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Baricitinib for Graft-Versus-Host Disease Prophylaxis after HLA-matched Hematopoietic Cell Transplantation

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Title:

Baricitinib for Graft-Versus-Host Disease Prophylaxis after HLA-matched Hematopoietic Cell Transplantation

Running title: Baricitinib for GVHD Prophylaxis

Key Points:

- Baricitinib when added to standard GVHD prophylaxis was safe and well tolerated in matched allogeneic hematopoietic cell transplantation.
- Baricitinib resulted in low rates of severe acute GVHD, disease relapse and graft failure.

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Deidentified individual participant data that underlie the reported results will be made available 3 months after publication for a period of 5 years after the publication date. Proposals for access should be sent to markschroeder@wustl.edu. The study protocol can be accessed on ClinicalTrials.gov as #NCT04131738

Abstract:

Acute graft-versus-host disease (aGVHD) is a leading cause of non-relapse mortality after allogeneic peripheral blood hematopoietic cell transplant (PB-HCT). The role of baricitinib, a balanced JAK1/2 inhibitor, for GvHD prophylaxis is unknown. Baricitinib after HLA-matched allogeneic PB-HCT was studied in a single-arm, open-label, phase I trial in 24 subjects. Subjects received baricitinib at 2 or 4 mg daily from days -3 to +100 post PB-HCT, followed by a taper. No primary graft failure, delayed engraftment or grade III/IV adverse events were observed. Overall incidence of grade III/IV aGVHD was 12.5% (3/24), of which 2/3 occurred after baricitinib discontinuation. Mild-moderate chronic GVHD occurred in 45.8% subjects, with no cases of severe chronic GVHD. Transplant-related mortality at day +180 was 8% (2/24). The 1-year OS was 70% (95% CI 48-85%), PFS 62% (95% CI 40-78%), and refined GVHD and relapse free survival (GRFS) 33% (95% CI 15-52%). Correlative studies confirmed on-target effect of baricitinib resulting in downregulation of JAK1/2 pathways. Baricitinib is safe and tolerable after HLA-matched allogeneic PB-HCT with no episodes of primary graft failure and low rates of grade III/IV acute GVHD while on therapy.

Introduction:

JAK inhibitors are being evaluated for both prophylaxis and treatment in acute and chronic graft-versus-host disease (GVHD). Acute GVHD (aGVHD) affects 40 – 50% of allogeneic hematopoietic cell transplant (allo-HCT) recipients and 10 – 15% of cases can be severe. Ruxolitinib is an oral, selective JAK1 and JAK2 inhibitor with FDA approval for treatment of steroid-refractory acute (SR-aGVHD) and chronic GVHD (cGVHD)[1,2]. Post-transplant ruxolitinib for cGVHD prophylaxis following reduced intensity matched donor allo-HCT is currently being studied in patients with acute myeloid leukemia (AML), myelodysplasia syndrome (MDS) and myelofibrosis, with encouraging preliminary results[3,4]. Recently, itacitinib has also been evaluated for GVHD prophylaxis in peripheral blood haploidentical HCT[5] and matched allo-HCT[6] . Baricitinib is being evaluated for treatment of cGVHD[7] but has not been studied for GVHD prophylaxis[7]. Preclinical results support the investigation of baricitinib as the best-in-class JAK inhibitor for aGVHD[8,9]. Despite evidence that JAK inhibitors can treat acute and cGVHD, their role in GVHD prophylaxis is understudied and the efficacy of baricitinib remains unknown in allo-HCT patients.

T-cell activation in aGVHD is in part driven by inflammatory cytokine interleukin 6 (IL-6) and interferon gamma (IFNγ) which signal through the JAK1/2 and down-stream STAT1/3 axis. JAK-STAT signaling is critically important for the development of aGVHD in mouse models[10,11]. We and others have published preclinical data highlighting the importance of IFNγR signaling in mediating the trafficking of effector T cells in aGVHD[12,13]. In addition, our group demonstrated that IFNγR/IL-6R or downstream JAK1/JAK2 are optimal targets to prevent or treat GVHD while preserving both GvL effect and donor cell engraftment following MHC-mismatched allo-HCT[8]. Baricitinib inhibits downstream IFNγR and IL-6R signaling through the JAK 1/2-STAT1/3 axis. In a major mismatched allo-HCT mouse model, baricitinib completely prevented GVHD[8], effectively inhibiting allogeneic effector T-cell activation, promoting regulatory T-cell expansion, reducing T-cell trafficking to GVHD target organs, and suppressing antigen presentation. GvHD prophylaxis with baricitinib outperformed ruxolitinib regardless of concurrent IL-6R inhibition. Moreover, baricitinib was also efficacious in treating ongoing GVHD and acute graft rejection[9,14]. Once GVHD was established, mice that received baricitinib had significantly lower clinical GVHD scores and all recovered from GVHD. Furthermore, Baricitinib did not impair neutrophil, lymphocyte or platelet engraftment[9]. Based on these preclinical results, we hypothesize that baricitinib is the best-in-class JAK inhibitor for the prevention and treatment of acute and chronic GVHD.

Currently, baricitinib is indicated for the treatment of adult patients with moderate to severe active rheumatoid arthritis (RA), who have had an inadequate response to TNF antagonist therapies. Although the FDA approved dose of baricitinib for RA in the United States is 2 mg, the higher 4 mg dosing showed higher efficacy in several RA trials[15]. The marginally higher incidence of thromboembolic events led to the FDA approval of the lower 2 mg dose of baricitinib[16].

The objective of this phase I trial was to evaluate safety and preliminary efficacy of baricitinib (2 mg and 4 mg) when combined with standard GVHD prophylaxis backbones in HLA matched allo-HCT. Based on preclinical and clinical data, we hypothesized that inhibition of the JAK-STAT axis downstream to IFNγR and IL-6R signaling by baricitinib will prevent GVHD following allo-HCT without affecting engraftment.

Methods:

Study design

This was a single-center, single-arm, open-label phase I study. Enrollment occurred from 7/2020-8/2021 at Siteman Cancer Center at Washington University School of Medicine in St. Louis and Barnes Jewish Hospital. The retrospective age/sex control cohort underwent HCT from 8/2016-3/2021 at the same institute. The trial received Washington University institutional review board's approval and was conducted in accordance with the Declaration of Helsinki. This trial was registered at ClinicalTrials.gov NCT04131738.

Patients

Patients with AML, acute lymphocytic leukemia (ALL) or MDS in complete morphological remission and Hodgkin or non-Hodgkin lymphoma ≥ 2nd complete or partial remission due to receive peripheral blood allo-HCT from HLA matched related or 8/8 matched unrelated donors (MUD) were eligible for study. Major exclusion criteria were prior allo-HCT, uncontrolled infection, end-organ dysfunction, myelofibrosis or other diseases known to delay engraftment, history of thrombosis or thrombophilia, recent myocardial infarction or embolic stroke (Figure 1A). All patients gave written informed consent.

Treatment

Recipients underwent institutionally standard myeloablative (MAC) or reduced intensity (RIC) conditioning followed by infusion of G-CSF mobilized peripheral blood stem cells on day 0 from related or unrelated HLA matched donors. A minimum CD34+ cells/kg dose of 2.0 x 10^6 was required. The GVHD prophylaxis backbone was either Tacrolimus/mini-Methotrexate (Tac/MTX), with Thymoglobulin (ATG) added for MUD allo-HCT (Figure 1B), or post-transplant cyclophosphamide (PTCy) followed by Tacrolimus/Mycophenolate mofetil (Tac/MMF) (Figure 1C, Supplementary Table 1)[17,18]. Baricitinib was given as either 2 mg or 4 mg PO daily from day -3 prior to HCT to +100, followed by a taper to 2 mg daily for 1 month, then 2 mg every other day for 1 month and then discontinued. Baricitinib dose reduction in the first 30 days after transplant was permitted only for non-hematological toxicities. Patients were recruited sequentially to the 2 and 4 mg cohorts. A 3-patient safety lead in phase was introduced at each dose level to confirm safety and tolerability before recruiting the remaining 9 patients. Acknowledging the change in standard of care in 12/2021, PTCy/Tac/MMF prophylaxis was allowed if preferred by the treating physician, once we confirmed engraftment in the 2 mg cohort with the previous Tac/MTX prophylaxis (+/- ATG) standard. Baricitinib had to be stopped for graft failure or severe infection. Dose modifications and guidance for supportive care are described in the supplemental material (Supplemental Table 2).

Study end points

The primary end points were the cumulative incidence of graft failure at day 30 ± 2 post-HCT and cumulative incidence of grade III-IV aGVHD by day 100. Secondary end points were transplant-related mortality and adverse events by day 180. Exploratory end points included platelet engraftment rates at day 30; median progression free survival (PFS), overall survival (OS), GVHD and relapse free survival (GRFS) at 6 months and 1 year, incidence of infectious complications by 1 year, grade II-IV and grade III-IV GVHD by day 180, and cumulative incidence of cGVHD at 1 year. Adverse events (AEs) were recorded and graded using National Cancer Institute Common Toxicity Criteria (NCI CTC) version 3.0. Incidence and severity of aGVHD and cGvHD were reported according to established criteria[19]. Patients were followed for 1 year after completion of baricitinib treatment or until death. Data collection concluded 6.1.2022. Definitions of terms and abbreviations (Supplementary Table 3)

Single cell RNA sequencing (scRNA seq) and flow cytometry

Correlative studies compared baricitinib group to contemporaneous retrospective controls that underwent matched allo-PB-HCT (Table 1). Blood samples from baricitinib-treated and control patients were collected at indicated time points (± 3 days) for peripheral blood mononuclear cells (PBMCs) isolation. PBMCs were evaluated utilizing scRNA-seq and spectral flow cytometry. Certain samples were unavailable or excluded due to poor viability, insufficient quantity, or failed quality control, as outlined in the consort diagram (Supplemental Figure 1A-B). Comprehensive methodologies, including sample processing, library preparation, and detailed bioinformatic and gating pipelines, are provided in the Supplementary Information.

Statistical Analysis

The sample size of n=24 was determined based on clinical feasibility, with an 86% chance to observe at least 1 event out of 12 patients by day +30 if the "true" incidence of primary graft failure was ≥ 15%. Exact binomial method was used to calculate the incidence of primary graft failure, grade III/IV aGVHD, and their 95% confidence intervals (CIs). The Gray sub-distribution method was used to estimate the cumulative incidences of aGVHD, cGVHD, platelet and neutrophil engraftment and relapse, while treating death as the competing event. Kaplan-Meier product limit estimator was used to describe the distribution of the RFS, GRFS and OS. Between-group differences in the time-to-event curves were compared using Fine-Gray test for cumulative incidences and log-rank test otherwise. All data from scRNA seq or flow cytometry were presented as mean ± standard deviation (SD). For all comparisons between groups, unpaired two-tailed Student's t-tests and ANOVA were used with a *p*-value <0.05 considered significant. Deidentified participant data are available on reasonable request at markschroeder@wustl.edu.

Results:

Baseline characteristics and Treatment

The overall population had a median age of 57 years and 50% were female (Table 1). No significant age or sex differences were appreciated between the dose cohorts. Underlying malignancies consisted predominantly of AML and MDS, which were equally represented in both cohorts. Morphologic remission was required for AML and MDS, but some recipients were minimal residual disease (MRD) positive by flow or cytogenetics at the time of transplant.

The 2 mg baricitinib group had fewer MRD positive patients pre-transplant compared to the 4 mg group (2/12 vs 6/12). Donor sources were matched sibling donors or MUD in equal proportions across all groups (MUD 6/12 vs 8/12). Overall, more patients underwent myeloablative rather than reduced intensity conditioning (19/24 vs 5/24). GVHD prophylaxis in the 2 mg group was exclusively Tac/MTX ± ATG (12/12) while patients in the 4 mg baricitinib group received either Tac/MTX ± ATG (7/12) or PTCy/Tac/MMF (5/12). Median follow up was 320 days (range 63-368).

Engraftment

No episodes of primary graft failure were observed in the 2 or 4 mg of baricitinib cohorts (Figure 2A). Neutrophil engraftment occurred prior to day 30 post allo-HCT in all patients with a median time to engraftment of 12.5 days (range 11-21), without significant differences amongst the 2 mg and 4 mg groups (12 vs 13.5 days). Platelet engraftment was delayed beyond day 30 in 1/12 patients from the 2 mg and 3/12 patients in the 4 mg group with a median time to platelet engraftment of 18.5 days for both groups (range 0-52). Retrospective controls had comparable platelet (14.5 days, range 9-31) and neutrophil (14.5 days, range 11-19) engraftment times regardless of GVHD prophylaxis backbone or conditioning consistent with previously published trials (Supplementary Figure 2A-F)[17]. Delayed engraftment in the 4 mg group was due in part to higher proportion of patients who received PTCy (Supplementary Figure 2D-E).

Graft vs Host Disease

Grade III-IV aGVHD occurred in 3 subjects, 1 in the 2 mg cohort and 2 in the 4 mg cohort (Figure 2B). In the 2 mg cohort, one patient developed grade IV SR-aGVHD of lower GI and liver (on day 169, off baricitinib). In the 4 mg cohort, two subjects developed grade IV lower GI aGVHD (day 289, off baricitinib) and grade III liver aGVHD (day 19, on baricitinib). Clinically significant grade II-IV aGVHD occurred in 8 out of 24 patients and was similar between the 2 mg and 4 mg dosing cohorts (4/12 vs 4/12) (Figure 2C). 5/8 cases of grade II-IV aGVHD occurred on baricitinib, of which 4/5 were grade II and did not progress beyond the stage established at the time of diagnosis. All cases with grade II GVHD were steroid responsive. The incidence of grade II-IV aGVHD in patients receiving PTCy was 40% and in patients receiving Tac-MTX ± ATG was 32% (Figure 2D). At one year follow up, 11 cases of cGVHD were identified,

8 moderate and 3 mild, evenly distributed amongst the 2 (6/12) and 4 mg (5/12) cohorts (Figure 2E). None of the cases developed severe cGVHD. Of the 8 moderate cases, 4 were *de novo* cGVHD. Of the 8 grade II-IV aGVHD patients, only 4 progressed to cGVHD on baricitinib (Supplementary Table 4). In these patients who had both acute and chronic GVHD, 3 out of 4 cases had discordant organ involvement from predominantly skin aGVHD to GI cGVHD.

Chimerism

Serial evaluation of relapse-censored T-cell chimerism in peripheral blood CD3 T-cells on day 100 was mixed in both RIC subjects (67.2%±22.7, mean±SD) and MAC subjects (83.0%±14.0) (Figure 2F). The frequency of peripheral blood CD3+ donor chimerism >95% (17% vs 16%) and >85% (33% vs 26%) by day 100 was similar between the 2 mg and 4 mg baricitinib dose cohorts. No difference in chimerism was seen between mini-MTX vs PTCy (79.9%±17.9 vs 70.2%±15.5) and acute GvHD vs no GvHD (78.1%±17.8 vs 74.0%±19.7) groups.

Efficacy

Median PFS was similar and not reached in either baricitinib cohort (Figure 2G, Supplemental Figure 2G). Median OS was similar amongst the 2 vs 4 mg baricitinib groups (5 vs 2.5 years, p=0.77) (Figure 2H, Supplemental Figure 2H). The overall incidence of disease relapse at 1 year was 21%, with 2 relapses occurring in the 2 mg cohort and 3 in the 4 mg cohort. All 5 relapses occurred in patients with AML, one of which was MRD+ at the time of HCT. Of the total 8 subjects with MRD+ MDS or AML, 2 relapsed within day 100 post-HCT, but others remained in complete remission at 1 year follow up. The composite endpoint of severe III/IV aGVHD, moderate/severe cGVHD, relapse or death was not statistically significant between 2 mg and 4 mg baricitinib dose cohorts (median GFRS 23.5 vs 7.5 months, p=0.29) (Figure 2I, Supplemental Figure 2I). Overall, the 1-year OS was 70% (95% CI 48-85%), PFS 62% (95% CI 40-78%), and refined GRFS 33% (95% CI 15-52%).

Safety and tolerability

No severe adverse events (SAE) related to baricitinib were reported. Grade 3-5 non-hematological SAE included mucositis (n=10), hypertension (n=4), febrile neutropenia (n=7), infections/sepsis (n=7), anorexia (n=5), diarrhea (n=4), thrombosis (n=4), BK cystitis (n=2), grade 5 intracerebral hemorrhage (n=1), and grade 5 pneumonia (n=1). AE

of special interest were thromboembolic events given the increased risk of thrombosis associated with baricitinib. Of the 4 venous thromboembolic events, 1 occurred in the 2 mg and 3 in the 4 mg baricitinib group. Two thromboses were deemed unrelated to baricitinib, given that one deep vein thrombosis (DVT) was chronic and the other occurred while off drug. The remaining two thromboembolic events, one in each of the 2 and 4 mg dose cohort were possibly related to baricitinib. Five deaths occurred on study and were secondary to disease relapse (3/6), intracerebral hemorrhage (1/6, not related to thrombocytopenia) and aGVHD (1/6), all unrelated to study drug. Thrombocytopenia was the principal reason for dose holds or reductions, occurring less frequently in the 2 mg cohort (43.9%; mean RDI 84.9%) than the 4 mg cohort (80%; mean RDI 77.3%). Consequently, a higher proportion of patients achieved an RDI >85% in the 2 mg cohort (58.3% vs 41.7%) (Supplementary Figure 3A-D). Treatment related mortality at day 180 was 8% (2/24) and deaths were unrelated to study drug.

Single Cell RNA Sequencing

To assess how baricitinib impacts GvHD, we performed scRNA-seq of PBMCs from transplant recipients who received standard-of-care immunosuppression with or without baricitinib. UMAP-based clustering of PBMCs identified 15 distinct cell clusters across all samples (Figure 3A). The representative UMAP plots revealed key differences in the naïve T-cell population between the baricitinib treated and control cohorts (Figure 3B-D). Patients on baricitinib had consistently higher frequency of naïve T cells (Figure 3E) relative to T effector cells (Figure 3F), with a higher naïve to effector T-cell ratio on day 30 and day 100 (Figure 3G). This pattern of high naïve to effector T-cell ratio on day 30 was predictive of patients without aGvHD, but not cGvHD (Figure 3H-I). We next performed differential gene expression (DGE) and gene set enrichment analyses (GSEA) to identify molecular mechanisms underlying differences in immune cell subsets between baricitinib and control group (Figure 4). Day 30 and day 100 GSE of naïve T cells, effector T cells and classical monocytes showed significant down-regulation of genes in the JAK1/2, Inflammation, and GvHD signaling pathway in the baricitinib group (Figure 4G-H). In contrast, relatively few pathways were differentially upregulated in the baricitinib group (Supplemental Figure 4). We show the representative genes of each enriched pathway on day 30 (Figure 4I) and day 100 (Figure 4J). While the day 30 and day 100 baricitinib group share the same enriched pathways, they are driven by a different set of genes and on day 100 predominantly represented by classical monocytes.

The significant downregulation of JAK1/2 pathways (IFNγ response, IFNα response, and IL6/JAK/STAT3 signaling) by scRNA seq (Figure 4I) and CXCR3 by flow cytometry (Figure 5A) indicates on-target activity of baricitinib. The transcriptomic profiles were similar between 2 mg and 4 mg baricitinib doses and were not affected by PTCy (Supplementary Figure 5) or the presence of aGvHD (Supplementary Figure 6). In summary scRNA seq analysis of PBMCs from baricitinib treated patients revealed an immune-regulatory cell profile consistent with JAK1/2 inhibition in T-cells and monocytes suppressing GvHD.

Immunophenotyping of PBMCs

To validate scRNA seq results, we immunophenotyped PBMCs by flow cytometry. Hierarchical clustering of 20 cell surface markers demonstrated a clear separation of baricitinib and control groups based on CXCR3 (CD183) expression (Figure 5A). UMAP showed distinct clustering of PBMCs in the baricitinib and control groups (Figure 5B-C). The relative frequency and absolute count of T cells across the groups was comparable (Figure 5D, Supplementary Figure 7A). Consistent with the scRNA-seq, the baricitinib group had significantly higher frequency of CD3 and CD4 naïve T-cells on day 100 compared to control (Figure 5E, Supplementary Figure 7B). Conversely, the relative and absolute frequency of activated T cells (CD38+HLADR+) was significantly lower in the baricitinib group on day 30 and day 100 (Figure 5F, Supplementary Figure 7C). The lower frequency of CD8 Tem cells on Day 30 in the baricitinib group was most notable within the activated T-cell subsets (Figure 5G-H, Supplementary Figure 7D-E). Consequently, the previously established naïve to activated T-cell ratio was significantly increased amongst CD3 T-cells in the baricitinib group on day 100 after allo-HCT (Figure 5I, Supplementary Figure 7F). This trend towards high naïve to activated T-cell ratio in patients without acute GvHD was observed early on day 30 (Figure 5J, Supplementary Figure 7G). Given the limited size of our cohort, low naïve to activated T-cell ratios were not predictive of acute or chronic GvHD on day 100. Early NK and cMo reconstitution following HCT has been associated with lower rates of acute GvHD [20,21]. In the baricitinib group early on day 30 we observed significantly higher relative and absolute frequency of NKs cells and cMo compared to control (Figure 5K-L, Supplementary Figure 7H-I) with cMo in the baricitinib group retaining their anti-inflammatory phenotype (Figure 4I-J).

In summary, immunophenotyping of PBMCs by flow cytometry showed baricitinib-driven differential cell counts with increased naive to activated T cell ratio, higher frequency of NKs and cMo with fewer CD8 Tem cells suggestive of a more immune-suppressed and regulatory environment.

Discussion:

This is the first prospective study evaluating the safety and preliminary efficacy of baricitinib for GVHD prophylaxis following HLA-matched, PB-HCT. In this single-arm, single-center, open-label design, 24 subjects were enrolled into 2 mg and 4 mg baricitinib dosing cohorts and treated in combination with either of two standard GVHD prophylaxis backbones. No primary graft failure was observed, with a short median time to neutrophil engraftment (12.5 days) and slightly delayed median time to platelet (18.5 days) engraftment compared to historical cohorts[17]. Thrombocytopenia, predominantly in the 4 mg cohort, required dose holds and dose adjustment, but no grade 3-4 non-hematological toxicities were observed. The incidence of grade III-IV aGVHD by day 100 was 4% (1/24) and overall 12.5% (3/24) with 2/3 cases occurring after baricitinib discontinuation. Treatment related mortality at day 180 was 8% (2/24) and deaths were unrelated to study drug. Incidence of DVTs was 12.5% (4/24), two of which may have been related to baricitinib. Overall, baricitinib was safe and well tolerated.

The incidence of aGVHD (grade II-IV 33%) and cGVHD (moderate 33%) was comparable to that reported in other contemporary GVHD prophylaxis trials with similar patient populations (aGVHD II-IV 26%, cGVHD 27-30%), conditioning (MAC 32%, RIC 29%), and prophylactic regimens (PTCy 26%, Tac/MTX 28%), as well as our previous institutional data[17,22–27] . The sites and median time of aGVHD onset were similar to prior studies[28–30]. aGVHD that occurred on baricitinib was predominantly low grade, steroid responsive, and did not progress beyond the stage at diagnosis. The more severe grade III-IV aGVHD cases occurred late after baricitinib taper and were steroid refractory. The incidence of SR- aGVHD on baricitinib (12.5%) was lower compared to prior studies (31%)[31]. Baricitinib did not seem to delay onset of cGVHD but may require prolonged course and taper to prevent later onset aGVHD. Along those lines, an extended maintenance with ruxolitinib was shown to be effective in preventing cGVHD[4] and in the itacitinib prophylaxis study, dosing was extended to day 180 followed by two-month taper because of flares in aGVHD during JAK inhibitor taper[5]. The overall incidence of moderate-severe cGVHD was 33% of which 50% were *de novo,* consistent with prior reports[23] . There were no cases of severe cGVHD during the 1 year follow up period.

Disease relapse rate of 21% at 1 year since allo-HCT is on par with prior studies, suggesting that graft-vs-leukemia effect is preserved with baricitinib.

The study was not adequately powered to account for differences in outcomes caused by donor selection (MUD vs matched sibling), various conditioning (RIC vs MAC) and GVHD prophylaxis (Tac/MTX vs PTCy) regimens. All 5/24 subjects that received PTCy were in the 4 mg baricitinib cohort at a time when PTCy surpassed Tac/MTX as standard of care GVHD prophylaxis. Mixed donor chimerism <85% is associated with lower rates of cGVHD at the expense of GVL effect[32–36]. Regardless of conditioning, complete donor chimerism >95% was achieved only in 35% of all subjects (Tac/mini-MTX 42%, PTCy 20%), which is significantly lower than reported in prior studies (Tac/MTX 68%, PTCy 68%)[37]. Subgroup analysis of 2 mg vs 4 mg baricitinib, Tac/MTX vs PTCy, patient with aGVHD vs cGVHD vs no GVHD did not show any significant differences in donor chimerism. Despite the high degree of mixed chimerism, GVHD and relapse rates remained consistent with prior studies.

Correlative studies confirmed robust on-target inhibition of JAK1/2 signaling pathway. Baricitinib led to an increase in naïve T cells with a relative decline in effector T-cell frequency resulting in high naïve to effector T-cell ratio. High naïve to effector ratios were notably found in patients without early acute GvHD early on day 30. Consistent with prior observations, we saw suppression of JAK1/2 dependent IFNγ, IFNα and IL6 signaling by scRNA seq across cell compartments, various conditioning intensities and GvHD prophylaxis regimens. The inhibition of JAK1/2 signaling had multiple downstream effects that may suppress alloreactivity. First, suppression of cytokine signals reliant on JAK1/2 impairs mutual activation of antigen presenting cells and allo-reactive T-cells, which was validated by gene expression profiles in both T-cells and monocytes. Furthermore, the relative increase in naive to effector T-cell ratios suggests impaired T-cell activation due to a direct effect of baricitinib on T-cells or an indirect effect on antigen presenting cells represented by monocytes. scRNA sequencing showed that these monocytes were less inflammatory, a finding unique to the current analysis that may help explain the low incidence of severe acute and chronic GVHD observed in both studies. Second, a downstream effect of JAK1/2 inhibition is impaired egress of T-cells to potential sites of GvHD by suppressing the CXCR3-CXCL9-11 chemokine axis. Specifically, baricitinib suppresses IFNγ-driven CXCR3 expression on T-cells and CXCL9-11 chemokine release by resident macrophages or stromal cells resulting in reciprocal failure in chemotaxis. This is reflected in the rise in peripheral blood monocytes with baricitinib and observed previously with itacitinib. Consistent with our itacitinib trial, we did not see increases

in peripheral blood regulatory T-cells on baricitinib. Based on our preclinical studies baricitinib increases frequency of regulatory T-cells in the spleen rather than blood.

In conclusion, the addition of baricitinib to standard GVHD prophylaxis in the peripheral blood HLA- matched allo-HCT setting regardless of conditioning intensity, is safe and tolerable, and results in low rates of severe grade III/IV aGVHD without compromising rates of disease relapse. Dose intensity of either 2 or 4 mg had similar effects on GVHD, although slightly longer platelet engraftment was observed at the 4 mg dose, along with 2 DVTs possibly related to baricitinib. Extended baricitinib prophylaxis beyond day 100 might be helpful to reduce late onset aGVHD. A prospective randomized trial is indicated to confirm these results.

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Authorship Contributions:

AC conducted final analysis of trial data and correlative studies and wrote the manuscript.

SK and RA designed correlative studies and subsequent analysis of all data and contributed to the writing of the manuscript.

SL, MH, JK, JE, CA, IP, MJ, PW, ZC, AFC, AG, GLU were essential in performing the clinical research study, collecting subject data and correlative studies, reviewed final study data, and contributed to the final manuscript.

FG designed the statistical analysis plan for the study, performed statistical analysis of data and contributed to the writing of the final manuscript.

JFD contributed to design of the clinical trial, performed the research, analyzed data and contributed to the writing of the manuscript.

MAS designed the clinical trial, developed the clinical protocol, supervised conduct of the clinical trial, designed and analyzed data and wrote the manuscript.

JC conducted pre-clinical studies supporting the clinical trial, helped in design of the clinical trial, designed correlative studies and performed subsequent analysis of all data and contributed to the writing of the manuscript.

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The authors declare no competing financial interests.

ZDC: Advisory Board-Vertex, Gamida Cell, BioLineRx, A2Bio.

AC: Advisory Board—Bristol Myers Squibb

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MAS: Adjudication and DSMB boards – GSK, Kura Oncology, Novo Nordisk

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	2 mg Baricitinib (n=12)	4 mg Baricitinib (n=12)	Total Baricitinib (n=24)	Historical Control (n=14)
Female, n (%)	7 (58)	5 (41)	12 (50)	6 (42)
Age at HCT, median (range)	54 (28-71)	61 (42-72)	58 (28-72)	67 (22-74)
HCT-CI score, n (%)				
0-1	11	7	18	10
2	0	2	2	2
3	1	3	4	2
4	0	0	0	0
³5	0	0	0	0
Diagnosis, n (%)				
AML	8	8	16	8
MDS	2	4	6	5
ALL	2	0	2	0
CMML	0	0	0	1
Measurable disease at HCT, n (%)				
None	2	5	7	8
NGS only positive	6	1	7	1
DAT mutation	3	1	4	1
Non-DAT mutation	3	0	3	0
MRD positive	2	4	6	5
Cytogenetics and NGS (non-DAT) positive	1	0	1	0
NGS (DAT) and flow MRD positive	1	0	1	5
Cytogenetics and flow MRD positive	0	2	2	0
Donor source				
MSib	6	4	10	0
MUD	6	8	14	14
MA conditioning, n (%)	11 (92)	7 (58)	18 (75)	8 (57)
Conditioning regimen, n (%)				
Cy-FxTBI	2	0	2	0
Bu-Cy	2	0	2	2
Flu-Bu4	7	7	14	4
Flu-Bu2	1	4	5	3
Flu-FxTBI	0	1	1	0
Flu-Cy-FxTBI	0	0	0	3
Flu-Mel	0	0	0	3
GvHD prophylaxis				
Tac-MTX	9	5	14	0
Tac-MTX-ATG	3	2	5	6
PTCy-Tac-MMF	0	5	5	8

Table 1. Patient characteristics

AML (Acute myelod leukemia), ALL (Acute lymphocytic leukemia), ATG (anti-thymoglobulin), Bu (Busulfan), CMML (Chronic myelomonocytic leukemia), Cy (Cyclophosphamide), Flu (Fludarabine), HCT (stem cell transplant), HCT-CI (HCT comorbidity index), MA (Myeloablative conditioning), MDS (Myelodysplastic syndrome), Mel (Melphalan), MMF (Mycophenolate mofetil), MRD (Measurable residual disease), MSib (Matched Sibling donor), MTX

474 (Methotrexate), MUD (Matched unrelated donor), NGS (Next generation sequencing), PTCy (Post-transplant

475 cyclophosphamide), Tac (Tacrolimus), TBI (Total body conditioning), Thymo (Thymoglobulin)

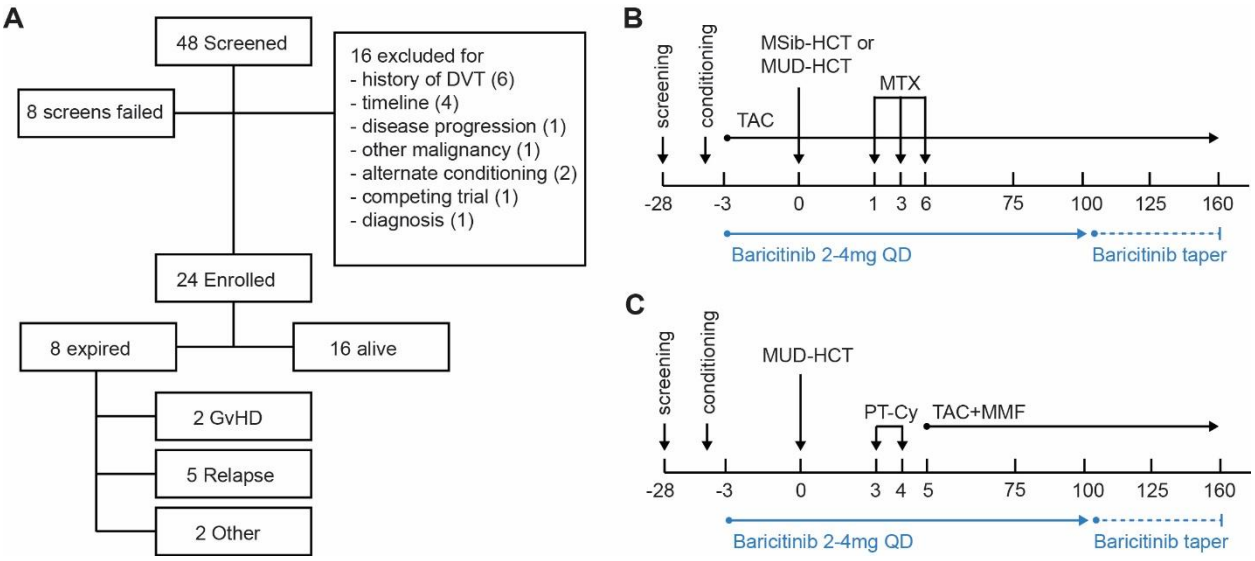


Figure 1. Study Design

(A) Consort diagram. **(B-C)** Patients with hematological malignancy due to receive allogeneic-HCT received GVHD prophylaxis with either 2 mg or 4 mg baricitinib PO daily from day -3 to +100 followed by a taper. Following myeloablative or reduced intensity conditioning patients were treated with either **(B)** Tacrolimus/Methotrexate (TAC/MTX) ± Thymoglobulin based or **(C)** post-transplant cyclophosphamide (PTCy) based GVHD prophylaxis as standard of care.

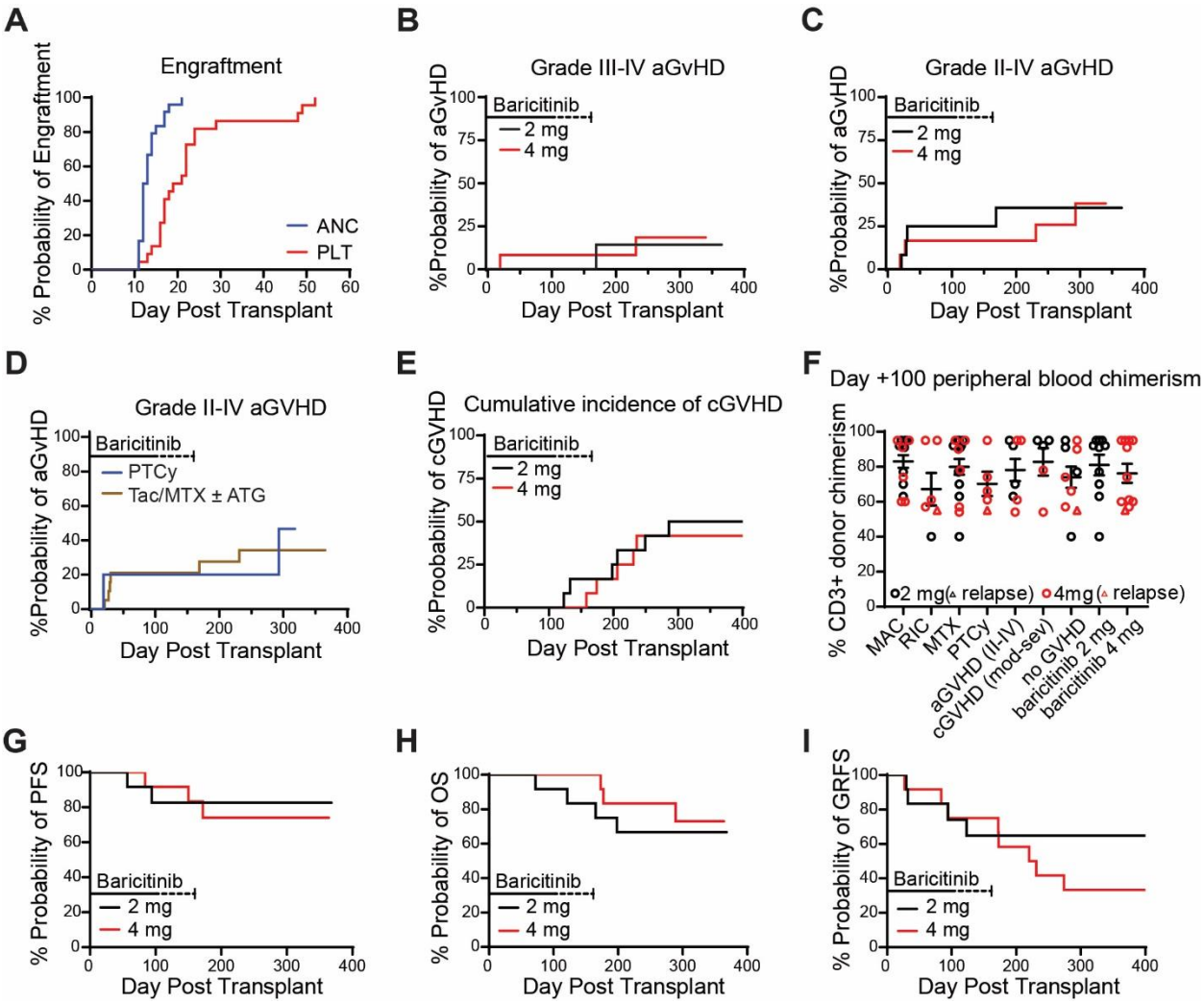


Figure 2. Engraftment, GvHD and outcome with baricitinib prophylaxis

(A) Platelet (PLT) and neutrophil (ANC) engraftment after allogeneic-HCT receiving baricitinib in addition to standard of care GVHD prophylaxis. **(B)** Cumulative incidence of Grade III-IV and **(C)** Grade II-IV acute GVHD (aGVHD) with 2 and 4 mg baricitinib. **(D)** Incidence of Grade II-IV acute GVHD in patients receiving PTCy vs Tac/MTX ± ATG. **(E)** Cumulative incidence of chronic GVHD (cGVHD) in the 2 and 4 mg baricitinib cohort. Duration of baricitinib taper outlined in plot insert. **(F)** Day +100 peripheral blood donor chimerism across indicated sub-groups. Cumulative rates of **(G)** progression free survival (PFS), **(H)** overall survival (OS) and **(I)** GVHD-free relapse-free survival (GFRS) in patients receiving 2 or 4 mg baricitinib for GvHD prophylaxis.

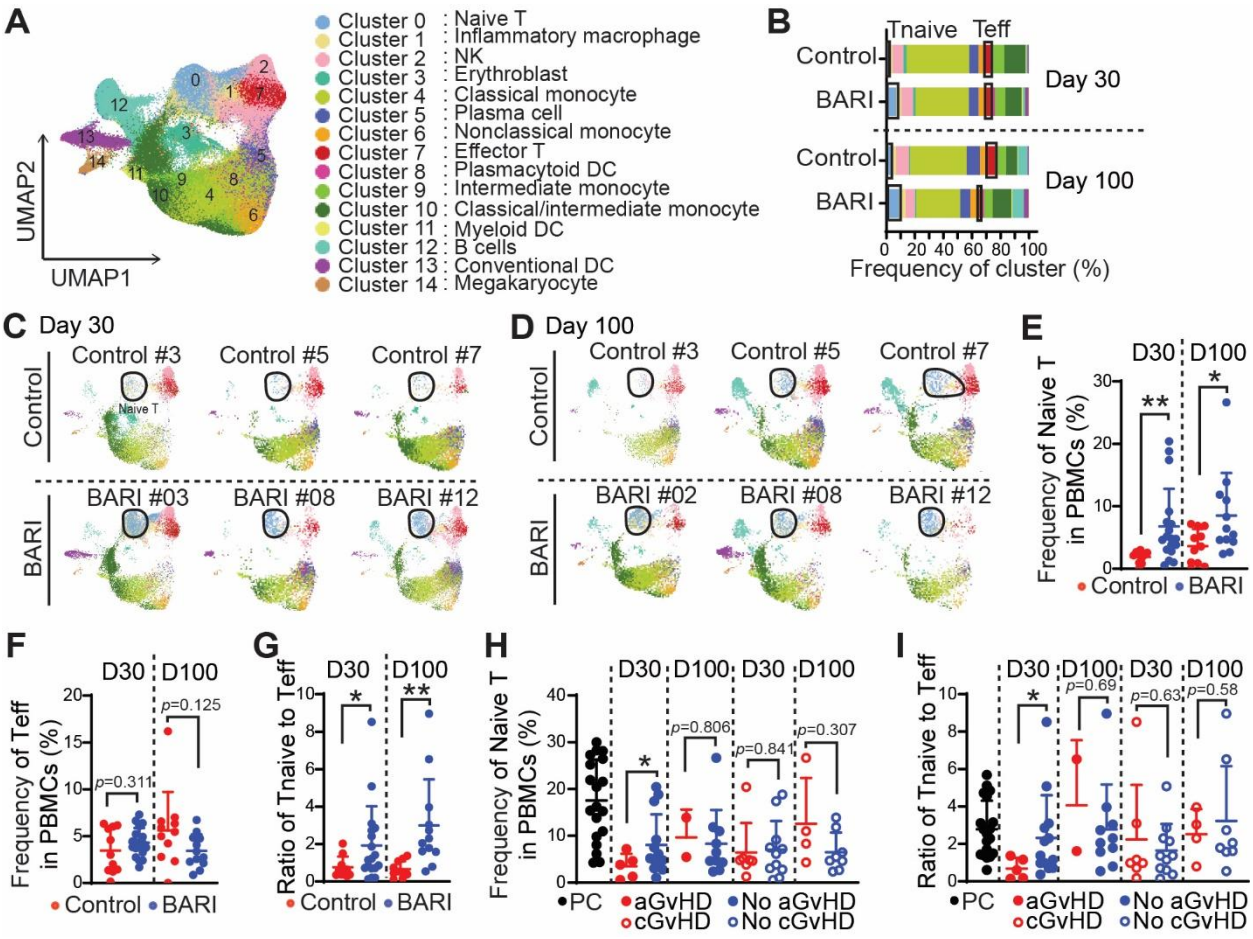


Figure 3. scRNA-seq analysis of PBMCs.

(A) UMAP plot of PBMCs identifying 15 clusters, with each cluster corresponding to a distinct cell type distinguished by color. **(B)** Bar graph showing the frequencies of identified clusters for control and baricitinib group on day 30 and day 100. **(C-D)** Representative UMAP plots from five individual samples each of control and baricitinib group on **(C)** day 30 and **(D)** day 100. **(E)** Frequencies of naïve T cells (clusters 0), **(F)** effector T cells (cluster 7) and **(G)** naïve to effector T-cell ratio in control and baricitinib group. **(H-I)** Within the baricitinib group, **(H)** Frequency of naïve T-cell and **(I)** Naïve to effector T-cell ratio in patients with aGvHD, cGvHD or no GvHD. PC indicates baseline samples prior to conditioning for allo-HCT. *p<0.05 and **p <0.01, mean ± SD shown.

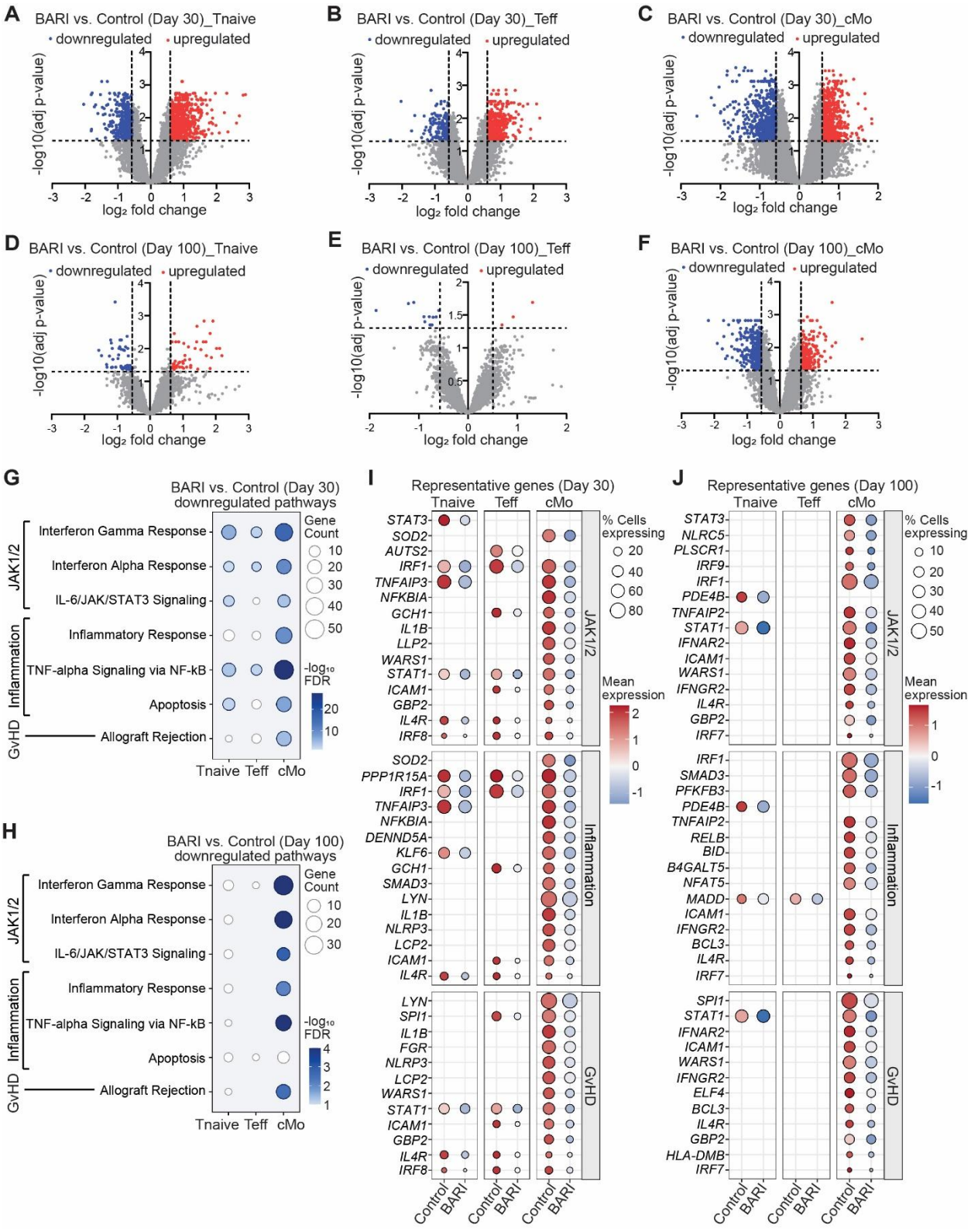


Figure 4. Differential Gene Expression (DGE) with gene set enrichment analysis (GSEA) of PBMCs after allo-HCT.

509 Volcano plots showing DGEs in naïve T, effector-T cells and classical monocytes from Day 30 **(A-C)** and Day 100 **(D-**
510 **F)** after HCT in control and baricitinib treated group. Significance of DGEs was defined as $p<0.05$ with a fold change
511 >1.5 (dotted line). GSEA of significantly down-regulated genes in baricitinib vs control group on **(G)** Day 30 and **(H)**
512 Day 100 after HCT using the Molecular Signatures Database (MSigDB). Representative genes of down-regulated
513 pathways in naïve T-cells, effector T-cells and classical monocytes on **(I)** Day 30 and **(J)** Day 100after HCT in control
514 and baricitinib treated group. Empty circles indicate non-significant.
515

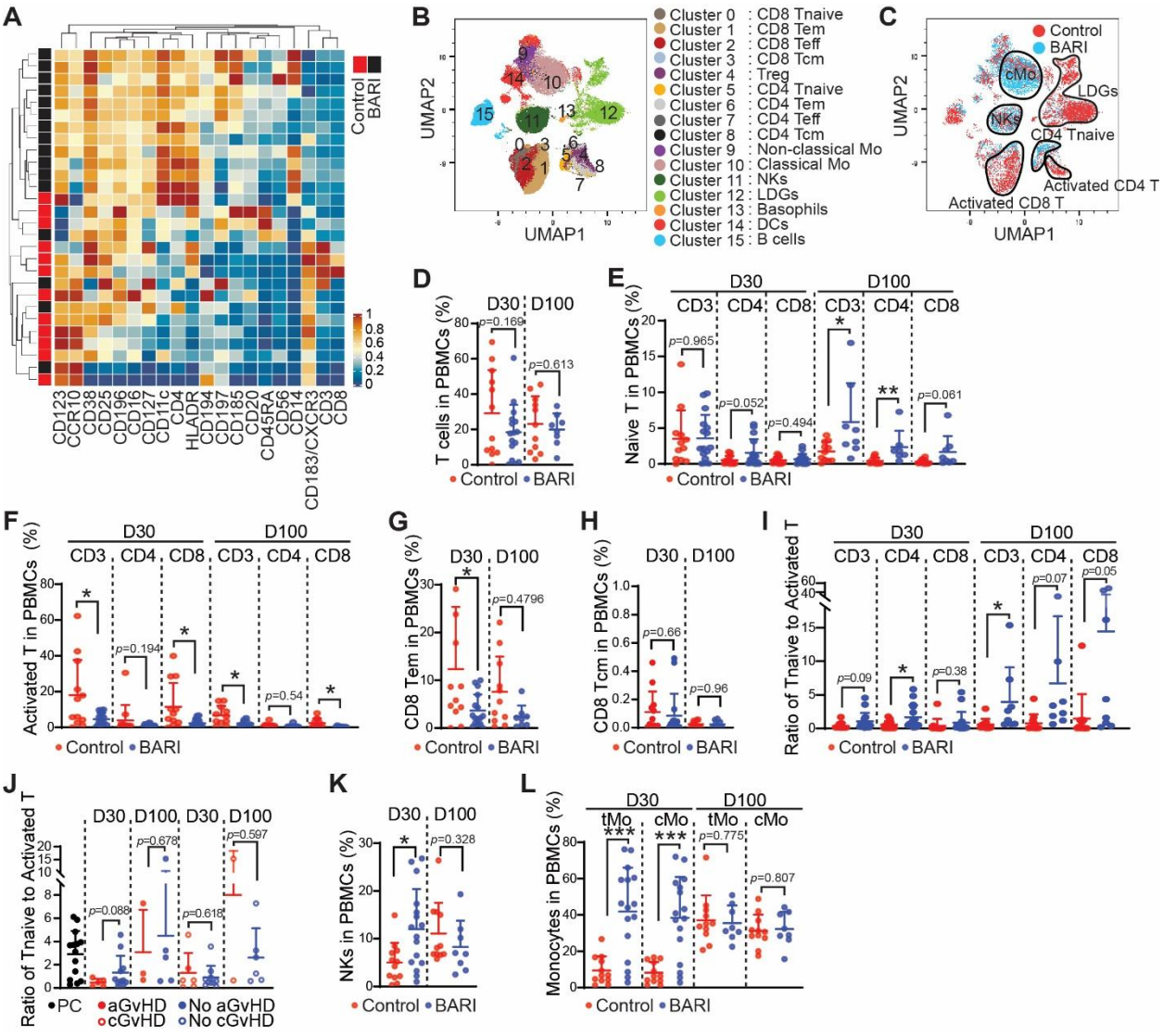


Figure 5. Immunophenotyping of PBMCs by flow cytometry.

(A) Heatmap with 20 cell surface marker intensities by flow cytometry on day 30. **(B-C)** UMAP showing manually defined cell populations on day 30. Frequency of **(D)** total T cells, **(E)** naive T-cells and **(F)** activated T cells subsets in control and baricitinib group on day 30 and 100. Frequency of **(G)** CD8 effector memory T cells (Tem), and **(H)** CD8 central memory T cells (Tcm). **(I)** Naïve to activated T-cell ratios within CD3, CD4 and CD8 T-cells subsets. **(J)** CD3 Naïve to activated T-cell ratio in patients with or without aGvHD within the baricitinib group. PC indicates pre-conditioning before allo-HCT. **(K)** Frequency of NK cells (NKs), **(L)** total (tMo) and classical monocytes (cMo). *p<0.05, **p <0.01, and ***p <0.001, mean ± SD shown.

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