HemaSphere
Powered by EHA

EHA EUROPEAN HEMATOLOGY ASSOCIATION

Letter

OPEN ACCESS

Sphingosine-1-phosphate Receptor-1 Agonist Averts the De Novo Generation of Autoreactive T-cells in Murine Acute Graft-versus-Host Disease

Madeleine Vollmer[1], Philip Smith[2], Christoph Bucher[3], Werner Krenger[1], Lukas T. Jeker[1,4]

Correspondence: Lukas T. Jeker (lukas.jeker@unibas.ch); Werner Krenger (werner.krenger@unibas.ch).

High-risk hematologic cancers are potentially cured by allogeneic hematopoietic stem cell transplantation (alloHSCT).[1] The success of this therapy is currently limited, however, by the occurrence of acute graft-versus-host disease (GVHD), an inflammatory process triggered by alloreactive donor T-cells.[2] The classical target organs of disease include skin, liver, and gastrointestinal tract. However, a prominent feature of patients with acute GVHD is also the disruption of regular posttransplantation T-cell immune recovery for which at least 2 mechanisms coalesce: GVHD itself[3] and also the contemporary measures employed to prevent this disease.[1,2] Improved modes for GVHD prophylaxis are needed that obviate the need for generalized immune suppression. Here, sphingosine-1-phosphate (S1P) receptor agonism may offer promise because it can efficiently prevent acute disease in preclinical models of GVHD.[4,5] S1P is a bioactive lysophospholipid metabolite that can signal agonistically via 5 different S1P receptors ($S1PR_1$ to $S1PR_5$).[6] As a result, an array of effects initiates functional antagonism. A key general function of S1PR signaling is the regulation of cell migration events, although the egress of T-cells from secondary lymphoid organs (SLO) into the circulation and peripheral tissues is selectively dependent on $S1PR_1$ activation.[7] While well studied, the compound FTY720 is only partially selective to $S1PR_1$. Newer compounds such as KRP203 are highly selective and are hence now being tested in patients at risk of developing acute GVHD (www.clinicaltrials.gov: #NCT01830010).[8]

To close gaps with regard to the mode of action of KRP203, we evaluated whether specific $S1PR_1$ agonism could avert the disruption of T-cell regeneration following alloHSCT. To this end, we studied murine transplantation models which had earlier also helped us and others to delineate a detrimental role of acute GVHD on thymus-dependent T-cell neogenesis.[9–11] Indeed, the recipient's thymus is a direct target of donor T-cell alloimmunity which results in severe depletion of immature T-cells and the destruction of nonhematopoietic cells of the thymic microenvironment.[9] Notably, central tolerance-inducing mature medullary thymic epithelial cells ($mTEC^{high}$ cells) are particularly sensitive to immune injury as a consequence of an acute GVH reaction.[10] For the present study, we reasoned that selective $S1PR_1$ agonism via KRP203 may avert thymic injury and its consequences by confinement of donor alloreactive T-cells to their initial activation sites in SLO.

We used different strains of mice as alloHSCT donors and recipients as described in more detail in the Supplemental Digital Content, Materials and Methods, http://links.lww.com/HS/A172. B6.RIP-mOVA mice express a membrane-bound form of ovalbumin (mOVA; $residues_{139\text{-}385}$) in mTEC.[11] Animals were purchased from The Jackson Laboratory (Bar Harbor, ME) and were kept in accordance with institutional regulations. The specific $S1PR_1$ agonist KRP203 was provided by Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). Route of administration and dosing regimen are given in the legends (Figures 1, 2). The efficacy of peripheral blood T-cell depletion by KRP203 was confirmed in dose-response experiments before initiation of our transplantation experiments (data not shown). Acute GVHD was induced both in haploidentical ($H\text{-}2^b \rightarrow H\text{-}2^{bd}$; designated as [b→bd]) and in fully major histocompatibility complex (MHC)-mismatched alloHSCT models ([b→d] and [d→b], respectively) with or without lethal irradiation, as indicated in the legends and as previously described by us.[9–11] Statistics, survival analysis, GVHD scoring, and histology were performed as described in the legends (Figures 1, 2) and Supplemental Digital Content, Materials and Methods, http://links.lww.com/HS/A172, and also as published.[9–11]

We first confirmed that the $S1PR_1$ agonist KRP203 reduced acute GVHD while preserving graft-versus-leukemia (GVL) activity, resulting in a survival benefit. In lethally irradiated and bone marrow transplanted (day 0) mice, we found that KRP203 given prophylactically from day −1 until day +21 posttransplant indeed lowered the severity of acute GVHD as demonstrated by prolonged survival and less injury of the gastrointestinal tract (Figure 1A, B). The beneficial effect was plausibly due to blockade of donor $CD4^+$ T-cell migration from SLO through the peripheral blood target organs of acute GVHD since lymphocyte counts, as assessed by flow cytometry,[9–11] were decreased in peripheral blood (Figure 1C, left panel). Concomitantly, donor

[1]Department of Biomedicine, Molecular Immune Regulation, University of Basel and University Hospital Basel, Switzerland
[2]Novartis Institutes for BioMedical Research, Autoimmunity, Transplantation & Inflammation Disease Area, Basel, Switzerland
[3]CMO, Anaveon AG, Technology Park, Basel, Switzerland
[4]Transplantation Immunology & Nephrology, Basel University Hospital, Basel, Switzerland
WK and LTJ are co-senior authors.
Supplemental digital content is available for this article.

HemaSphere (2021) 5:8(e613).
http://dx.doi.org/10.1097/HS9.0000000000000613.
Received: 11 March 2021 / Accepted: 2 June 2021

mature CD4+ T-cells (H-2b-positive) had accumulated in peripheral lymph nodes of H-2d mice (Figure 1C, right panel). The beneficial effect was comparable to the effects of the less receptor-selective agonist FTY720 with regard to reducing the severity of acute GVHD.[4,5,12]

We then tested the effect of KRP203 on the progression of lymph node-resident lymphoma using Luciferase+ (luc+) A20 lymphoma cells[13] that were injected directly into 1 inguinal lymph node. In vivo bioluminescence imaging revealed that an antitumor effect against A20 cells was preserved by KRP203 (Figure 1D). This fact was indicated by using mice that had received alloreactive T-cells together with 1 × 10⁴ luc+A20 tumor cells and KRP203 from day –1 until day +24. Consistent with the sequestration of donor T-cells in SLO, our data demonstrated collectively that KRP203 given before alloHSCT efficiently reduced the severity of acute GVHD while functional

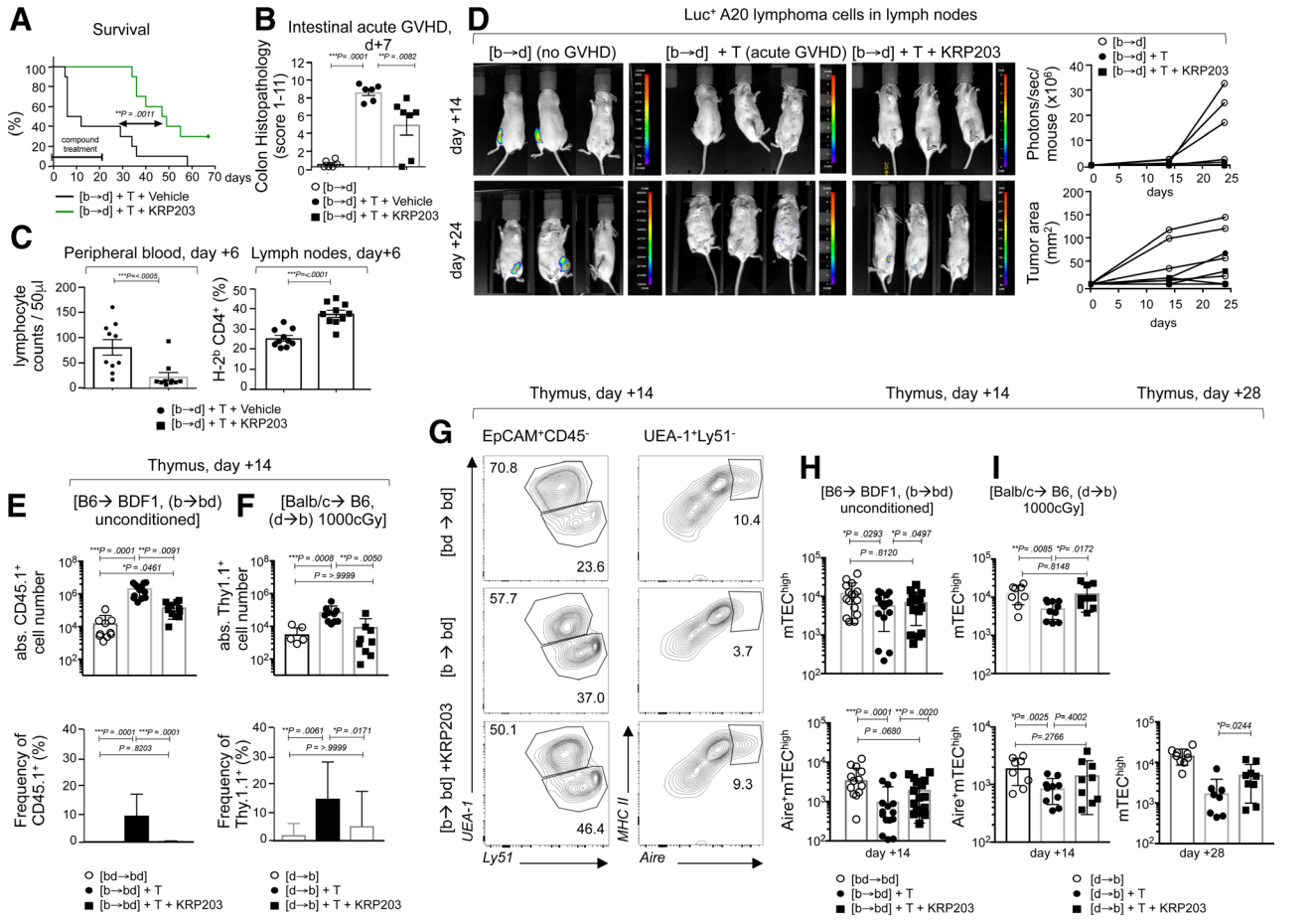

Figure 1. KRP203 inhibits alloreactive T-cell migration to the host thymus and prevents injury of the thymic medullary cell compartment. The effects of KRP203 were studied in 2 fully MHC-mismatched alloHSCT models and 1 unirradiated haploidentical model. In the fully MHC-disparate B6→Balb/c model (H-2b→H-2d; A–D), 5 × 10⁶ TCDBM from C57BL/6 mice and 1.2 × 10⁷ splenocytes (containing 3 × 10⁶ donor T cells) from congenic B6.CD45.1 mice were transplanted on day 0 into lethally irradiated (800 cGy) Balb/c mice. AlloHSCT recipients received either KRP203 in 0.5% methylcellulose vehicle prophylactically from day –1 (designated [b→d] + T + KRP203) or vehicle alone ([b→d] + T + vehicle). An additional control group received TCDBM without alloreactive T cells and did not develop acute GVHD ([b→d]). In some experiments, the control cohorts did not receive vehicle. In the Balb/c→B6 model (H-2d→H-2b; F, I), thymic acute GVHD was induced by transplantation on day 0 of 7 × 10⁶ TCDBM from Balb/c mice (Thy1.2+) and 2 × 10⁷ purified CD3+ T cells from congenic Balb/c.Thy1.1+ mice into lethally irradiated (1000 cGy) B6 mice. Transplant groups in this [d→b] alloHSCT model were the same as in the above model. In the haploidentical setting (E, G, H), thymic acute GVHD was induced in unirradiated 8-wk-old female BDF1 recipients (CD45.2+) by injection on day 0 of 35 × 10⁶ splenocytes from parental B6.CD45.1 donors (H-2b→H-2bd). AlloHSCT recipients received either KRP203 prophylactically from day –1 until the end of experiment (designated [b→bd] + T + KRP203) or did not receive the drug ([b→bd] + T). As non-GVHD controls, BDF1 mice received syngeneic splenocytes and no KRP203 ([bd→bd]). (A), Survival was measured in alloHSCT recipients receiving KRP203 (1 mg/kg/d) daily until day +21. Kaplan-Meier plot with Gehan-Breslow-Wilcoxon statistical analysis to compare survival curves. The data shown are from 1 experiment that is representative of a total of 3 experiments; with n = 6 BM + T cells and n = 7 BM +T cells + KRP203. (B), Colon histopathology was scored at day +7 in the same cohorts. One-way ANOVA; data are representative of 2 experiments. (C), Donor T-cells (H-2b-positive CD4+ and CD8+) were analyzed by flow cytometry in lymph nodes at day +6 in the same cohorts. *t* test; data are from 2 pooled experiments. (D), Antitumor activity. The A20 lymphoma cell line (1 × 10⁴ luciferase+ cells) was injected into the left inguinal lymph node. Mice were then treated with KRP203 (3 mg/kg, i.p., every second day) until the end of the experiment. One representative bioluminescence image is shown for each tested group at days +14 and +24 after alloHSCT. Tumor area and signal intensity data of luc+A20 cells were obtained from 2 pooled experiments with n = 3 mice per group (right panel). (E, F), Donor T-cell infiltration into recipient thymi was analyzed in the 2 alloHSCT models by flow cytometry assessment of CD45.1+ cells at day +14 and is given as absolute cell numbers and also frequencies among total thymic cells. In these experiments, mice received KRP203 (3 mg/kg, i.p., every second day) from day –1 until the end of experiment. (G), mTEChigh cells were identified as EpCAM+CD45-UEA-1+Ly51- mTEC cells that express MHCIIhigh and Aire. (H, I), Absolute cell numbers of mTEChigh and Aire+mTEChigh cells were examined at days +14 and +28 in mice without acute GVHD, mice that developed acute GVHD without or with treatment with KRP203 (3 mg/kg, i.p., every second day from day-1 until the end of experiment). Statistical analyses for pooled experiments (E, F, H, I) were done with 2-way ANOVA and Fisher's LSD test. The graphs represent data from 2 to 4 independent experiments with 3 to 5 mice per group and experiment and statistical significance is given as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BM = bone marrow; GVHD = graft-versus-host disease; HSCT = hematopoietic stem cell transplantation; i.p. = intraperitoneal; LSD = least significant difference; MHC = major histocompatibility complex; mTEC = medullary thymic epithelial cell; TCDBM = T-cell depleted bone marrow cells.

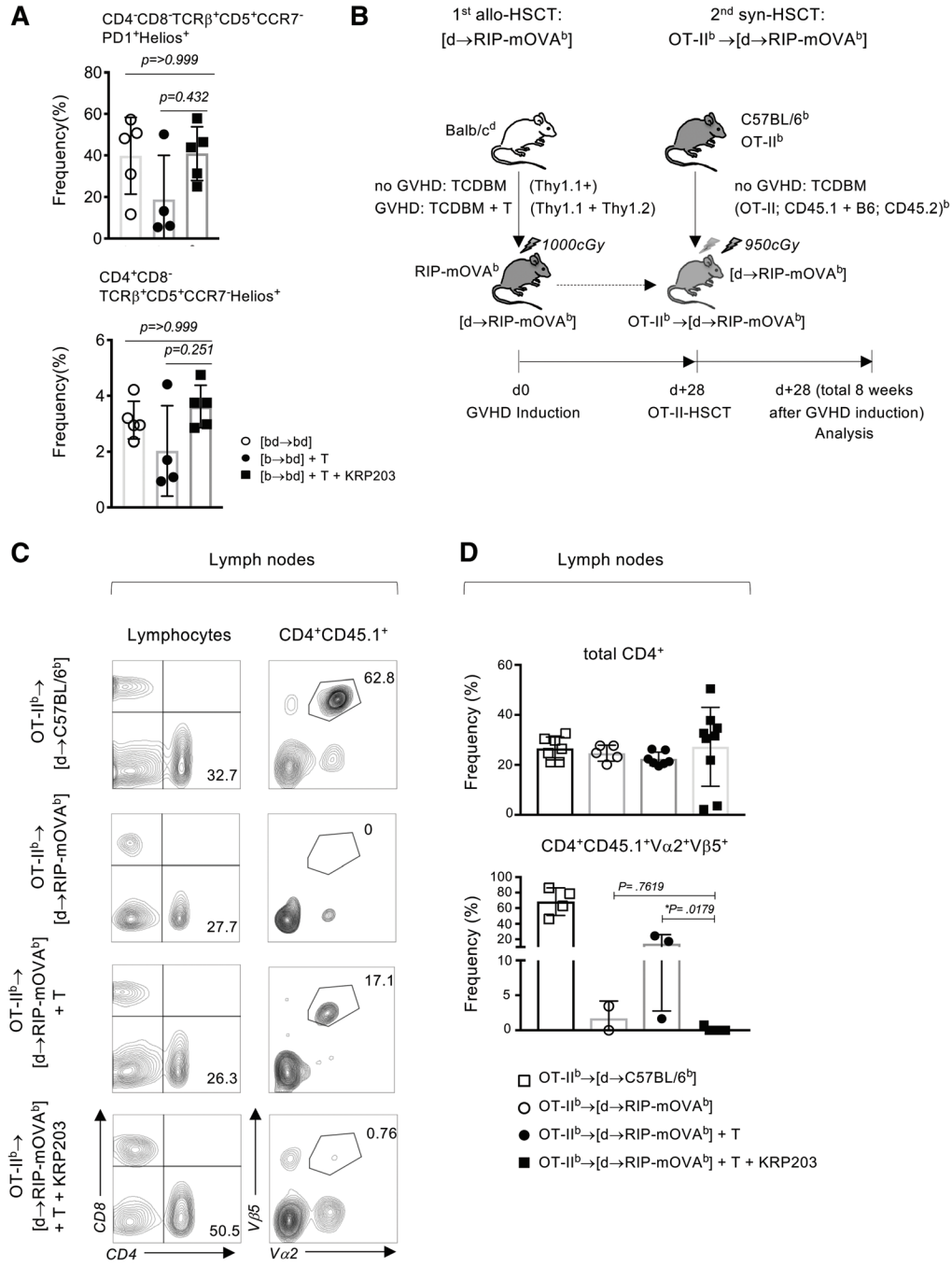

Figure 2. KRP203 inhibits thymic de novo generation of autoreactive T-cells following alloHSCT. The effects of KRP203 were studied in 2 alloHSCT models. In an unirradiated haploidentical transplantation model (A), acute GVHD (H-2^{b}→H-2^{bd}) was induced as described in the legend to Figure 1E, G, H. AlloHSCT recipients received either KRP203 (3 mg/kg, i.p., every second day) prophylactically from day −1 until the end of the experiment (designated [b→bd] + T+ KRP203) or did not receive the drug ([b→bd] + T). As non-GVHD controls, BDF_1 mice received syngeneic splenocytes and no KRP203 ([bd→bd]). Negatively selected T cells were detected among CD4-single-positive mature thymocytes (A, lower panel). Thymocytes that underwent negative selection were TCR^{+}CD5^{-}CCR7^{-} and PD-1^{+}/Helios^{+} or PD-1^{-}/Helios^{+}. Absolute cell numbers of cells marked to become negatively selected and express PD1 and Helios or Helios alone were determined. The graphs show representative data from 1 experiment with n = 5 mice per group. *P < 0.05, Kruskal-Wallis, and Dunnetts's multiple comparison test. To study the effects of KRP203 on thymic negative selection, B6.RIP-mOVA mice were used as recipients in a transgenic murine [d→b] alloHSCT model (B–D) as described by Dertschnig et al.[10] To this end, acute GVHD was induced at day 0 in 8 wk old, lethally irradiated (950 cGy) B6.RIP-mOVA mice (H-2^{b}) by transfer of Thy1.1^{+} T-cell depleted bone marrow cells together with Thy1.2^{+} splenic T-cells (H-2^{d}) from Balb/c donors (designated [d→RIP-mOVA^{b}] + T). These recipients received either KRP203 from day −1 to +28 or were not treated. As controls without acute GVHD, KRP203 untreated mice received Balb/c-Thy1.1^{+} TCDBM only (designated [d→RIP-mOVA^{b}]). On cessation of KRP203 treatment at day +28, all mice were lethally reirradiated and retransplanted in a second syngeneic HSCT with hematopoietic stem cells from CD45.1^{+} OT-II mice (H-2^{b}) mixed at a 1:4 ratio with TCDBM from wild-type CD45.2^{+} C57BL/6 mice (H-2^{b}). This approach generated chimeric mice (designated OT-II^{b}→[d→RIP-mOVA^{b}]) as detailed in (B) and as described.[3] (C, D), OVA-specific OT-II CD4^{+} T cells in transplanted chimeric mice were identified as CD4^{+}CD45.1^{+} T cells in lymph nodes at day +28 later in all cohorts treated or not treated with KRP203. An untreated and nontransgenic alloHSCT recipient cohort without acute GVHD was included as positive controls that did not delete OVA-specific T cell due to the absence of the negative selector OVA on mTEC^{high} cells (designated OT-II^{b}→[d→B6^{b}]). To authenticate that CD4^{+}CD45.1^{+} cells accurately represented OT-II T-cells, the population of Vα2^{+}Vβ5^{+} T cells was analyzed in lymph nodes by flow cytometry (C; 1 representative experiment is depicted). Frequencies of OT-II cells among total CD4^{+} CD45.1^{+} T cells are given in (D). The figure represents data from 3 combined experiments. *P < 0.05, Mann-Whitney U Test. GVHD = graft-versus-host disease; HSCT = hematopoietic stem cell transplantation; i.p. = intraperitoneal; mOVA = membrane-bound form of ovalbumin; mTEC = medullary thymic epithelial cell; TCDBM = T-cell depleted bone marrow cells.

antitumor alloreactivity in the peripheral lymphoid tissues could be preserved.

We then tested both unconditioned haploidentical and lethally irradiated fully MHC-mismatched alloHSCT models for infiltration of donor-derived allogeneic mature T-cells into the host thymus, an universal manifestation of experimental thymic acute GVHD.[3] We could demonstrate that thymic donor T-cell infiltration was diminished on day +14 on prophylactic KRP203 administration in both alloHSCT models (Figure 1E, F) and remained low even at 2 weeks after drug withdrawal (Supplemental Digital Contents, Figure 1, http://links.lww.com/HS/A173, http://links.lww.com/HS/A172). In contrast, a therapeutic administration of KRP203 (starting at day +7) was not advantageous since numbers of thymus-infiltrating donor T-cells were not lower than in untreated mice with acute GVHD (Supplemental Digital Contents, Figure 1, http://links.lww.com/HS/A173, http://links.lww.com/HS/A172).

We were next interested to learn whether KRP203 could also provide an advantage for thymic mTEC^{high} cells, including the subset which expresses the transcription factor Aire.[11] Pool sizes of these thymic stromal cells are diminished by the intrathymic donor T-cell alloresponse during untreated acute GVHD in all models tested[11] (Figure 1G–I). These cells of the thymic microenvironment are indispensable for negative selection of self-reactive T-cells as they display peripheral tissue-restricted antigens to developing lymphocytes.[11] We found that in both haploidentical and fully MHC-mismatched alloHSCT models, the prophylactic KRP203 administration indeed maintained normal numbers of mTEC^{high} and Aire$^+$mTEC^{high} at days +14 and +28 (Figure 1H, I). Consistent with low donor T-cell infiltration into host thymi, the Aire$^+$mTEC^{high} compartment remained protected for as long as 1 week after drug withdrawal but cell numbers were decreased at 2 weeks after withdrawal (Supplemental Digital Contents, Figure 1, http://links.lww.com/HS/A173, http://links.lww.com/HS/A172).

Loss of mTEC^{high} in mice due to acute GVHD allows escape of autoreactive T-cells into the periphery which can be detected 2-5 weeks after the onset of disease.[11] Thus, the development of a chronic autoimmune form of GVHD may be mechanistically linked to prior alloimmunity via functional impairment of the thymus medulla that in turn causes breakdown of central self-tolerance induction.[14] As Helios may serve as a marker of thymic negative selection, we tested its expression in the different experimental groups. While frequencies of Helios$^+$ cells appeared to be slightly lower in untreated mice with GVHD in our introductory experiments, the reduction was not statistically significant (Figure 2A). More importantly, however, we observed that treatment with KRP203 had no negative effect on Helios expression, suggesting normal thymic selection. Therefore, to conclusively establish whether KRP203 indeed averted the de novo production of autoreactive T-cells, we next used mOVA as a surrogate self-antigen that is expressed by mTEC^{high} cells.[11] In these experiments, transgenic B6.RIP-mOVA recipients of Balb/c mature T-cells (designated [d→RIP-mOVA^b]) developed acute GVHD following a primary alloHSCT. These mice then further received hematopoietic stem cells from CD45.1$^+$ B6.OT-II mice in a secondary alloHSCT (designated OT-II^b→[d→RIP-mOVA^b] mice; Figure 2B). The mTEC^{high} compartment and thymic negative selection to OVA in these alloHSCT recipients was analyzed by flow cytometry as detailed before.[11] Consistent with our previous report,[11] the loss of mOVA expression negatively affected central deletion of OVA-specific T-cells during acute GVHD (Figure 2C, D). In contrast, KRP203 administration during the induction and propagation phases of acute GVHD significantly reduced the frequency of OVA-specific T-cells (CD45.1$^+$CD4$^+$ cells expressing the Vα2 and Vβ5 TCR chains) that were detectable in recipient lymph nodes (Figure 2C, D). Hence, our data showed that KRP203-mediated protection of OVA$^+$ Aire$^+$mTEC^{high} could reduce the escape of "forbidden" OVA-specific OT-II cells from thymic negative selection. Contrary to what one would have expected from reports that S1PR$_1$ functional antagonism inhibits intrathymic T-cell development and export of mature T-cells,[7] KRP203 allowed for normal T-cell export to the periphery: First, in the B6.RIP-mOVA model, the frequencies of lymph node–resident total CD4$^+$ T-cells were not different in KRP203-treated from those in untreated recipients at the end of the observation period (8 weeks; Figure 2D). Second, in the nontransgenic alloHSCT settings tested, intrathymic T-cell development (Supplemental Digital Contents, Figure 2, http://links.lww.com/HS/A174, http://links.lww.com/HS/A172) and posttransplantation peripheral T-cell numbers were similar in treated mice when compared to untreated mice in both GVHD$^-$ and GVHD$^+$ settings (Supplemental Digital Contents, Figures 2, 3, http://links.lww.com/HS/A174, http://links.lww.com/HS/A175, http://links.lww.com/HS/A172).

All data taken together, we conclude that S1PR$_1$ functional antagonism via the selective KRP203 represents a promising avenue for acute GVHD prophylaxis as it ameliorates the typical deficits in posttransplantation immune recovery: by preserving an integral thymus, this approach retains a sizeable posttransplantation peripheral T-cell compartment that is capable to induce immune responses to SLO-resident tumors but that harbors a smaller autoreactive T-cell compartment due to diminished thymus-dependent neogenesis of autoreactive T-cells. To facilitate the clinical implementation of highly selective S1PR$_1$ agonists, we advocate a further in-depth study of KRP203 or related mediators as a GVHD prophylactic principle. Our results were also consistent with other recent findings which have suggested a new combination of short-term KRP203 and posttransplant cyclophosphamide to constitute a promising novel GVHD prophylaxis in haploidentical HSCT.[15]

Acknowledgments

We thank Dr Gang Zhou (Georgia Health Sciences University, GA) for providing the luc$^+$A20 cell line.

Disclosures

CB was employee of Novartis Inc. as study coordinator. He currently serves as head of the clinical advisory board at Priothera SAS France.

Sources of funding

This work was supported by Swiss National Science Foundation (SNSF) grants 310030-162602 (WK), SNSF Professorship PP00P3_144860 (LTJ), NIH grant R56/R01AI106923 (LTJ) and by a grant from the Stiftung Krebsforschung Schweiz (KFS-3237-08-2013), Basel, Switzerland (WK).

References

1. Blazar BR, Hill GR, Murphy WJ. Dissecting the biology of allogeneic HSCT to enhance the GvT effect whilst minimizing GvHD. *Nat Rev Clin Oncol.* 2020;17:475–492.
2. Zeiser R, Blazar BR. Acute graft-versus-host disease. *N Engl J Med.* 2018;378:585–586.
3. Krenger W, Blazar BR, Holländer GA. Thymic T-cell development in allogeneic stem cell transplantation. *Blood.* 2011;117:6768–6776.
4. Kim YM, Sachs T, Asavaroengchai W, Bronson R, Sykes M. Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720. *J Clin Invest.* 2003;111:659–669.
5. Taylor PA, Ehrhardt MJ, Lees CJ, et al. Insights into the mechanism of FTY720 and compatibility with regulatory T cells for the inhibition of graft-versus-host disease (GVHD). *Blood.* 2007;110:3480–3488.
6. Chun J, Goetzl EJ, Hla T, et al. International Union of Pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol Rev.* 2002;54:265–269.

7. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature.* 2004;427:355–360.
8. Song J, Matsuda C, Kai Y, et al. A novel sphingosine 1-phosphate receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukin-10 gene-deficient mice. *J Pharmacol Exp Ther.* 2008;324:276–283.
9. Hauri-Hohl MM, Keller MP, Gill J, et al. Donor T-cell alloreactivity against host thymic epithelium limits T-cell development after bone marrow transplantation. *Blood.* 2007;109:4080–4088.
10. Dertschnig S, Nusspaumer G, Ivanek R, et al. Epithelial cytoprotection sustains ectopic expression of tissue-restricted antigens in the thymus during murine acute GVHD. *Blood.* 2013; 122:837–841.
11. Dertschnig S, Hauri-Hohl MM, Vollmer M, et al. Impaired thymic expression of tissue-restricted antigens licenses the de novo generation of autoreactive CD4+ T cells in acute GVHD. *Blood.* 2015;125:2720–2723.
12. Song J, Ito T, Matsuda C, et al. Inhibition of donor-derived T cells trafficking into target organs by FTY720 during acute graft-versus-host disease in small bowel transplantation. *Clin Exp Immunol.* 2006;146:85–92.
13. Marabelle A, Kohrt H, Sagiv-Barfi I, et al. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. *J Clin Invest.* 2013;123:2447–2463.
14. Parkman R. A 2-hit model for chronic GVHD. *Blood.* 2013;122:623–624.
15. Emi Y, Hashimoto D, Hayase E, et al. Short-term KRP203 and post-transplant cyclophosphamide for graft-versus-host disease prophylaxis. *Bone Marrow Transplant.* 2020;55:787–759.