive value. These tools, currently undergoing extensive validation, show great promise in mitigating inter-centre variability and traditional scale biases, ultimately helping identify patients who could benefit from risk-adapted strategies.^{14,16,19,20,36}.

However, most studies analysing ST2, REG3 α and the MAGIC algorithm involved patients with HLA identical or non-identical donors, with only two studies focused on the haplo-HSCT setting^{22,33}. Moreover, most studies conducted their analysis on days +15, +30 and/or at the onset of aGVHD, but lacked data from earlier time-points^{13,17,18,20,25,27,33,37,38}. Early assessment of these biomarkers could help tailor prophylaxis strategies based on the risk of specific complications.

The present study provides novel insights by comprehensively exploring the early predictive capacity of ST2 and REG3 α , alongside the MAGIC algorithm, assessed prior to infusion (pre-conditioning and Day 0) and during the early post-transplant period (days +7, +14, and +21), specifically within the context of haplo-HSCT with PTCy. In addition, this study also incorporates GRFS, a composite endpoint not previously analysed in this setting.

We observed consistent associations between elevated biomarker levels and adverse outcomes, several of which persisted after adjustment for clinical variables. ST2 predicted OS across all time points (days 0, +7, +14, +21), NRM on days +7 and +14, EFS on days 0, +7, +14, and +21, and grade II–IV aGVHD at days +14 and +21. REG3 α independently predicted OS (days +7, +14, +21), NRM (days +14, +21), EFS (days +7, +14, +21), and GRFS (days 0, +14, +21). Notably, although no consistent association was observed with aGVHD incidence at early time points, ST2 showed an association with aGVHD at later time points (day +14 and day +21). This suggests that ST2 and REG3 α may capture underlying biological processes, particularly tissue injury and systemic inflammatory vulnerability, not fully reflected by clinical GVHD grading. Longitudinal trajectory analyses further revealed progressive elevations of both biomarkers over time, with ST2 dynamics significantly discriminating between

patients with and without events for NRM, OS, EFS, and grade II–IV aGVHD, however, these dynamic trajectory evaluations remain exploratory and hypothesis-generating, requiring independent validation. The MAGIC algorithm showed early prognostic capacity, especially for composite survival endpoints, by discriminating prognostic groups at baseline and day +14 in univariate analyses, although its independent prognostic value was limited when accounting for individual biomarkers and clinical variables.

Elevated serum biomarkers levels before transplantation have been scarcely studied. Metafuni *et al.* demonstrated that higher baseline levels of ST2 were associated with higher NRM and lower OS, whereas REG3 α was not predictive; however, their cohort included only 12 haplo-HSCT setting³⁹. Gjørde *et al.* reported an association between higher pre-transplant ST2 levels and an increased NRM and 1-year mortality; however measurements were obtained at a median of 23 days prior to infusion—failing to capture conditioning toxicity—and included only 9 haplo-HSCT recipients⁴⁰. Rowan *et al.* also reported an association between higher pre-transplantation ST2 levels (day -7) and an increased NRM, although haplo-SCT patients were not included³⁰. Weber *et al.* demonstrated that REG3 α concentrations measured at day 0 but not before conditioning, were significantly influenced by conditioning-related mucosal injury and early systemic antibiotic use leading to intestinal dysbiosis, predicting NRM, although their cohort did not include haplo-HSCT recipients²⁶.

Significantly, our day 0 baseline findings expand these reports. While consistent with Metafuni *et al.*, Gjørde *et al.*, and Rowan *et al.* in confirming the prognostic value of pre-infusion ST2, our univariate analysis linked high ST2 levels to adverse NRM, OS, GRFS, and EFS^{30,39,40}. Furthermore, unlike Metafuni

et al., we identified that REG3 α levels at day 0 were also predictive of OS, GRFS, and EFS in the univariate setting. The predictive utility of these biomarkers persisted into the multivariate analysis: ST2 retained its independent significance for OS and EFS, while REG3 α remained independently predictive of GRFS. Severe grade ≥ 3 infections were not associated with transplant endpoints, suggesting that biomarker elevations may reflect true tissue damage and pro-inflammatory vulnerability rather than confounding infectious events (Supplementary Table S20). Notably, to our knowledge, this is the first study to demonstrate a significant surge in both biomarkers between pre-conditioning baseline and day 0, suggesting biological changes and tissue injury occurring during the conditioning period prior to graft infusion, although no significant differences could be confirmed between conditioning regimens, likely due to sample size constraints. These results highlight the importance of baseline assessment to detect an underlying pro-inflammatory state or heightened vulnerability to conditioning toxicity, suggesting that incorporating pre-conditioning assessments in future studies may be useful to better characterize early tissue damage kinetics. Confirmation in larger, independent series is required to validate the analysis of day 0 ST2 and REG3 α levels as a tool to guide preemptive strategies to mitigate early severe complications.

Importantly, our findings regarding the early post-transplant period suggest that the prognostic significance of the biomarkers may emerge earlier in the PTCy setting. Previous analyses by Kanakry *et al.*³³ and Solán *et al.*²² identified the predictive value of ST2 and REG3 α for NRM at day +30. In contrast, our data reveal a broader and earlier predictive window: ST2 demonstrated significant prognostic value for multiple adverse outcomes (NRM, OS, GRFS,

EFS) as early as day +7, predicting grade II–IV and III–IV aGVHD at days +14 and +21. Day +21 biomarker evaluations precede the median onset of aGVHD grades II–IV in our cohort, reflecting early subclinical mucosal and endothelial injury. REG3 α also showed this early prognostic relevance, predicting NRM, OS, and EFS at day +7 and maintaining robust associations at days +14 and +21.

These discrepancies likely stem from methodological and cohort-related differences: Kanakry *et al.*³³ evaluated biomarkers only from day +30 onward focused on NRM, whereas Solán *et al.*²² evaluated day +15 samples but was underpowered to adequately evaluate REG3 α at this specific time point and included a high proportion of bone marrow grafts. Although associations between these biomarkers and NRM have been reported in other studies, the vast majority of patients in those cohorts were not haplo-HSCT recipients^{30,40}.

Another strong point of our study, in addition to the pre-infusion and early measurement of individual ST2 and REG3 α levels, is the evaluation of the MAGIC algorithm in a homogeneous haplo-HSCT cohort. While previous studies have included limited numbers of haplo-HSCT recipients in their mixed cohorts^{29,31,41}, this represents, to our knowledge, the first comprehensive evaluation of the MAGIC algorithm in an exclusively haplo-HSCT cohort receiving PTCy. In our cohort, the MAGIC algorithm predicted NRM, EFS, and OS at the time of infusion (day 0), and EFS and GRFS at day +14 in univariate analyses, retaining independent prognostic significance for EFS and GRFS at day +14 in multivariate models. Previous reports by Hartwell *et al.*²⁷ and Hotta *et al.*³⁸ identified associations between the MAGIC algorithm at day +7 post-HSCT and NRM and OS; however, these findings could not be confirmed in our study. Notably, those analyses were restricted to transplants from matched or

mismatched related or unrelated donors, highlighting potential differences in biomarker kinetics and immune reconstitution between haploidentical and non-haploidentical transplant platforms. We hypothesize that the use of PTCy in our haplo-HSCT platform could modulate the early inflammatory response, as several patients experience cytokine release due to the infusion of donor cells, resulting in early inflammation and exposure to antibiotics, which provides an additional explanation for these differences and which shifts the predictive window of the MAGIC algorithm when contrasted with series using conventional prophylaxis. This finding opens the door to further investigation into the algorithm's utility at alternative temporal windows and for different transplant endpoints.

Our analysis has some limitations. First, this is a single-center data collection which, despite the benefits of cohort homogeneity, could limit the number of patients included in the study. Estimates derived from endpoints with few events should be interpreted cautiously because of the potential for overfitting and limited precision. This limitation underscores the critical need for confirmation of our results in larger, multicentre studies before these findings can be generalized. Second, even though ST2 and REG3 α are widely known to be associated with transplant and aGVHD outcomes, there is no consensus regarding the optimal cut-offs; the cut-offs identified in our study should therefore be considered exploratory and hypothesis-generating, requiring external and independent validation before clinical implementation. Moreover, the MAGIC algorithm has not been specifically validated for PTCy haplo-HSCT. Therefore, a standardization of the methodology, as well as confirmation of our results in larger

series in a multicentre study is required prior to their inclusion in the clinical routine.

In conclusion, ST2 and REG3 α demonstrate significant predictive value for NRM, EFS, OS, and GRFS as early as day 0, +7, and +14 specifically in PTCy haplo-HSCT. Furthermore, this study provides the first evaluation of the MAGIC algorithm in an exclusively PTCy haplo-HSCT cohort, demonstrating prognostic utility at day 0 and independent significance for day +14 EFS and GRFS. Beyond static thresholds, our findings suggest biological changes and tissue injury occurring during the conditioning period, with longitudinal trajectory models offering valuable dynamic insights. This study highlights the early, weekly association of ST2 and REG3 α levels, as well as the MAGIC algorithm, in predicting transplant outcome in PTCy haplo-HSCT. Given the widespread adoption of PTCy haplo-HSCT, future prospective studies in larger, multicentre cohorts are needed to further explore these dynamic models and evaluate early biomarker-guided interventions, such as modifying aGVHD prophylaxis. Additionally, these results suggest that the prognostic performance and optimal timing of the MAGIC algorithm warrant further evaluation specifically in the PTCy haplo-HSCT setting.

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TABLES AND TABLE LEGENDS

CHARACTERISTICS	Cohort (n=81)
Age at transplant, y, median (range)	57 (18-70)
Donor age, y, median (range)	36 (14-75)
Recipient sex male/female, n (%)	45 (55.6) / 36 (44.4)
Indication for HSCT: n (%)	
Acute myeloid leukaemia	35 (43.3)
Non-Hodgkin lymphoma	12 (14.8)
Hodgkin lymphoma	13 (16.0)
Myelodysplastic Syndrome	11 (13.6)
Acute lymphoblastic leukaemia	3 (3.7)
Myeloproliferative syndrome	2 (2.5)
Chronic myelomonocytic leukaemia	4 (4.9)
Multiple Myeloma	1 (1.2)
Blood group mismatch, n (%)	
No mismatch	61 (75.3)
Minor mismatch	7 (8.6)
Major mismatch	13 (16.1)
Prior treatment regimens, n (%)	
0-1	52 (64.2)
≥2 n	29 (35.8)
Previous autologous transplantation, n (%)	20 (24.7)
Previous allogenic transplantation, n(%)	7 (8.6)
Disease risk index, n (%)	
Low/ Intermediate/	23 (28.3) / 45 (55.6)
Very high/high	2 (2.5) / 11 (13.6)
HCT-CI, %	
0-2	59 (72.8)
≥3	22 (27.2)
Donor-recipient sex match, n (%)	
No mismatch	42 (51.8)
Female to male	16 (19.8)
Male to female	23 (28.4)
Donor-recipient relationship, n (%)	
Child to parent	43 (53.1)
Sibling to sibling	30 (37.0)
Mother to child	3 (3.7)
Father to child	5 (6.2)
Conditioning regimen, n (%)	
Myeloablative regimen	21 (25.9)
TBF-MAC	20 (95.2)
FluBux3-MAC	1 (4.8)
Reduced intensity regimen	57 (70.4)
FluBux2-RIC	47 (82.5)
FluBux1-RIC	4 (7.0)
TBF-RIC	4 (7.0)
FluBu-TBI-RIC	2 (3.5)
Sequential regimen	3 (3.7)
GvHD prophylaxis	
Tacro + MMF + Cy	81 (100)
Graft source, n (%)	
Peripheral blood	81 (100)
CD34+×10 ⁶ /kg infused dose, median (range)	6,25 (3,42-9,27)

HSCT: Hematopoietic Stem Cell Transplantation; HCT-CI: Hematopoietic Transplantation-specific Comorbidity Index, TBF: Thiotepa, Busulfan and Fludarabine; MAC: myeloablative conditioning; FluBu; Fludarabine and Busulfan; RIC: reduced intensity conditioning; TBI: Total Body Irradiation; GVHD: Graft-versus-host disease; Tacro: Tacrolimus; CsA: Cyclosporin A; MMF: mycophenolate mofetil; Cy: Cyclophosphamide.

Table 1. Patient and transplant characteristics.

Day 0 (n=71)	NRM		OS		GRFS		EFS		aGVHD II-IV	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.075 (1.007-1.147)	0.029	1.042 (0.990-1.098)	0.109	0.998 (0.968-1.030)	0.937	1.024 (0.980-1.070)	0.276	1.002 (0.981-1.024)	0.793
Sex recipient male	2.282 (0.510-10.193)	0.280	1.255 (0.373-4.226)	0.712	0.552 (0.234-1.300)	0.174	0.858 (0.277-2.654)	0.791	2.206 (1.069-4.552)	0.032
HCT-CI ≥ 3	5.717 (1.331-24.541)	0.019	3.792 (1.155-12.455)	0.028	3.123 (1.343-7.266)	0.008	6.978 (2.295-21.221)	0.000	1.187 (0.550-2.561)	0.661
Myeloablative conditioning	0.887 (0.652-1.207)	0.446	0.824 (0.611-1.113)	0.207	0.927 (0.710-1.212)	0.582	0.810 (0.603-1.088)	0.161	0.985 (0.808-1.202)	0.886
DRI $\geq$ intermediate	0.404 (0.112-1.458)	0.166	0.597 (0.183-1.945)	0.392	0.866 (0.358-2.095)	0.750	0.421 (0.135-1.310)	0.135	0.777 (0.387-1.558)	0.477
Previous Allogenic transplantation	0.776 (0.068-8.821)	0.839	0.880 (0.085-9.113)	0.914	1.628 (0.438-6.046)	0.466	1.248 (0.205-7.595)	0.809	1.419 (0.482-4.177)	0.524
ST2	3.468 (0.983-12.226)	0.053	3.890 (1.284-11.780)	0.016	2.146 (0.973-4.733)	0.058	5.456 (1.853-16.068)	0.002	0.798 (0.307-2.073)	0.643
REG3 α	8449 (NE)	0.998	3.131 (0.904-10.847)	0.071	2.349 (1.032-5.349)	0.041	3.199 (0.968-10.569)	0.056	0.389 (0.150-1.013)	0.053

NRM: Non-relapse mortality; OS: Overall survival; GRFS: graft-versus-host-free relapse-free survival; EFS: event-free survival, aGVHD: acute graft-versus-host disease; NE: not evaluable.

Table 2. Multivariate analysis for NRM, OS, GRFS, EFS and aGvHD II-IV on day 0.

Predictor	Endpoint	Time point	HR (95% CI)	p-value
ST2	NRM	Day 7	5.794 (1.523-22.041)	0.009
		Day 14	4.963 (1.439-17.118)	0.011
		Day 21	5.568 (0.887-34.956)	0.066
	OS	Day 7	6.208 (2.010-19.172)	0.001
		Day 14	4.710 (1.588-13.974)	0.005
		Day 21	5.125 (1.301-20.194)	0.019
	GRFS	Day 7	2.091(0.895-4.886)	0.080
		Day 14	2.335 x10 ⁸ (NE)	0.997
		Day 21	2.112 (0.714-6.245)	0.176
	EFS	Day 7	7.003 (2.417-20.466)	<0.001
		Day 14	4.977 (1.785-13.870)	0.002
		Day 21	4.945 (1.295-18.880)	0.019
REG3α	NRM	Day 7	0.529 (0.285-0.984)	0.064
		Day 14	2.704 (1.227-5.957)	0.013
		Day 21	4.822 (2.449-9.498)	<0.001
	OS	Day 7	3.246 (0.639-16.468)	0.155
		Day 14	4.084 (1.162-14.353)	0.028
		Day 21	10.695 (1.374-83.250)	0.023
	GRFS	Day 7	4.802 (1.029-22.406)	0.045
		Day 14	4.393 (1.485-12.991)	0.007
		Day 21	6.343 (1.309-30.736)	0.021
	EFS	Day 7	1.958 (0.651-5.887)	0.231
		Day 14	3.327 (1.371-8.068)	0.007
		Day 21	5.155 (1.575-16.868)	0.006
REG3α	aGVHD II-IV	Day 7	4.030 (1.095-14.824)	0.035
		Day 14	3.355 (1.217-9.251)	0.019
		Day 21	5.046 (1.155-22.048)	0.031
	aGVHD II-IV	Day 7	0.292 (0.145-1.587)	0.080
		Day 14	0.373 (0.133-1.047)	0.061
		Day 21	0.204 (0.079-1.525)	0.075

NRM: Non-relapse mortality; OS: Overall survival; GRFS: graft-versus-host-free relapse-free survival; EFS: event-free Survival; aGVHD: acute graft-versus-host disease; NE: not evaluable.

Table 3. Multivariate analysis summary of ST2 and REG3α for days 7, 14 and 21. Summary of the independent prognostic value of serum ST2 and REG3α levels at early post-transplant time points (+7, +14, and +21).

Predictor	Endpoint	Time point	HR (95% CI)	p-value
MAGIC	NRM	Day 0	2.700 (0.787-9.264)	0.114
		Day 7	1.504 (0.372-6.083)	0.566
		Day 14	5.932 (0.730-48.201)	0.095
		Day 21	5.774x10 ⁷ (NE)	0.999
	OS	Day 0	2.922 (0.982-8.692)	0.053
		Day 7	1.211 (0.371-3.949)	0.750
		Day 14	6.552 (0.848-50.600)	0.071
		Day 21	8.117x10 ⁷ (NE)	0.998
	GRFS	Day 0	2.008 (0.901-4.471)	0.087
		Day 7	0.901 (0.363-2.235)	0.822
		Day 14	10.010 (1.325-75.592)	0.025
		Day 21	8.324x10 ⁷ (NE)	0.998
	EFS	Day 0	3.490 (1.293-9.423)	0.013
		Day 7	1.688 (0.528-5.398)	0.377
		Day 14	8.326 (1.065-65.070)	0.043
		Day 21	8.324x10 ⁷ (NE)	0.998
	aGVHD II-IV	Day 0	0.828 (0.438-1.565)	0.562
		Day 7	0.529 (0.272-1.031)	0.061
		Day 14	1.543 (0.722-3.298)	0.265
		Day 21	7.971x10 ⁷ (NE)	0.997

NRM: Non-relapse mortality; OS: Overall survival; GRFS: graft-versus-host-free relapse-free survival; EFS: event-free survival; aGVHD: acute graft-versus-host disease; NE: not evaluable.

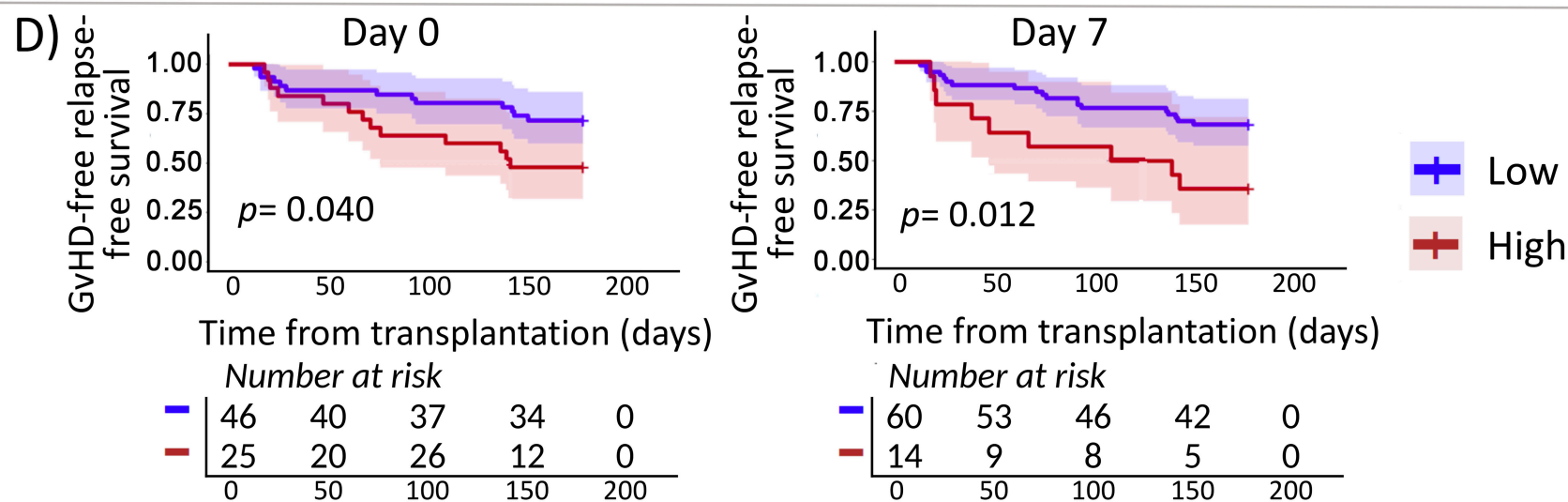
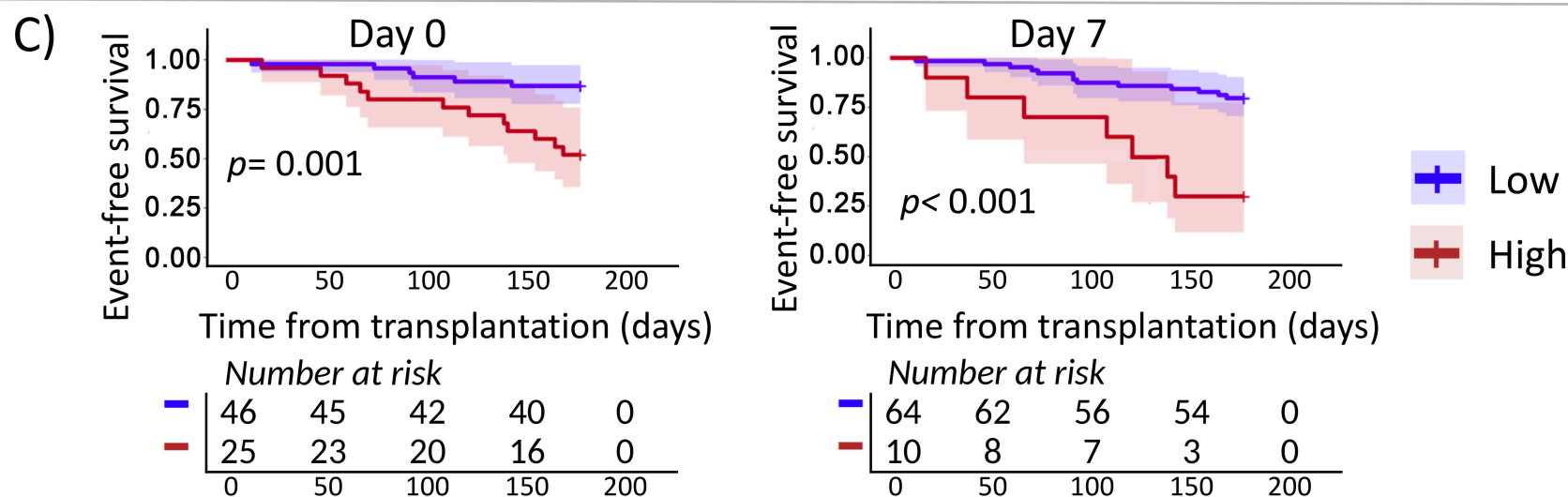
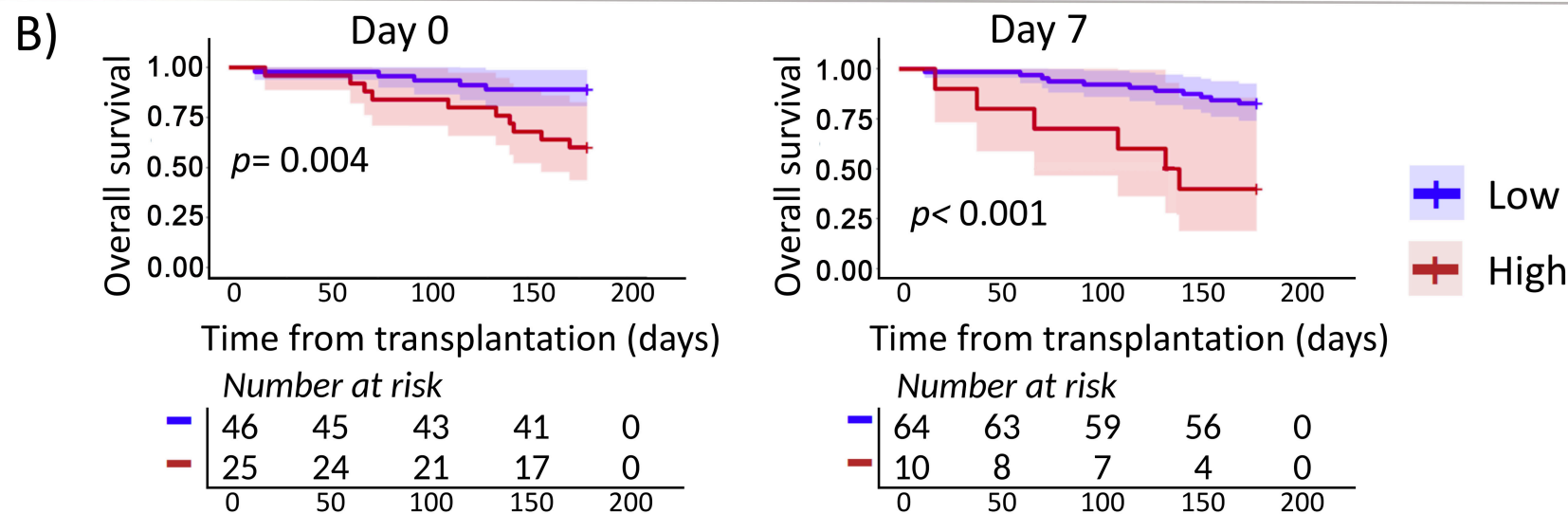
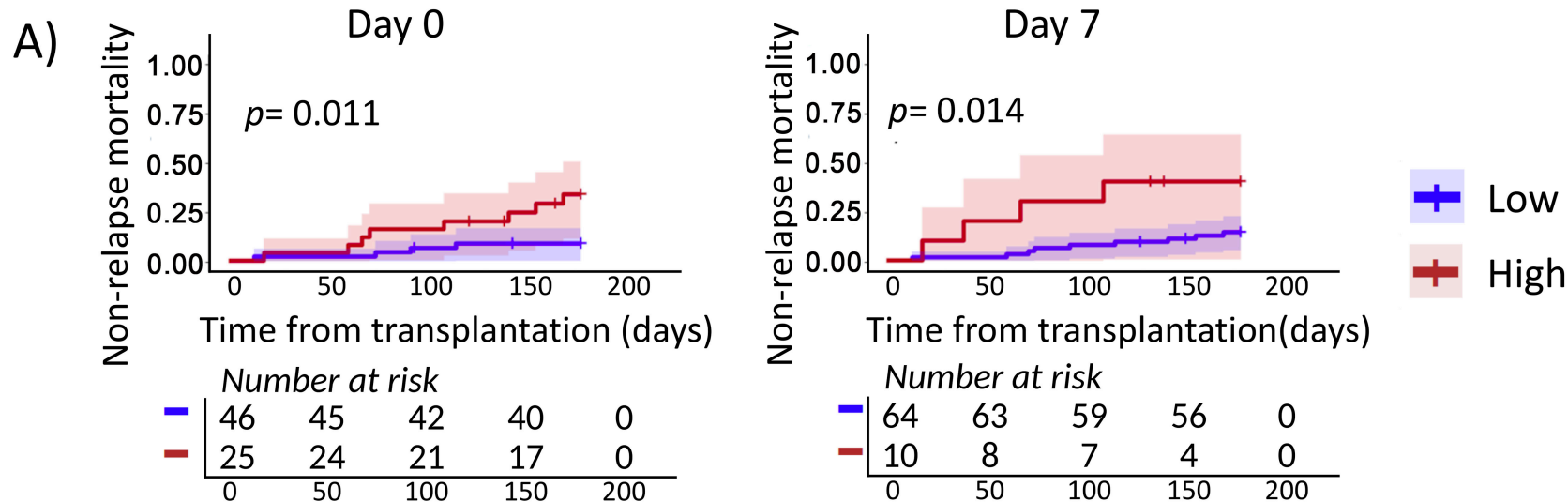
Table 4. Multivariate analysis summary of MAGIC algorithm for days 0, 7, 14 and 21. Summary of the independent prognostic value of MAGIC algorithm at early post-transplant time points (+7, +14, and +21).

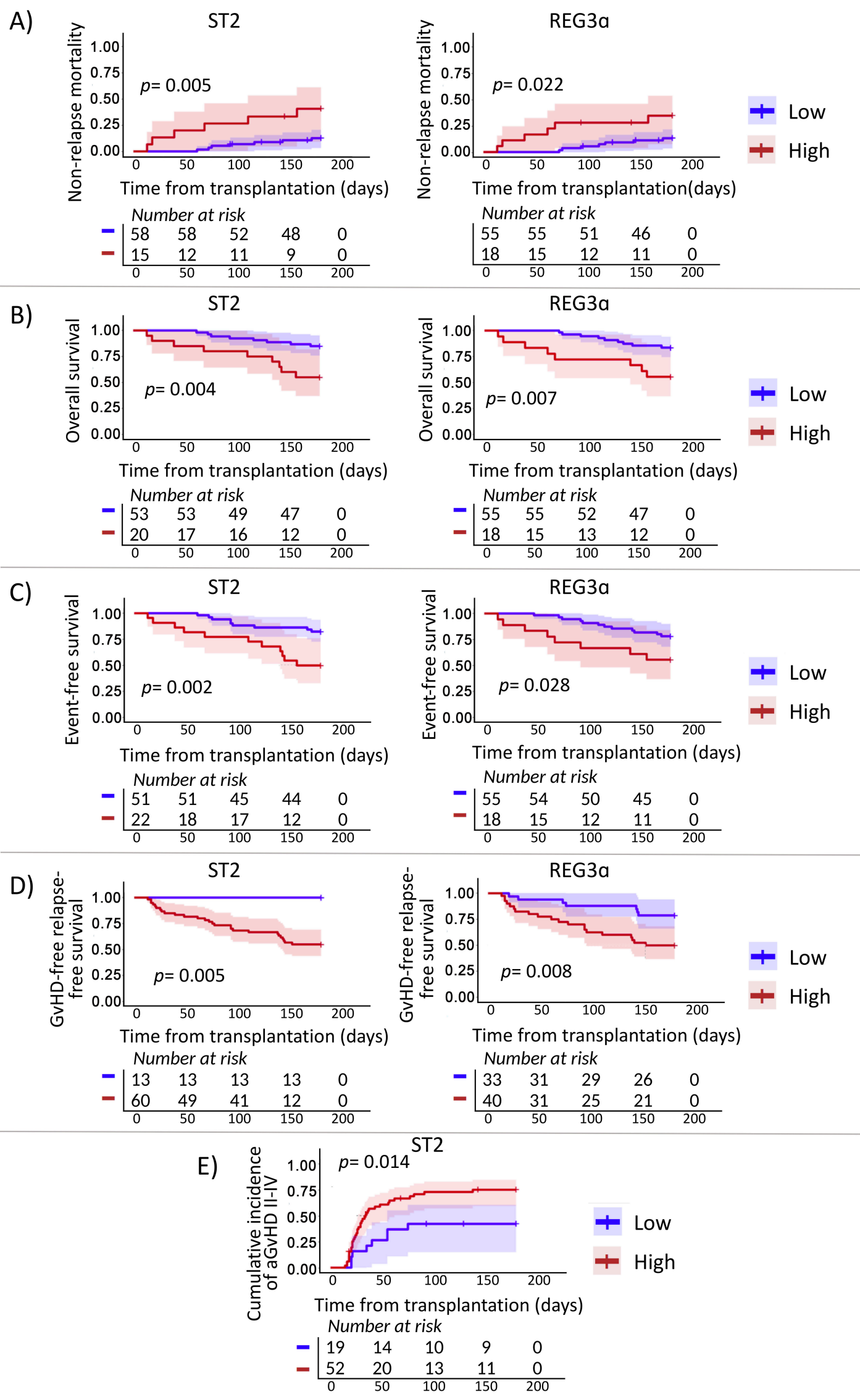
FIGURE LEGENDS

Figure 1. Impact of early ST2 levels on post-transplant outcomes at day 0 and day +7. (A) Non-relapse mortality (NRM), (B) Overall survival (OS), (C) Event-free survival (EFS), and (D) GVHD-free relapse-free survival (GRFS). Stratified by high (red) and low (blue) ST2 levels at day 0 (left) and day +7 (right). High and low biomarker groups were defined using endpoint-specific cut-offs, as detailed in Supplementary Table S3.

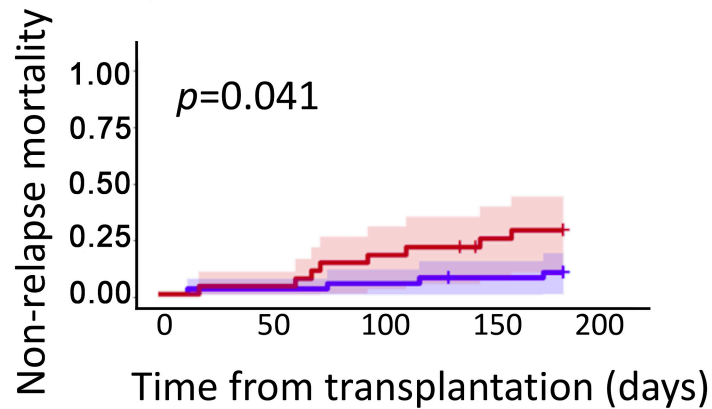
Figure 2. Impact on post-transplant outcomes at day +14 of ST2 and REG3 α . (A) Non-relapse mortality (NRM), (B) Overall survival (OS), (C) Event-free survival (EFS), (D) GVHD-free relapse-free survival (GRFS) and (E) Cumulative incidence of aGvHD grades II-IV. Stratified by high (red) and low (blue) ST2 levels. ST2 (left) and REG3 α (right). High and low biomarker groups were defined using endpoint-specific cut-offs, as detailed in Supplementary Table S3.

Figure 3. Impact of the MAGIC algorithm on post-transplant outcomes. Univariate analysis showing the prognostic value of the MAGIC algorithm risk groups at different time points: (A) Day 0: Non-relapse mortality (NRM), Overall Survival (OS) and event-free survival (EFS). (B) Day +14: GVHD-free relapse-free survival (GRFS) and EFS. (C) Day +21: grade II-IV acute GVHD. Patients are stratified into high-risk (red) and low-risk (blue) groups

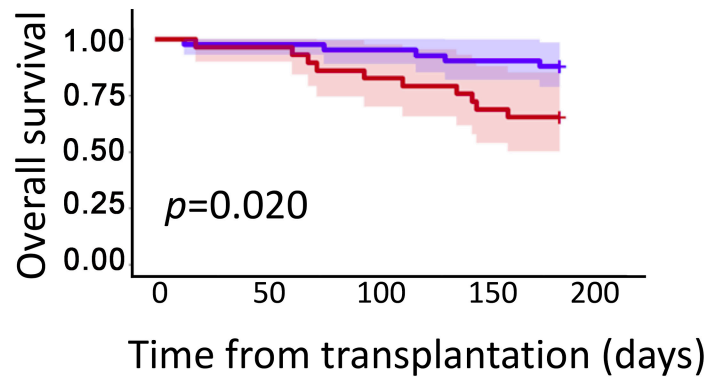




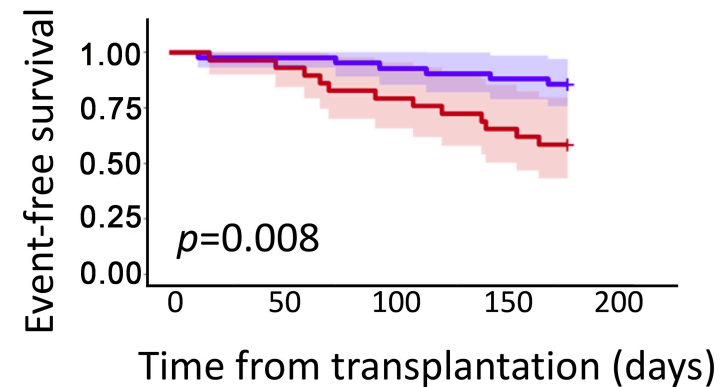
A) Day 0



Number at risk					
	0	50	100	150	200
Low	58	58	52	48	0
High	15	12	11	9	0



	0	50	100	150	200
Low	42	41	40	38	0
High	29	28	24	20	0

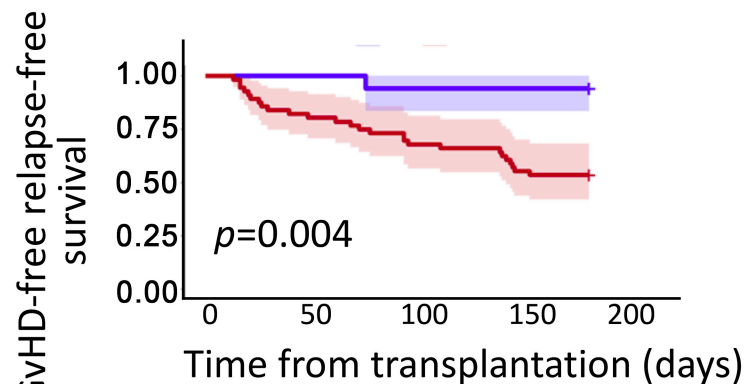


	0	50	100	150	200
Low	42	41	39	37	0
High	29	27	23	19	0

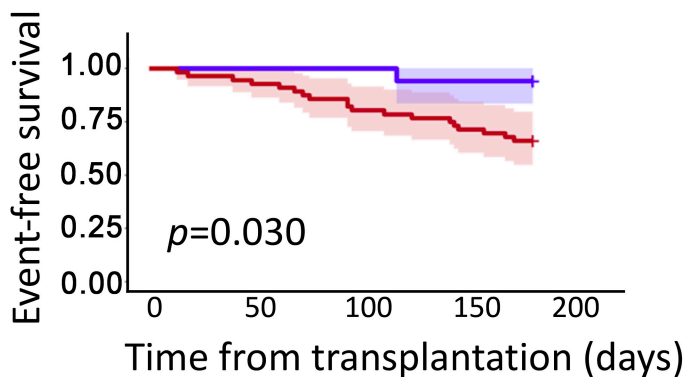
Low

High

B) Day 14



Number at risk					
	0	50	100	150	200
Low	17	17	16	16	0
High	56	45	38	31	0

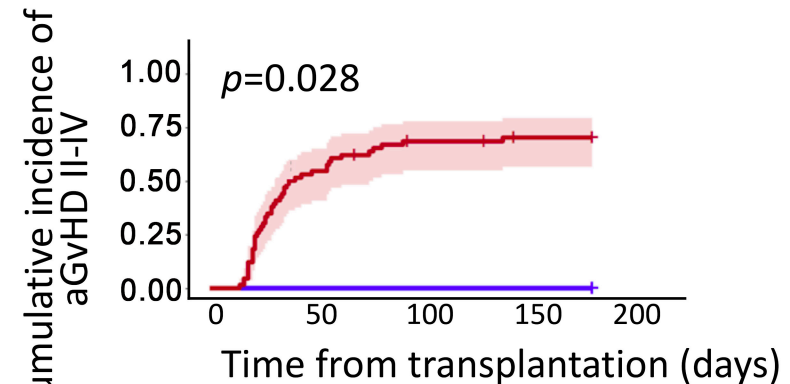


Number at risk					
	0	50	100	150	200
Low	17	17	17	16	0
High	56	52	45	40	0

Low

High

C) Day 21



Number at risk					
	0	50	100	150	200
Low	4	4	4	4	0
High	66	30	19	16	0

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Table S20. Association between grade ≥ 3 infections and study endpoints.

CHARACTERISTICS	Sample Cohort (n=81)	Non-sample cohort (n=69)	P
Age at transplant, y, median (range)	57 (18-70)	54 (17-73)	0.950
Donor age, y, median (range)	36 (14-75)	39 (16-75)	0.357
Recipient sex male/female, n (%)	45 (55.6) / 36 (44.4)	39 (56.5) / 30 (43.5)	0.950
Indication for HSCT: n (%)			0.548
Acute myeloid leukemia	35 (43.3)	27 (39.1)	
Non-Hodgkin lymphoma	12 (14.8)	13 (18.8)	
Hodgkin lymphoma	13 (16.0)	9 (13.0)	
Myelodysplastic Syndrome	11 (13.6)	8 (11.6)	
Acute lymphoblastic leukemia	3 (3.7)	6 (8.7)	
Myeloproliferative syndrome	2 (2.5)	5 (7.2)	
Chronic myelomonocytic leukemia	4 (4.9)	1 (1.4)	
Multiple Myeloma	1 (1.2)	0 (0)	
Blood group mismatch, n (%)			0.061
No mismatch	61 (75.3)	46 (66.7)	
Minor mismatch	7 (8.6)	14 (20.3)	
Major mismatch	13 (16.1)	8 (11.6)	
Bidirectional	0 (0)	1 (1.4)	
Prior treatment regimens, n (%)			0.367
0-2	52 (64.2)	43 (62.3)	
>2	29 (35.8)	26 (37.7)	
Previous autologous transplantation, n (%)	20 (24.7)	15 (21.7)	0.991
Previous allogeneic transplantation, n(%)	7 (8.6)	6 (8.7)	0.670
Disease risk index, n (%)			0.146
Very high/high	2 (2.5)/11 (13.6)	3 (4.3) / 11 (15.9)	
Intermediate/low	45 (55.6)/ 23 (28.3)	43 (62.3) / 12 (17.4)	
HCT-CI, %			0.263
0-2	59 (72.8)	47 (68.1)	
≥3	22 (27.2)	22 (31.9)	
Donor-recipient sex match, n (%)			0.313
No mismatch	42 (51.8)	35 (50.7)	
Female to male	16 (19.8)	20 (29.0)	
Male to female	23 (28.4)	14 (20.3)	
Donor-recipient relationship, n (%)			0.058
Child to parent	43 (53.1)	35 (50.7)	
Sibling to sibling	30 (37.0)	21 (30.4)	
Mother to child	3 (3.7)	5 (7.2)	
Father to child	5 (6.2)	8 (11.6)	
Conditioning regimen, n (%)			0.313
Myeloablative regimen	21 (25.9)	13 (18.9)	
TBF-MAC	20 (95.2)	8 (61.5)	
FluBux3-MAC	1 (4.8)	5 (38.5)	
Reduced intensity regimen	57 (70.4)	55 (79.7)	
FluBux2-RIC	47 (82.5)	42 (76.4)	
FluBux1-RIC	4 (7.0)	6 (10.9)	
TBF-RIC	5 (7.0)	4 (7.3)	
FluBu-TBI-RIC	2 (3.5)	3 (5.4)	
Sequential regimen	3 (3.7)	1 (1.4)	
GvHD prophylaxis			0.123
Tacro + MMF + Cy	81 (100)	67 (97.1)	
CsA + MMF + Cy	0 (0)	2 (2.9)	
Graft source, n (%)			0.58
Peripheral blood	81 (100)	66 (95.7)	
Bone Marrow	0 (0)	3 (4.3)	
CD34+x106/kg infused dose, median (range)	6,25 (3,42-9,27)	6,24 (2,80-9,34)	0.164

Table S1. Sensitivity analysis comparing baseline clinical characteristics between patients with available longitudinal biomarker samples (sample cohort, n=81) and those without available samples (non-sample cohort, n=69).

Transplant outcomes	Sample Cohort (n=81)	Non-sample cohort (n=69)	<i>P</i>
aGvHD at day +180			
grades II-IV	65.3% (IC 95%, 54.7% – 75.9%)	53.9% (IC 95%, 41.6% – 66.3%)	0.175
grades III-IV	14.3% (IC 95%, 6.5 % – 22.1%)	8.0% (IC 95%, 1.3% – 14.7%)	0.242
Survival and transplant events at day +180			
OS	76.5% (IC 95%, 67.3% – 85.7%)	81.2% (IC 95%, 72.0% – 90.4%)	0.936
NRM	17.5% (IC 95%, 9.1% – 25.9%)	18.8% (IC 95%, 9.6% – 28.0%)	0.752
EFS	72.8% (IC 95%, 63.2% – 82.4%)	75.1% (IC 95%, 64.9% – 85.3%)	0.958
Relapse incidence	10.0% (IC 95%, 2.9% – 17.1%)	8.6% (IC 95%, 1.4% – 15.9%)	0.971
GRFS at 2 years	49.2% (IC 95%: 38.2% – 60.2%)	55.1% (IC 95%: 43.3% – 66.9%)	0.257

Table S2. Sensitivity analysis comparing transplant outcomes between patients with available longitudinal biomarker samples (sample cohort, n=81) and those without available samples (non-sample cohort, n=69).

Endpoint	Time point	Cut-off for ST2	Cut-off for REG3 α
aGVHD II-IV	Day 0	13.918	13.693
	Day 7	13.283	12.931
	Day 14	13.495	13.721
	Day 21	14.954	13.414
NRM	Day 0	13.145	12.108
	Day 7	15.256	13.219
	Day 14	15.376	13.265
	Day 21	15.631	13.847
EFS	Day 0	13.145	13.881
	Day 7	15.256	13.219
	Day 14	14.667	13.265
	Day 21	15.631	13.847
OS	Day 0	13.145	13.881
	Day 7	15.256	13.219
	Day 14	14.719	13.265
	Day 21	15.631	13.847
GRFS	Day 0	13.145	13.373
	Day 7	14.553	13.056
	Day 14	13.271	12.482
	Day 21	15.619	13.847

aGVHD; acute graft-versus-host disease; NRM: non-relapse mortality; EFS: event-free survival; GRFS: GVHD-free, relapse-free survival; OS: overall survival.

Table S3. Optimal biomarker cut-off thresholds by endpoint and time point.

Outcome	%, (95% CI)
aGvHD grades II-IV	
+100	63.7%, (52.9–74.5)
+180	65.3%, (54.7–75.9)
aGvHD grades III-IV	
+100	12.8%, (5.4–20.2)
+180	14.3%, (6.5–22.1)
cGvHD overall	
+1-year	26.9%, (15.9–37.9)
+2-year	39.6%, (26.7–52.5)
cGvHD moderate-severe	
+1-year	10.6%, (3.2–18.0)
+2-year	14.7%, (5.7–23.7)
NRM	
+100	13.6%, (6.2–21.0)
+180	17.5%, (9.1–25.9)
+1-year	21.5%, (12.5–30.5)
+2-year	22.9%, (13.5–32.3)
Relapse	
+100	4.1%, (0.0–8.6)
+180	10.0%, (2.9–17.1)
+1-year	17.9%, (8.7–27.1)
+2-year	17.9%, (8.7–27.1)
EFS	
+100	84.0%, (76.0%–92.0%)
+180	72.8%, (63.2%–82.4%)
+1-year	62.9%, (52.4%–73.4%)
+2-year	62.9%, (52.4%–73.4%)
OS	
+100	88.9%, (82.0 – 95.8)
+180	76.5%, (67.3 – 85.7)
+1-year	71.6%, (61.8 – 81.4)
+2-year	67.9%, (57.7 – 78.1)
GRFS	
+1-year	55.6% (44.8 – 66.4)
+2-year	49.2% (38.2 – 60.2)

aGVHD; acute graft-versus-host disease; cGVHD; chronic graft-versus-host disease; NRM: non-relapse mortality; EFS: event-free survival; GRFS: GVHD-free, relapse-free survival; OS: overall survival.

Table S4. Overall outcomes.

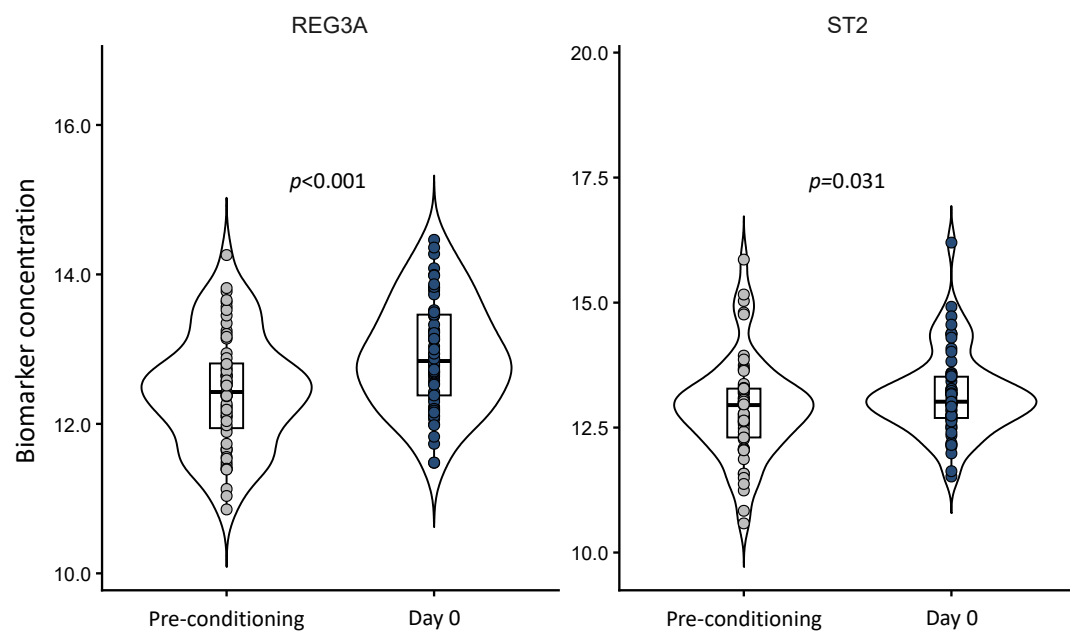


Figure S1. Paired comparison of ST2 and REG3 α concentrations between pre-conditioning baseline and day 0 (pre-infusion).

Transplant variables	ST2			REG3 α		
	Down (n=25)	Up (n=36)	p- value	Down (n=12)	Up (n=49)	p- value
Age at transplant (years), median ± SD	58 ± 12.38	52.5 ± 14.43	0.370	60.0 ± 11.28	55.0 ± 14.01	0.148
Recipient sex male/female, n (%)	10 (40)	19 (52.8)	0.435	9 (75.0)	23 (46.9)	0.111
HCT-CI ≥3, n (%)	17 (68.0)	27 (75.0)	0.574	8 (66.7)	36 (73.5)	0.723
DRI ≥ intermediate, n (%)	15 (60)	25 (69.4)	0.584	8 (66.7)	32 (65.3)	1.000
Previous transplant, n (%)	3 (12.0)	1 (2.8)	0.295	1 (8.3)	3 (6.1)	1.000
CD34+ ≥ 6 × 10 ⁶ /kg, n (%)	19 (76.0)	24 (66.7)	0.570	9 (75.0)	34 (69.4)	1.000
Myeloablative conditioning, n (%)	7 (28)	10 (29.4)	1.000	1 (8.3)	16 (34.0)	0.150

Table S5. Clinical and transplant characteristics according to ST2 and REG3 α early post-conditioning dynamic.

	ST2		REG3 α	
	HR (95% CI)	p-value	HR (95% CI)	p-value
NRM	< or $\geq$ 13.145		< or $\geq$ 12.108	
	4.175 (1.255-13.886)	0.011	2480 (0-Inf)	0.099
OS	< or $\geq$ 13.145		< or $\geq$ 13.881	
	4.189 (1.430-12.269)	0.004	3.486 (1.187-10.230)	0.015
GRFS	< or $\geq$ 13.145		< or $\geq$ 13.373	
	2.191 (1.093-4.739)	0.040	2.091 (0.958-4.565)	0.058
EFS	< or $\geq$ 13.145		< or $\geq$ 13.881	
	4.421 (1.656-11.801)	0.001	2.733 (0.971-7.690)	0.047
aGVHD	< or $\geq$ 13.400		< or $\geq$ 13.666	
	0.613 (0.314-1.195)	0.146	0.413 (0.185-1.920)	0.075
aGVHD II-IV	< or $\geq$ 13.918		< or $\geq$ 13.693	
	0.676 (0.285-1.601)	0.371	0.440 (0.185-1.044)	0.055
aGVHD III-IV	< or $\geq$ 12.608		< or $\geq$ 12.063	
	0.590 (0.166-2.091)	0.408	2.526 (0.000-2.310)	1.223

aGVHD; acute graft-versus-host disease; cGVHD; chronic graft-versus-host disease; NRM: non-relapse mortality; EFS: event-free survival; GRFS: GVHD-free, relapse-free survival; OS: overall survival.

Table S6. Univariate analysis of ST2 and REG3 α regarding different survival-endpoints on day 0.

ST2						
	Day 7		Day 14		Day 21	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
NRM	< or ≥ 15.256		< or ≥ 15.376		< or ≥ 15.631	
	4.185 (1.258-13.919)	0.012	4.153 (1.393-12.377)	0.005	2.655 (0.776-9.080)	0.105
OS	< or ≥ 15.256		< or ≥ 14.719		< or ≥ 15.631	
	5.089 (1.868-13.865)	0.000	3.661 (1.410-9.505)	0.004	3.191 (1.134-8.975)	0.020
GRFS	< or ≥ 14.553		< or ≥ 13.271		< or ≥ 15.619	
	2.641 (1.191-5.856)	0.012	2.695 (NS)	0.005	1.772 (0.739-4.245)	0.193
EFS	< or ≥ 15.256		< or ≥ 14.667		< or ≥ 15.631	
	5.074 (2.004-12.844)	0.000	3.568 (1.475-8.629)	0.002	2.300 (0.862-6.134)	0.086
aGVHD	< or ≥ 13.529		< or ≥ 13.595		< or ≥ 14.954	
	0.587 (0.345-0.999)	0.077	1.603 (0.843-3.045)	0.145	1.891 (1.097-3.261)	0.019
aGVHD II-IV	< or ≥ 13.283		< or ≥ 13.495		< or ≥ 14.954	
	0.598 (0.329-1.087)	0.088	2.513 (1.169-5.402)	0.014	2.533 (1.411-4.544)	0.001

NS: not stimable due to the lack of events.

aGVHD; acute graft-versus-host disease; cGVHD; chronic graft-versus-host disease; NRM: non-relapse mortality; EFS: event-free survival; GRFS: GVHD-free, relapse-free survival; OS: overall survival. NE: not evaluable

Table S7. Univariate analysis of ST2 regarding different survival- endpoints on days +7, +14 and +21.

REG3 α						
	Day 7		Day 14		Day 21	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
NRM	< or $\geq$ 13.219		< or $\geq$ 13.265		< or $\geq$ 13.847	
	4.460 (0.987-20.156)	0.033	3.355 (1.156- 10.003)	0.021	4.039 (1.181- 13.810)	0.015
OS	< or $\geq$ 13.219		< or $\geq$ 13.265		< or $\geq$ 13.847	
	6.269 (1.432-27.444)	0.005	3.427 (1.320-8.895)	0.007	3.547 (1.211- 10-386)	0.013
GRFS	< or $\geq$ 13.056		< or $\geq$ 12.482		< or $\geq$ 13.847	
	2.612 (0.991- 6.883)	0.043	2.999 (1.266 - 7.107)	0.008	3.265 (1.354 - 7.870)	0.005
EFS	< or $\geq$ 13.219		< or $\geq$ 13.265		< or $\geq$ 13.847	
	4.907 (1.436 - 16.770)	0.004	2.620 (1.070 - 6.418)	0.028	2.673 (0.952 - 7.501)	0.051
aGVHD	< or $\geq$ 12.931		< or $\geq$ 13.099		< or $\geq$ 13.244	
	0.577 (0.321-1.037)	0.062	0.637 (0.355- 1.143)	0.127	0.564 (0.306-1.039)	0.063
aGVHD II-IV	< or $\geq$ 12.931		< or $\geq$ 13.721		< or $\geq$ 13.414	
	1.384 (0.208-1.708)	0.064	0.506 (0.199- 1.283)	0.143	0.499 (0.223-1.119)	0.085
aGVHD III-IV	< or $\geq$ 12.616		< or $\geq$ 12.655		< or $\geq$ 13.847	
	0.275 (0.077-0.974)	0.082	3.923 (0.841-18.902)	0.065	3.534 (0.880-14-194)	0.057

aGVHD; acute graft-versus-host disease; cGVHD; chronic graft-versus-host disease; NRM: non-relapse mortality; EFS: event-free survival; GRFS: GVHD-free, relapse-free survival; OS: overall survival. NE: not evaluable

Table S8. Univariate analysis of REG3 α regarding different survival- endpoints on days +7, +14 and +21.

	Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.053 (0.993-1.116)	0.079	1.070 (1.013-1.131)	0.015	1.109 (1.025-1.199)	0.009
Sex recipient male	2.368 (0.599-9.351)	0.218	2.529 (0.607-10.531)	0.202	1.192 (0.217-6.533)	0.838
HCT-CI ≥ 3	6.506 (1.584-26.717)	0.009	5.188 (1.269-21.212)	0.021	7.650 (1.262-46.381)	0.026
Myeloablative conditioning	0.948 (0.701-1.284)	0.0.73 4	0.756 (0.545-1.050)	0.095	0.824 (0.586-1.157)	0.264
DRI $\geq$ intermediate	0.531 (0.154-1.823)	0.314	0.355 (0.093-1.349)	0.128	0.312 (0.065-1.494)	0.145
Previous allogenic transplantation	0.637 (0.053-7.605)	0.721	1.197 (0.105-13.649)	0.884	3.517 (0.242-50.932)	0.356
ST2	5.794 (1.523-22.041)	0.009	4.963 (1.439-17.118)	0.011	5.568 (0.887-34.956)	0.066
REG3 α	3.246 (0.639-16.468)	0.155	4.084 (1.162-14.353)	0.028	10.695 (1.374-83.250)	0.023

Table S9. Multivariate analysis for NRM including ST2 and REG3 α .

	Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.042 (0.995-1.092)	0.079	1.063 (1.014-1.115)	0.011	1.076 (1.014-1.141)	0.014
Sex recipient male	1.608 (0.526-4.911)	0.404	1.580 (0.504-4.955)	0.432	0.668 (0.177-2.519)	0.552
HCT-CI ≥ 3	3.787 (1.184-12.115)	0.024	2.747 (0.849-8.880)	0.091	3.762 (0.939-15.073)	0.061
Myeloablative conditioning	0.934 (0.706-1.236)	0.633	0.765 (0.564-1.036)	0.083	0.848 (0.612-1.177)	0.325
DRI $\geq$ intermediate	0.791 (0.259-2.413)	0.681	0.611 (0.192-1.938)	0.403	0.652 (0.184-2.300)	0.506
Previous Allogenic transplantation	0.549 (0.054-5.590)	0.613	1.294 (1.129-12.991)	0.826	1.559 (0.159-15.274)	0.702
ST2	6.208 (2.010-19.172)	0.001	4.710 (1.588-13.974)	0.005	5.125 (1.301-20.194)	0.019
REG3 α	4.802 (1.029-22.406)	0.045	4.393 (1.485-12.991)	0.007	6.343 (1.309-30.736)	0.021

Table S10. Multivariate analysis for OS including ST2 and REG3 α .

	Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	0.999 (0.969-1.031)	0.980	1.000 (0.973-1.036)	0.791	1.026 (0.989-1.065)	0.167
Sex recipient male	0.675 (0.287-1.584)	0.366	9.400 (0.374-2.358)	0.895	0.552 (0.223-1.367)	0.199
HCT-CI ≥ 3	2.222 (0.963-5.126)	0.061	2.735 (1.105-6.771)	0.029	2.788 (1.025-7.578)	0.044
Myeloablative conditioning	0.991 (0.764-1.287)	0.951	0.967 (0.749-1.249)	0.800	0.895 (0.687-1.168)	0.416
DRI $\geq$ intermediate	0.680 (0.286-1.618)	0.383	0.838 (0.337-2.086)	0.705	0.796 (0.313-2.024)	0.632
Previous Allogenic transplantation	1.253 (0.307-5.107)	0.753	0.677 (0.147-3.111)	0.616	2.137 (0.524-8.711)	0.289
ST2	2.091 (0.895-4.886)	0.080	2.335x10 ⁸ (NS)	0.997	2.112 (0.714-6.245)	0.176
REG3 α	1.958 (0.651-5.887)	0.231	3.327 (1.371-8.068)	0.007	5.155 (1.575-16.868)	0.006

NS: not stimable due to the lack of events.

Table S11. Multivariate analysis for GRFS including ST2 and REG3 α .

	Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.026 (0.984-1.070)	0.225	1.047 (1.004-1.093)	0.030	1.0526 (1.002-1.106)	0.040
Sex recipient male	1.114 (0.379-3.275)	0.843	1.352 (0.453-4.033)	0.588	0.597 (0.1875-1.906)	0.384
HCT-CI ≥ 3	6.043 (2.057-17.754)	0.001	4.373 (1.505-12.707)	0.006	6.430 (1.839-22.487)	0.003
Myeloablative conditioning	0.945 (0.715-1.251)	0.696	0.768 (0.572-1.031)	0.079	0.843 (0.613-1.159)	0.293
DRI $\geq$ intermediate	0.612 (0.213-1.757)	0.361	0.574 (0.195-1.690)	0.313	0.615 (0.200-1.893)	0.397
Previous Allogenic transplantation	0.994 (0.161-6.113)	0.995	1.971 (0.333-11.669)	0.454	1.971 (0.348-11.139)	0.442
ST2	7.003 (2.417-20.466)	0.000	4.977 (1.785-13.870)	0.002	4.945 (1.295-18.880)	0.019
REG3 α	4.030 (1.095-14.824)	0.035	3.355 (1.217-9.251)	0.019	5.046 (1.155-22.048)	0.031

Table S12. Multivariate analysis for EFS including ST2 and REG3 α .

	Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.010 (0.989-1.032)	0.351	0.996 (0.975-1.018)	0.751	0.999 (0.978-1.020)	0.932
Sex recipient male	1.399 (0.729-2.687)	0.312	2.063 (1.071-3.974)	0.030	2.564 (1.240-5.301)	0.011
HCT-CI ≥ 3	1.289 (0.617-2.695)	0.498	1.166 (0.536-2.537)	0.698	1.417 (0.701-2.860)	0.331
Myeloablative conditioning	0.943 (0.748-1.188)	0.618	1.006 (0.817-1.239)	0.954	1.037 (0.844-1.275)	0.724
DRI $\geq$ intermediate	0.859 (0.433-1.702)	0.663	0.924 (0.467-1.829)	0.820	1.060 (0.531-2.113)	0.868
Previous Allogenic transplantation	2.858 (1.060-7.706)	0.037	1.156 (0.393-3.400)	0.792	1.184 (0.441-3.179)	0.737
ST2	0.529 (0.285-0.984)	0.064	2.704 (1.227-5.957)	0.013	4.822 (2.449-9.498)	0.000
REG3 α	0.292 (0.145-1.587)	0.080	0.373 (0.133-1.047)	0.061	0.204 (0.079-1.525)	0.075

Table S13. Multivariate analysis for aGVHD II-IV including ST2 and REG3 α .

ST2 and REG3 α dynamics on NRM

NRM No Yes

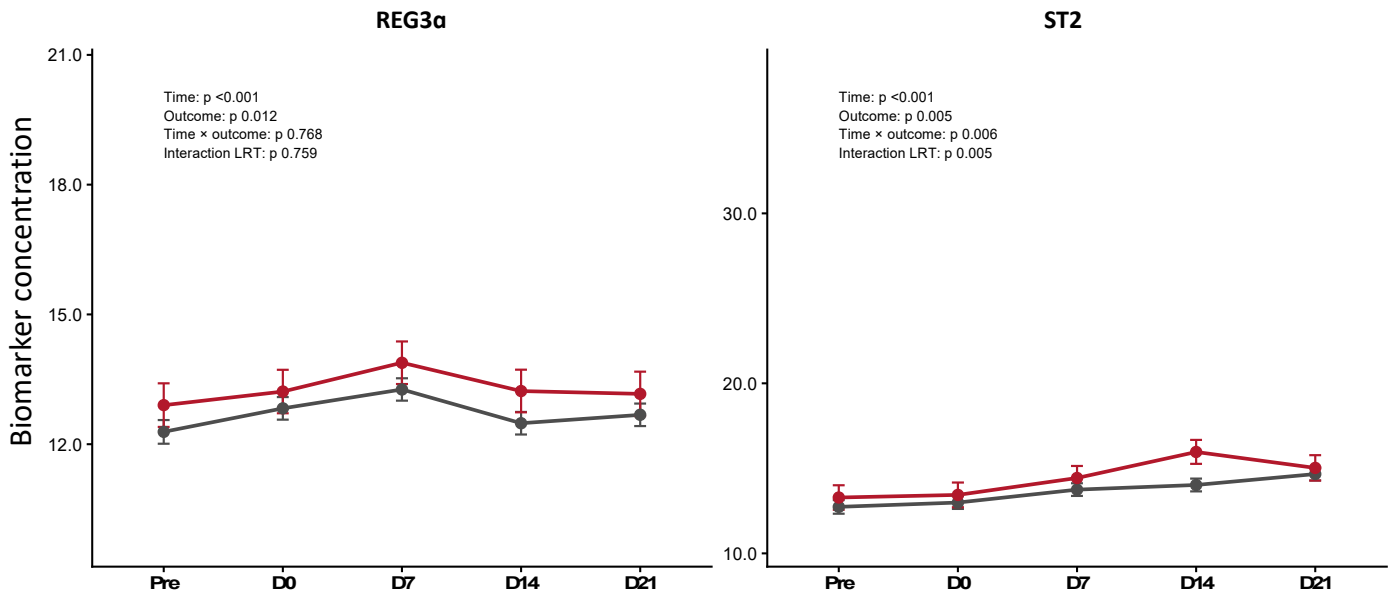


Figure S2. Dynamic model of ST2 and REG3 α for NRM.

ST2 and REG3 α dynamics on OS

Death — No — Yes

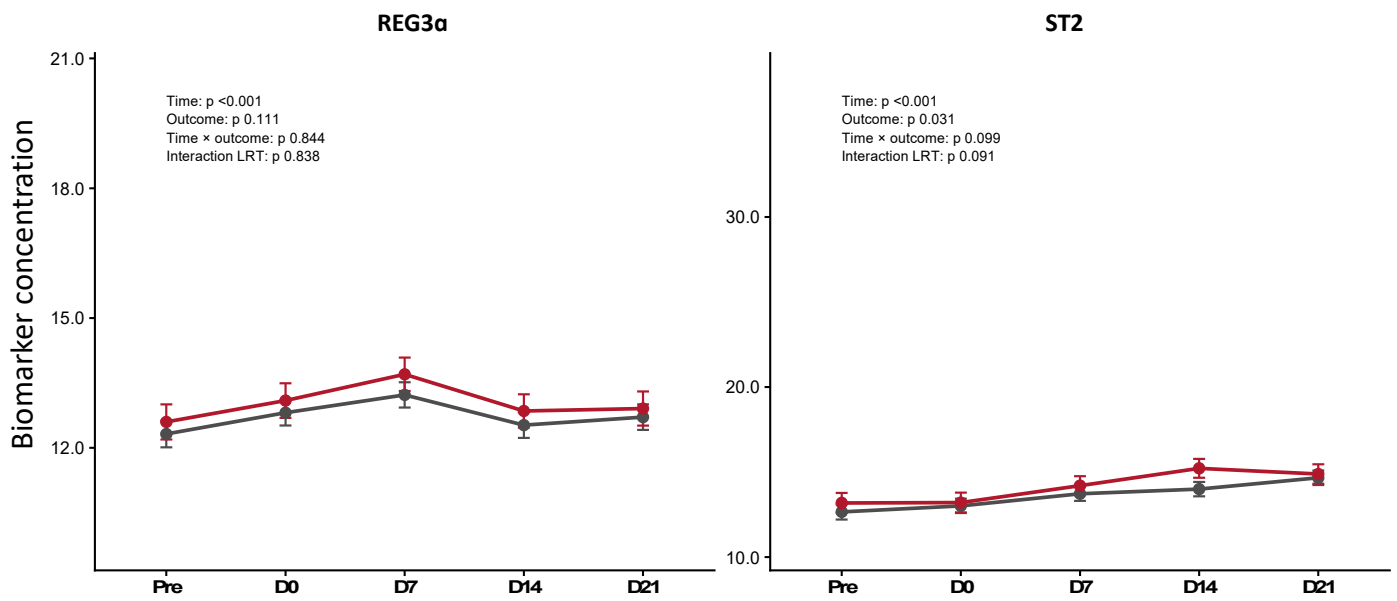


Figure S3. Dynamic model of ST2 and REG3 α for OS.

ST2 and REG3 α dynamics on EFS

Event ● No ● Yes

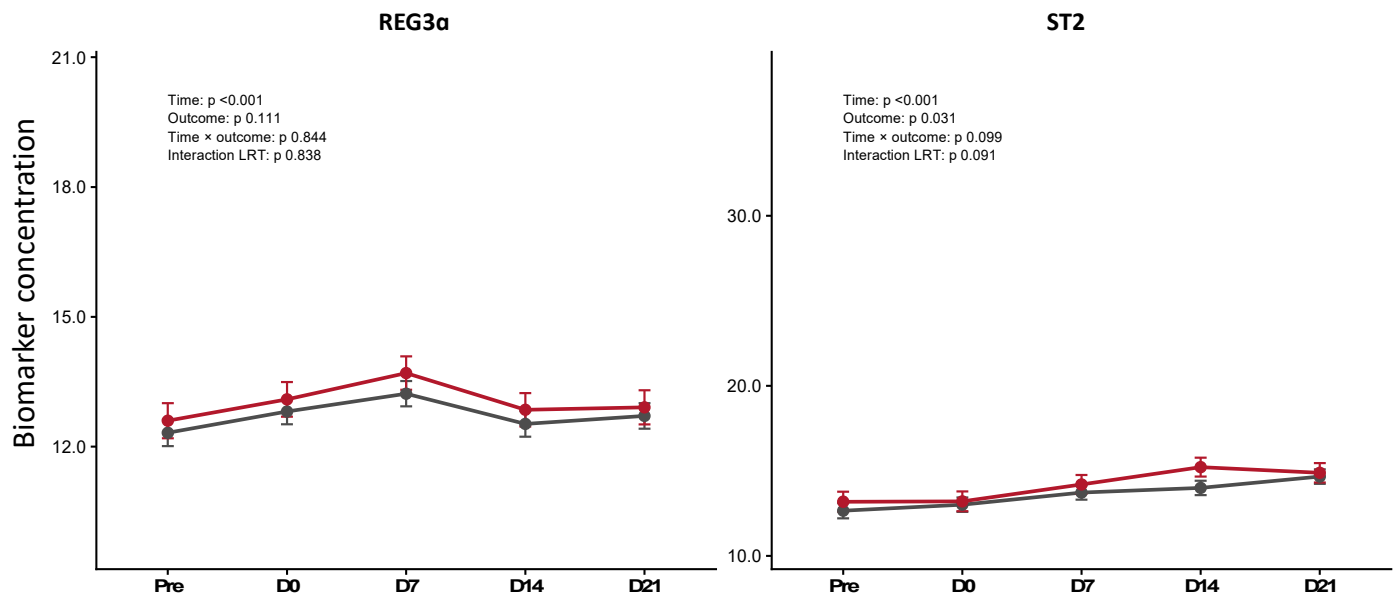


Figure S4. Dynamic model of ST2 and REG3 α for EFS.

ST2 and REG3 α dynamics on GRFS

GRFS No Yes

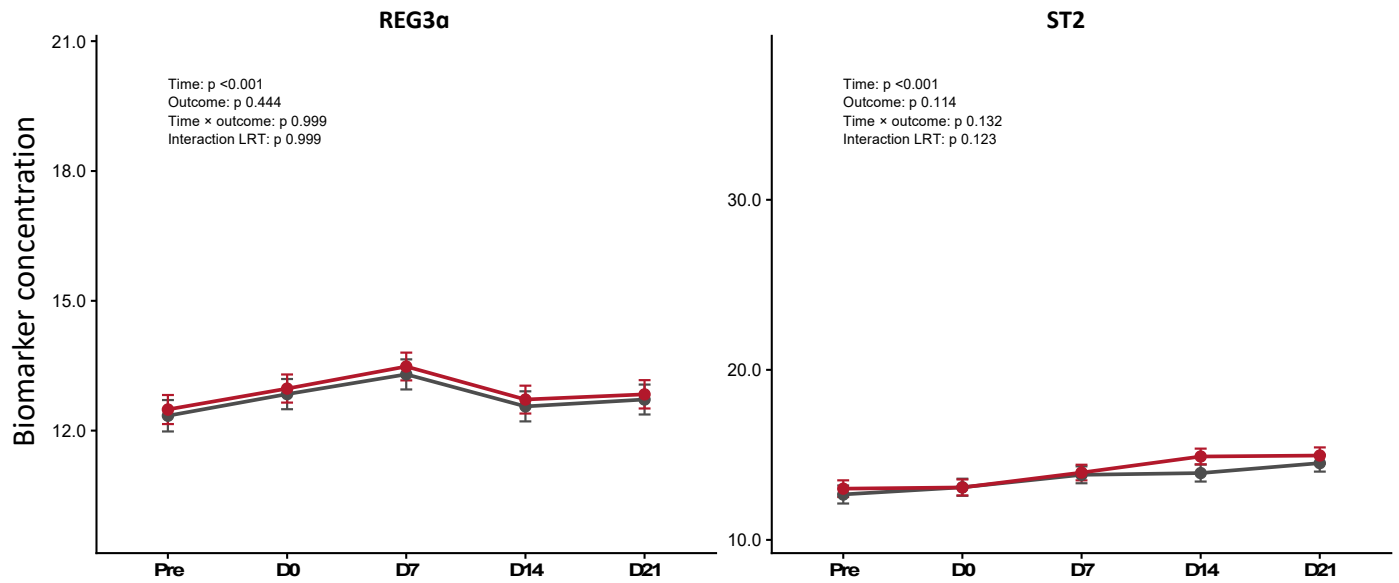


Figure S5. Dynamic model of ST2 and REG3 α for GRFS.

ST2 and REG3 α dynamics on aGVHD grades II-IV

aGVHD II-IV No Yes

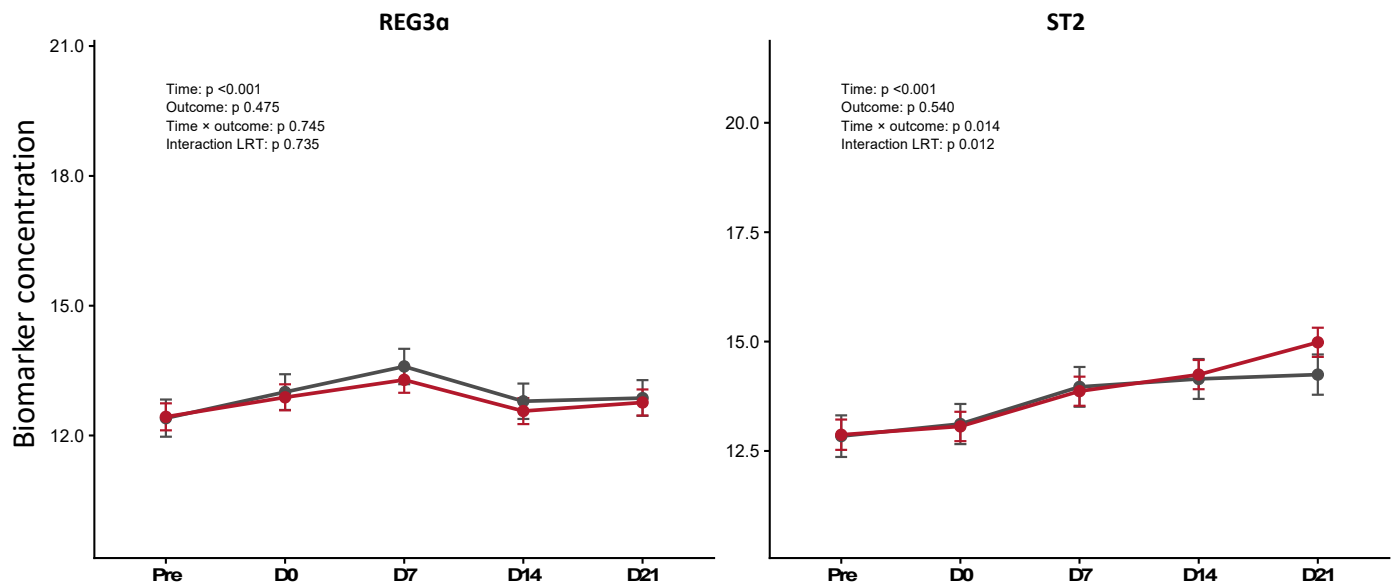


Figure S6. Dynamic model of ST2 and REG3 α for aGVHD grades II-IV.

	MAGIC							
	Day 0		Day 7		Day 14		Day 21	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
NRM	3.283 (0.987 - 10.921)	0.041	1.324 (0.364 - 4.814)	0.668	4.102 (0.533 - 31.562)	0.141	NS	0.394
OS	3.303 (1.127 - 9.675)	0.020	1.280 (0.417 - 3.928)	0.664	5.515 (0.731 - 41.601)	0.062	NS	0.316
GRFS	1.929 (0.890 - 4.181)	0.089	1.020 (0.433 - 2.400)	0.961	10.153 (1.376 - 74.885)	0.004	NS	0.173
EFS	3.456 (1.295 - 9.224)	0.008	1.625 (0.543 - 4.864)	0.380	6.821 (0.912 - 50.974)	0.029	NS	0.265
Grade II-IV aGVHD	0.990 (0.545- 1.801)	0.970	0.608 (0.329- 1.124)	0.110	1.563 (0.754- 3.242)	0.223	NS	0.028

NS: not stimable due to the lack of events

Table S14. Univariate analysis of the MAGIC algorithm and post-transplantation outcomes.

	MAGIC							
	Day 0 (n=71)		Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.078 (1.007-1.154)	0.030	1.065 (1.001-1.135)	0.045	1.070 (1.005-1.139)	0.032	1.091 (1.009-1.179)	0.029
Sex recipient male	2.627 (0.587-11.745)	0.206	2.232 (0.599-8.321)	0.231	2.468 (0.661-9.218)	0.178	2.722 (0.640-11.564)	0.175
HCT-CI $\geq$ 3	6.009 (1.451-24.884)	0.013	4.630 (1.356-15.801)	0.014	4.799 (1.336-17.240)	0.016	2.702 (0.676-10.793)	0.160
Myeloablative conditioning	0.892 (0.656-1.241)	0.469	0.931 (0.691-1.253)	0.637	0.921 (0.680-1.247)	0.594	0.984 (0.721-1.343)	0.920
DRI $\geq$ intermediate	0.472 (0.134-1.658)	0.241	0.501 (0.149-1.628)	0.263	0.374 (0.108-1.286)	0.118	0.489 (0.130-1.836)	0.289
Previous Allogenic transplantation	0.829 (0.074-9.295)	0.879	0.654 (0.064-6.685)	0.720	0.563 (0.055-5.720)	0.627	1.154 (0.114-11.594)	0.903
MAGIC algorithm	2.700 (0.787-9.264)	0.114	1.504 (0.372-6.083)	0.566	5.932 (0.730-48.201)	0.095	NS	0.999

NS: not stimable due to the lack of events

Table S15. Multivariate analysis for NRM including MAGIC algorithm.

	MAGIC							
	Day 0 (n=71)		Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.048 (0.996-1.102)	0.066	1.048 (0.998-1.100)	0.055	1.052 (1.002-1.105)	0.041	1.061 (1.003-1.121)	0.035
Sex recipient male	1.471 (0.457-4.729)	0.516	1.374 (0.474-3.980)	0.558	1.571 (0.547-4.514)	0.400	1.399 (0.464-4.210)	0.550
HCT-CI ≥ 3	3.186 (1.019-9.963)	0.046	2.652 (0.956-7.354)	0.060	2.5144 (0.874-7.236)	0.087	1.542 (0.471-5.043)	0.474
Myeloablative conditioning	0.906 (0.679-1.209)	0.504	0.919 (0.686-1.233)	0.576	0.917 (0.678-1.240)	0.574	0.995 (0.735-1.347)	0.975
DRI $\geq$ intermediate	0.787 (0.251-2.464)	0.681	0.831 (0.279-2.469)	0.739	0.653 (0.215-1.977)	0.451	0.851 (0.260-2.787)	0.790
Previous Allogenic transplantation	0.686 (0.071-6.553)	0.744	0.553 (0.063-4.861)	0.593	0.454 (0.051-3.985)	0.476	0.783 (0.092-6.644)	0.823
MAGIC algorithm	2.922 (0.982-8.692)	0.053	1.211 (0.371-3.949)	0.750	6.552 (0.848-50.600)	0.071	NS	0.998

NS: not stimable due to the lack of events

Table S16. Multivariate analysis for OS including MAGIC algorithm.

	MAGIC							
	Day 0 (n=71)		Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	0.999 (0.969-1.031)	0.984	1.003 (0.974-1.033)	0.830	1.01096 (0.980-1.043)	0.492	1.013 (0.986-1.046)	0.439
Sex recipient male	0.611 (0.255-1.464)	0.269	0.647 (0.277-1.513)	0.315	0.98914 (0.413-2.368)	0.980	0.7367 (0.312-1.737)	0.485
HCT-CI $\geq$ 3	2.858 (1.223-6.679)	0.015	2.681 (1.201-5.986)	0.016	2.6956 (1.159-6.269)	0.021	1.916 (0.790-4.642)	0.150
Myeloablative conditioning	0.977 (0.751-1.272)	0.865	0.956 (0.722-1.266)	0.756	0.97085 (0.740-1.272)	0.830	1.046 (0.792-1.381)	0.751
DRI $\geq$ intermediate	0.783 (0.327-1.876)	0.583	0.801 (0.347-1.846)	0.602	0.707 (0.289-1.725)	0.446	0.901 (0.361-2.248)	0.823
Previous Allogenic transplantation	1.553 (0.395-6.094)	0.527	1.373 (0.354-5.326)	0.646	0.898 (0.227-3.550)	0.878	1.464 (0.392-5.466)	0.570
MAGIC algorithm	2.008 (0.901-4.471)	0.087	0.901 (0.363-2.235)	0.822	10.010 (1.325-75.592)	0.025	NS	0.998

NS: not stimable due to the lack of events

Table S17. Multivariate analysis for GRFS including MAGIC algorithm.

	MAGIC							
	Day 0 (n=71)		Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	1.030 (0.987-1.075)	0.161	1.032 (0.990-1.075)	0.130	1.038 (0.995-1.082)	0.080	1.043 (0.996-1.092)	0.071
Sex recipient male	1.0121 (0.341-3.004)	0.982	1.092 (0.399-2.984)	0.863	1.231 (0.455-3.321)	0.682	1.071 (0.389-2.949)	0.894
HCT-CI ≥ 3	5.225 (1.850-14.751)	0.001	4.208 (1.648-10.746)	0.002	4.141 (1.157-10.917)	0.004	2.685 (0.996-7.231)	0.050
Myeloablative conditioning	0.9032 (0.680-1.199)	0.481	0.907 (0.678-1.215)	0.515	0.906 (0.671-1.223)	0.518	0.968 (0.718-1.306)	0.834
DRI $\geq$ intermediate	0.618 (0.212-1.798)	0.377	0.696 (0.249-1.941)	0.489	0.500 (0.173-1.440)	0.199	0.766 (0.260-2.251)	0.628
Previous Allogenic transplantation	1.553 (0.395-6.094)	0.927	0.831 (0.159-4.333)	0.827	0.735 (0.142-3.796)	0.713	1.174 (0.237-5.819)	0.843
MAGIC algorithm	3.490 (1.293-9.423)	0.013	1.688 (0.528-5.398)	0.377	8.326 (1.065-65.070)	0.025	NS	0.998

NS: not stimable due to the lack of events

Table S18. Multivariate analysis for EFS including MAGIC algorithm.

	MAGIC							
	Day 0 (n=71)		Day +7 (n=74)		Day +14 (n=73)		Day +21 (n=71)	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Recipient higher age	0.999 (0.979-1.021)	0.97	1.003 (0.983-1.024)	0.751	1.001 (0.979-1.022)	0.940	1.001 (0.979-1.023)	0.942
Sex recipient male	1.716 (0.861-3.420)	0.125	1.498 (0.778-2.886)	0.226	1.743 (0.908-3.344)	0.094	1.464 (0.768-2.792)	0.247
HCT-CI $\geq$ 3	1.099 (0.516-2.340)	0.806	0.975 (0.459-2.068)	0.947	1.077 (0.497-2.337)	0.849	9.034 (0.409-1.995)	0.802
Myeloablative conditioning	0.963 (0.791-1.172)	0.707	0.974 (0.798-1.189)	0.794	0.961 (0.792-1.166)	0.686	1.021 (0.831-1.255)	0.841
DRI $\geq$ intermediate	0.769 (0.380-1.555)	0.465	0.804 (0.407-1.585)	0.528	0.887 (0.449-1.751)	0.729	0.897 (0.454-1.772)	0.755
Previous Allogenic transplantation	2.155 (0.783-5.934)	0.137	2.873 (0.988-8.355)	0.052	1.818 (0.626-5.274)	0.271	2.308 (0.818-6.511)	0.114
MAGIC algorithm	0.828 (0.438-1.565)	0.562	0.529 (0.272-1.031)	0.061	1.543 (0.722-3.298)	0.265	NS	0.997

NS: not stimable due to the lack of events

Table S19. Multivariate analysis for aGVHD II-IV including MAGIC algorithm.

Endpoint	HR (95% CI), p-value
NRM	2.309 (0.895-5.959) 0.084
OS	1.309 (0.613-2.794) 0.487
GRFS	1.458 (0.756-2.813) 0.261
EFS	0.802 (0.232-2.774) 0.727
aGVHD II-IV	1.196 (0.628-2.279) 0.586

Table S20. Association between grade ≥ 3 infections and study endpoints.