ELSEVIER

Blood 142 (2023) 476–477

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

722.ALLOGENEIC TRANSPLANTATION: ACUTE AND CHRONIC GVHD, IMMUNE RECONSTITUTION

Clonal GZMK $^{+}$CD8 $^{+}$ t Cells Are Identified As a Hallmark of Pathogenesis of Cgvhd-Induced Bronchiolitis Obliterans Syndrome after Allogeneic Hematopoietic Stem Cell Transplantation

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Chronic graft-versus-host disease (cGVHD), as the main cause of non-relapse mortality (NRM), remains the major complication leading to reduced patient-reported quality of life (QoL) and increased incidence of secondary malignancies in long-term transplant recipients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Bronchiolitis obliterans syndrome (BOS) is a late-stage complication of cGVHD involving the lungs and one of its most devastating outcomes. BOS currently has no satisfactory therapeutic approaches partly due to the limited understanding of pathogenic mechanisms.

To generate a comprehensive immune atlas of patients with cGVHD, we have enrolled 22 patients with cGVHD and 11 patients without cGVHD following allo-HSCT and collected their peripheral blood mononuclear cell (PBMC) samples, respectively. According to the organs involved in cGVHD, 10 patients had cGVHD with only skin involvement (abbreviated as cGVHD-skin here), 12 patients had lung involvement with diagnosed cGVHD-BOS. By applying mass cytometry, bulk RNA-sequencing and cytokine array, we revealed that circulating Granzyme K (GZMK)-expressing CD8 $^{+}$ T cells were accumulated in cGVHD-BOS patients. As lung fibrosis is one of the hallmarks of cGVHD-BOS, we next explore whether it is related to the increase of our identified GZMK $^{+}$ CD8 $^{+}$ T cells. We performed *in vitro* culture of human embryonic lung fibroblast HFL-1 cells in the presence or absence of 100ng/ml human GZMK. Supporting our hypothesis, GZMK dramatically boosted the expression levels of fibrosis-related genes including collagen type I alpha 1 chain (COL1A1), fibronectin (FN1), detected by both RT-qPCR and western blotting, suggesting an important role of GZMK in the pathogenesis of cGVHD-BOS.

In order to validate the role of GZMK $^{+}$CD8 $^{+}$ T cells in cGVHD-BOS, we established murine cGVHD-BOS model after allo-HSCT. We next performed 10×genomics single-cell RNA sequencing (scRNA-seq) and paired single-cell TCR sequencing analysis of engrafted living donor mouse immune cells (FVS510 $^{-}$ H2 $^{b+}$ H2 $^{k-}$ CD45 $^{+}$), which were isolated from lung tissues of cGVHD-BOS mice (n=3) and of control mice (n=3), respectively. We identified three T cell clusters that highly expressed *Gzmk* as well as *CCR5*, namely CD8 EX Gzmk, CD8 EM CCL4 and CD8 EFF Gzma. Total Gzmk $^{+}$CD8 $^{+}$ T cells were significantly enriched in lungs of cGVHD-BOS mice compared with those of control mice ($p < 0.01$), in which CD8 EX Gzmk and CD8 EM CCL4 cells were significantly dominant in lung tissues of cGVHD-BOS mice. We further explored the unique transcriptional states of Gzmk $^{+}$ CD8 $^{+}$ T cells in lungs of cGVHD-BOS mice. Compared with Gzmk $^{-}$ CD8 $^{+}$ T cells in cGVHD-BOS lungs, GO pathway enrichment analysis showed that Gzmk $^{+}$ CD8 $^{+}$ T cells are highly cytotoxic subtypes with migratory features. Notably, Gzmk $^{+}$ CD8 $^{+}$ T cell subsets, CD8-EX-Gzmk and CD8-EM-CCL4, also exhibited significantly higher numbers of TCR clonotypes in BOS lungs compared to controls, which confirmed that GZMK $^{+}$CD8 $^{+}$ T cells infiltrated and expanded in lungs of

cGVHD-BOS mice. Furthermore, we observed that three Gzmk $^{+}$ CD8 $^{+}$ T cell subsets had highly overlapping TCR repertoires, indicating that similar immunodominant epitopes may drive the clonal expansion of Gzmk $^{+}$ CD8 $^{+}$ T cells.
Finally, to further explore novel promising drugs to treat cGVHD-BOS by inhibition of the expression of GZMK in T cells, we screened 40 small molecular drugs, which were selected from a library containing 932 drugs targeting epigenetic regulators, to treat T cells for 48h. We revealed that Bosutinib, the second-generation BCR-ABL1-targeting tyrosine kinase inhibitor (TKI), could inhibit GZMK expression on CD8 $^{+}$ T cells *in vitro*. More importantly, the reduced lung resistance with restored lung compliance were observed in cGVHD-BOS mice treated with Bosutinib, suggesting that Bosutinib had effect on reducing of lung stiffness and pulmonary fibrosis. Multiple immunofluorescence staining confirmed that Bosutinib treatment could reduce the infiltration of Gzmk $^{+}$CD8 $^{+}$T cells and collagen deposition in lungs of cGVHD-BOS mice. Our data shed light on the new therapeutic approaches for cGVHD-BOS.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-189930