学位論文（要約）

Exploration of Maximizing the Graft-Versus-Leukemia Effect Following Allogeneic Hematopoietic Cell Transplantation Utilizing Novel Agents

（新規薬剤による同種造血細胞移植後の移植片対白血病効果最大化の検討）

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Background and Purpose

Allogeneic hematopoietic cell transplantation (allo-HCT) stands as a pivotal curative treatment modality for hematologic malignancies. The therapeutic impact of allo-HCT encompasses myeloablation, reconstitution of donor hematopoiesis, and the establishment of a new immune system. This leads to the graft's allo-reactivity, which can be directed either against leukemia/lymphoma cells (graft-versus-leukemia/lymphoma effect; GVL effect) or against the recipient's own tissues, resulting in graft-versus-host disease (GVHD).

GVHD is one of the most significant and life-threatening complications after allo-HCT, and GVHD prophylaxis is indispensable for successful allo-HCT. While the standard GVHD prophylaxis using calcineurin inhibitors (CNIs) has improved safety of allo-HCT by reducing the occurrence of severe acute GVHD. CNIs, such as cyclosporine (CSP) and tacrolimus, function by suppressing calcium-dependent gene transcription in T cells and by inhibiting the production of IL-2. However, profound immunosuppression induced by CNIs is associated with the development of a range of infectious events and may lead to leukemia/lymphoma relapse after allo-HCT. This dilemma becomes particularly critical in patients undergoing allo-HCT for malignancies at a high risk of relapse, emphasizing the clinical need to optimize GVHD prevention while preserving the GVL effect. In the current study, we have explored two strategies to potentiate GVL effects against leukemia. First, we tried to promote GVL effects by stimulating FMS-like tyrosine kinase 3 (FLT3)-mutated acute myeloid leukemia (AML) cells to produce immune-stimulatory cytokine, interleukin-15 (IL-15). Second, we attempted to develop a novel GVHD prophylaxis using a sphingosine-1-phosphate receptor 1 (S1PR1) modulator, mocravimod (KRP203). This approach will preserve more significant GVL effects compared to CNIs.

In the first part, we have tested whether selective FLT3 inhibitor, gilteritinib, could promote GVL effects against FLT3 internal tandem duplication (FLT3-ITD) positive AML after allo-HCT. The FLT3-ITD mutation constitutively activates FLT3 signaling, promotes proliferation and survival of AML cells, and is closely associated with a higher leukemia burden and a poor prognosis. While allo-HCT holds promise for improving outcomes in FLT3-ITD$^+$ AML, it is of note that relapse rates in this subset are significantly higher compared to FLT3-ITD-negative AML following allo-HCT.

FLT3 inhibitors inhibit FLT3-ITD signaling and are approved for the treatment of newly diagnosed and relapsed/refractory FLT3-ITD positive AML. First generation FLT3 inhibitors such as sorafenib, exhibit a lack of specificity for FLT3 and fall under the category of multikinase inhibitors. In contrast, second-generation FLT3 inhibitors such as gilteritinib and quizartinib display a higher degree of specificity in their targeting of mutated FLT3. In murine models of allo-HCT, sorafenib has demonstrated the capacity to trigger interferon regulatory factor 7 (IRF7)-dependent production of interleukin-15 (IL-15) within FLT3-ITD expressing leukemia cells. This, in turn, fosters the expansion of cytotoxic donor T cells and augments the GVL effects directed against FLT3-ITD^{+} leukemia. It remains to be clarified whether second-generation FLT3 inhibitors could enhance GVL effects after allo-SCT. In the current study, we delved into the effects of the short-term administration of gilteritinib on GVHD and GVL effects after mouse allo-SCT.

In the second part, we have tested whether S1P1 modulator, mocravimod could ameliorate chronic GVHD and enhance GVL effects by enabling the early cessation of CNIs. S1PRs have emerged as potential therapeutic targets for various diseases based on their involvement in the regulation of lymphocyte trafficking, brain and cardiac function, vascular permeability, and the modulation of vascular and bronchial tone. S1PR modulators have been developed as a novel class of immunosuppressants capable of sequestering T cells within lymph nodes (LNs) by downregulating S1PRs. A multi-S1PR modulator, fingolimod (FTY720), inhibits signaling of S1P1, 3, 4, 5 receptors amd has been approved for the treatment of multiple sclerosis. Although fingolimod was tested for GVHD prophylaxis in the clinical trials, serious adverse effects such as macular edema and bradycardia fingolimod led to the cessation of clinical trial. Mocravimod selectively inhibits S1PR1 signaling and is expected to offer a superior safety profile compared to multi-S1PR modulators. In clinical studies, mocravimod demonstrated its safety after allo-HCT. We have previously reported that short-term administration of mocravimod can ameliorate acute GVHD while preserving better GVL effects compared to CNIs (Yokoyama, BMT 2020). However, it remains to be clarified whether mocravimod could ameliorate chronic GVHD. In the current study, we investigated whether long-term administration of mocravimod could ameliorate chronic GVHD after allo-SCT.

Our study aimed to assess the administration of novel agents with the goal of

optimizing the GVL effects, all the while minimizing the exacerbation of GVHD effects, by using murine models of allo-HCT.

Materials and Methods

The effects of gilteritinib upon GVL effects and GVHD were tested using MHC haplo-identical HCT model; B6 into B6C3F1. B6C3F1 (H-$2^{b/k}$) mice were lethally irradiated (10.5 Gy) and transplanted with T cell-depleted bone marrow cells only (TCD-BM group) or in combination with purified T cells from allogeneic B6 (H-$2^{b/b}$) mice (TCD-BM+T group) on day 0. Recipients were i.v. injected with 5×10^4 Ba/F3 leukemia cells (H-$2^{k/k}$) transfected with FLT3-ITD and luciferase (Ba/F3-FLT3-ITD-luc^+) on day 0 and followed by daily oral administration of gilteritinib (10 mg/kg/day) or diluent from day +5 to +14 after allo-HCT. In vivo bioluminescent imaging (BLI) with luciferin injection was performed weekly after allo-SCT to assess leukemia-cell expansion. The Q-PCR analysis of IL-15 in Ba/F3-FLT3-ITD cells that were purified from the spleen and bone marrow. Furthermore, flowcytometric analyses of donor T cell exhaustion and donor cytotoxic T lymphocyte (CTL) responses in the spleen were performed after SCT.

The effects of mocravimod on chronic GVHD were evaluated using a minor histocompatibility antigen (MiHA)-mismatched HCT model. BALB/c (H-2^d) mice were exposed to irradiation at a dosage of 5.5 Gy and then injected with 8×10^6 bone marrow cells along with 15×10^6 splenocytes obtained from MiHA-mismatched allogeneic B10.D2 (H-2^d) donors on day 0. The recipients were administered orally with 3 mg/kg/day mocravimod or diluent from day -1 to day +42 of allo-HCT. The clinical scores for skin chronic GVHD were evaluated twice weekly after allo-HCT. Volume of lachrymal secretion was evaluated on day +42 by Schirmer-test. Furthermore, flowcytometric analyses of donor T cell expansion and exhaustion in the lymphoid and target organs, and bone marrow were performed after HCT. To evaluate the effects of mocravimod on GVL effects, B6D2F1 recipients were injected with luciferase-expressing P815 leukemia cells on day 0 of allo-HCT.

Results

First, we confirmed that gilteritinib induced a dose-dependent upregulation of IL-15 expression in mouse and human leukemia cell lines expressing FLT3-ITD, in vitro. We also found that gilteritinib administered from day +5 to day +8 after allo-HCT

significantly upregulated IL-15 expression in Ba/F3-FLT3-ITD-luc$^+$ cells isolated from allogeneic recipients on day +8, compared to those from vehicle-treated recipients. This enhanced IL-15 production in gilteritinib-treated recipients was associated with a significant reduction in the expression of inhibitor receptors, such as PD-1 and TIGIT on donor CD8$^+$ T cells on day +8 after allo-HCT. Notably, gilteritinib significantly enhanced recipient-specific CTL responses after allo-HCT. While short-term gilteritinib from day +5 to day +12 exerted minimal anti-leukemia effects in the recipients of TCD-BM, it significantly suppressed leukemia expansion, as assessed through BLI, and leukemia-associated mortalities in the recipients which transplanted with TCD-BM and T cells. Importantly, administration of gilteritinib did not exacerbate GVHD after allo-HCT.

Next, mocravimod administered from day -1 to day +42 significantly reduced chronic GVHD skin scores and increased of lachrymal secretion volume compared to vehicle-treated controls on day +42 after allo-SCT. Moreover, the pathological skin chronic GVHD scores and fibrotic areas in the liver and salivary glands were significantly reduced in recipients treated with mocravimod compared to those treated with vehicle. Flow cytometric analysis on day +28 demonstrated that mocravimod significantly reduced IL-17A$^+$IFNγ^+CD4$^+$ pathogenic Th17 cells in the spleen. Subsequently, on day +42, flow cytometric analysis demonstrated that long-term administration of mocravimod resulted in a significant reduction of both CD4$^+$ and CD8$^+$ donor T cells in the mesenteric lymph nodes (MLN), indicating that mocravimod induced activation-induced cell death of donor T cells as demonstrated in previous studies (Hashimoto Eur J Haematol 2007, Yokoyama BMT 2019). Importantly, mocravimod significantly reduced donor CD4$^+$ T cells while sparing CD8$^+$ T cells in other organs, such as spleen, bone marrow, and liver. It is expected that the CD8$^+$ T cells that persist in mocravimod-treated recipients could contribute to GVL effects. Thus, we evaluated GVL effects using another mouse allo-SCT model, in which lethally irradiated B6D2F1 recipients were transplanted from MHC-haploidentical B6 donors, and recipient-type luciferace-expressing leukemia cells, P815 were i.v. injected on day0 of transplantation. Leukemia-injected recipients were administrated with mocravimod alone or in combination with short-term (day 0-+14) or long-term (day 0-+42) CSP. In vivo bioluminescence imaging demonstrated that the recipients treated with either vehicle or mocravimod alone were able to reject the leukemia cells. Addition of CSP to mocravimod significantly suppressed GVL effects, whereas early cessation of CSP

significantly blunted leukemia expansion compared to long-term administration of CSP without exacerbation of GVHD.

Discussion

Maintaining or enhancing GVL effects while suppressing GVHD has remained a critically important goal in the research of allo-SCT. In the current study, we developed two strategies to accomplish this purpose.

First, we found that a selective FLT3-inhibtor, gilteritinib, enhances GVL effects without exercerbation of GVHD. It has been well-described that the IL-15/IL-15Rα complex is shuttled to the cell membrane and directly presented to surrounding T cells expressing IL-15Rβ/γc, a process known as 'trans-presentation.' FLT3-ITD inhibition using a multikinase inhibitor, sorafenib, promotes leukemia-cell production of both IL-15 and IL-15Rα, suggesting that FLT3 inhibitors activate donor T cells localizing around leukemia cells more potently than those are away from leukemia cells, thereby mitigating the risk of exacerbating GVHD. Furthermore, gilteritinib likely activates immunity only in the presence of leukemia cells, as it requires IL-15 production by leukemia cells. This enables gilteritinib to elicit a precisely calibrated immune response, eradicating leukemia cells while avoiding undue exacerbation of GVHD after allo-HCT.

In the latter part of the current study, we found that the S1PR1 modulator, mocravimod ameliorates chronic GVHD and allows for the early cessation of CNIs while preserving significant GVL effects. Cytokines play a pivotal role in the pathophysiology of both acute and chronic GVHD, and pathogenic Th17/Tc17 cells, which produce both IL-17A and IFN-γ, have particular importance in chronic GVHD; IL-17A, primarily produced from Th17/Tc17 cells, has been identified as a factor exacerbating skin pathology in chronic GVHD. In chronic GVHD models, mocravimod significantly reduced pathogenic Th17/Tc17 cells, thereby ameliorating pathological GVHD in skin, liver, and salivary glands in mice.

We also found mocravimod enables the early cessation of CNIs which is associated with better GVL effects compared to prolonged administration of CNIs. Combined with our previous paper, we concluded that CNIs suppressed GVL effects much more profoundly compare to mocravimod. We have previously shown that S1PR modulators selectively induce activation-induced cell death in the LNs, while CNIs more generally

suppress T cell activation and proliferation. Furthermore, we found that mocravimod spares $CD8^+$ T cells in the bone marrow and GVHD target organs. It has been reported that S1PR modulators sequester $CD4^+$ T cells more efficiently compared to $CD8^+$ T cells. $CD8^+$ T cells persist in mocravimod-treated recipients may contribute to better GVL effects.

Conclusion

1. Gilteritinib, a selective inhibitor of FLT3, promoted IL-15 expression in FLT3-ITD^+ leukemia cells, reduced co-inhibitory molecules on donor T cells, enhanced donor anti-leukemia CTL responses, and promoted GVL effects without exacerbating GVHD after allo-HCT.
2. Mocravimod, a selective S1PR1 modulator, reduced both donor $CD4^+$ and $CD8^+$ donor T cells in the LNs and IL-$17A^+IFN\gamma^+$ Th17 cells in the spleen and ameliorated mouse chronic GVHD, while it spared $CD8^+$ T cells in other organs. Mocravimod enabled early cessation of CSP without exacerbation of GVHD, which led to improved GVL effects.

Our data will pave the way for clinical studies in which gilteritinib and mocravimod will be tested to enhance GVL effects.