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学位論文内容の要旨

博士の専攻分野の名称　博士（医　学）　氏 名　Zhang Zixuan

学 位 論 文 題 名

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)

[Background and Purpose]

Allogeneic hematopoietic cell transplantation (allo-HCT) is a pivotal curative treatment modality for hematologic malignancies. Graft-versus-host disease (GVHD) is one of the most significant and life-threatening complications after allo-HCT. The standard GVHD prophylaxis using calcineurin inhibitors (CNIs), like cyclosporine (CSP) has improved safety of allo-HCT whereas profound immunosuppression may lead to leukemia/lymphoma relapse, emphasizing the clinical need to optimize GVHD prevention while preserving the graft-versus-leukemia (GVL) effect. In the current study, we have explored two strategies to potentiate GVL effects against leukemia.

FLT3 inhibitors are approved for the treatment of newly diagnosed and relapsed/refractory FLT3 internal tandem duplication (FLT3-ITD) positive AML, which is associated with a higher leukemia burden and relapse rate. First-generation FLT3 inhibitors like sorafenib, a multikinase inhibitor, has demonstrated the capacity to trigger interferon regulatory factor 7 (IRF7)-dependent production of IL-15 within FLT3-ITD expressing leukemia cells in murine model of allo-HCT. This, in turn, fosters the expansion of cytotoxic donor T cells and augments the GVL effects directed against FLT3-ITD^{+} leukemia. The second-generation FLT3 inhibitors demonstrate a higher degree of specificity in targeting mutated FLT3. However, whether this enhances the GVL effect following allo-HCT remains to be clarified. In **Chapter 1**, we delved into the effects of the short-term administration of gilteritinib on GVHD and GVL effects after mouse allo-HCT.

S1PR modulators is a novel class of immunosuppressants capable of sequestering T cells within lymph nodes (LN) by downregulating S1PRs. A multi-S1PR modulator, fingolimod (FTY720) has been approved for the treatment of multiple sclerosis while serious adverse effects such as macular edema and bradycardia fingolimod led to the cessation of clinical trial for GVHD prophylaxis. Mocravimod selectively inhibits S1PR1 signaling and is expected to offer a superior safety profile compared to multi-S1PR modulators after allo-HCT. We have previously reported that mocravimod can ameliorate acute GVHD while preserving better GVL effects compared to CNIs. However, it remains to be clarified whether mocravimod could ameliorate chronic GVHD. In the current study of **Chapter 2**, we investigated whether long-term administration of mocravimod could ameliorate chronic GVHD and enhance GVL effects by enabling the early cessation of CNIs.

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.

[Methods]

B6C3F1 (H-2$^{b/k}$) mice were lethally irradiated (10.5 Gy) and transplanted with T cell-depleted bone marrow cells only or in combination with purified T cells from allogeneic B6 (H-2$^{b/b}$) mice on day 0. Recipients were i.v. injected with luciferase expressing Ba/F3-FLT3-ITD cells on day 0 and followed by oral administration of gilteritinib or diluent from day +5 to +14 after allo-HCT. In vivo bioluminescent imaging with luciferin injection was performed weekly after allo-HCT to assess leukemia-cell expansion.

The effects of mocravimod on chronic GVHD were evaluated using a minor histocompatibility antigen (MiHA)-mismatched HCT model, B10.D2 (H-2^{d}) into BALB/c (H-2^{d}). Recipients were lethally irradiated (5.5 Gy) and transplanted with bone marrow cells and splenocytes on day 0. The recipients were administered orally with mocravimod or diluent from day -1 to day +42 of allo-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.

[Result]

In Chapter 1, 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 significantly

upregulated IL-15 expression in Ba/F3-FLT3-ITD-luc$^+$ cells isolated from allogeneic recipients on day +8 after allo-HCT, 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. Gilteritinib significantly suppressed leukemia expansion and leukemia-associated mortalities without exacerbate GVHD in the recipients which transplanted with TCD-BM and T cells.

In Chapter 2, we found that mocravimod significantly reduced chronic GVHD skin scores and increased of lachrymal secretion volume compared to vehicle-treated controls on day +42 after allo-HCT. Moreover, the pathological skin chronic GVHD scores and fibrotic areas in the liver and salivary glands were significantly reduced in recipients treated with mocravimod. 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 LN. Importantly, mocravimod significantly reduced donor CD4$^+$ T cells while sparing CD8$^+$ T cells in other organs. It is expected that the CD8$^+$ T cells that persist in mocravimod-treated recipients could contribute to GVL effects. In GVL model, 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-HCT. 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 exacerbation 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. 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 localizing away from leukemia cells, thereby mitigating the risk of exacerbating GVHD. Furthermore, as gilteritinib requires IL-15 that produced by leukemia cells to activate T cells, it 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 the early cessation of CNIs while preserving significant GVL effects. In chronic GVHD models, which play a pivotal role in chronic GVHD, 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 apoptosis of allo-reactive T cells 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. These may contribute to better GVL effects.

[Conclusion]

①Gilteritinib, 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. ②Mocravimod, a selective S1PR1 modulator, reduced both donor CD4$^+$ and CD8$^+$ donor T cells in the LNs and IL-17A$^+$IFNγ$^+$ Th17/Tc17 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.