BCL2 as a new target for the treatment of SR-GvHD

Paola Frisone | Apr 24, 2020

Graft-*versus*-host disease (GvHD) is the major complication observed after allogeneic hematopoietic stem cell transplant (allo-HSCT). Steroids represent the first-line treatment but approximately one-third of patients are refractory to steroid and have poor outcomes. Novel therapies are needed for this difficult to treat population of patients with steroid-refractory GvHD (SR-GvHD).

This month the GvHD Hub is focusing on novel targets in the treatment of GvHD, here we present the results of a study, recently published in *The Journal of Investigative Dermatology* by Johanna Strobl and colleagues, evaluating BCL2 expression levels in T cells of patients with GvHD.

Methods

Patients who underwent allo-HSCT were sampled in four cohorts:

- Cohort 1, stratified in “no GvHD” (n = 6) and “GvHD developers” (GvHDdev; n = 5) with appearance of GvHD after Day +14 post allo-HSCT. In this cohort, peripheral blood (PB) samples were collected at four time points (-7, 0, +14, and +100) for transcriptome analysis
- Cohort 2, patients with acute GvHD (aGvHD). Samples from PB mononuclear cells (PBMC; n = 6), skin (n = 31), or gastrointestinal (GI) tract (GIT; n = 17) were collected at the time of aGvHD onset
- Cohort 3, patients with chronic GvHD (cGvHD). PBMC (n = 10), skin (n = 21), liver (n = 4) or lung (n = 3) samples were collected at the time of cGVHD onset

- Cohort 4, PB samples were collected from patients with chronic SR-GvHD (SR; n = 9) and patients with healed steroid-sensitive GvHD in the past (non-SR; n = 10)
- Analysis was performed using RNA sequencing, to identify genes dysregulated in GvHD patients, and quantitative PCR to examine the mRNA levels

Results

- Comparison of gene expression in T cells from no GvHD group and GvHDdev group in Cohort 1 revealed a dysregulation of ten genes. Of these, *BCL2* and *PMAIP1* were up-regulated on the day of allo-HSCT (Day 0) and remained up-regulated in the GvHDdev group (Day +14 and +100) but not in the no GvHD group
- The levels of BCL2 mRNA were compared between Cohort 2 (aGvHD) and Cohort 3 (cGvHD), and healthy controls (non-inflamed respective organ)
    - A significant increase in BCL2 mRNA levels was found in PBMC (n = 12; p <01), skin (n = 23; p < 0.001), and GIT (n = 17) of patients with aGvHD as well as in PBMC (n = 10; p < 0.05) and skin (n = 20; p < 0.001) of patients with cGvHD
    - A trend of elevated BCL2 mRNA was also observed in liver (n = 3) and lung (n = 4) of patients with cGvHD
    - BCL2 mRNA levels of GIT aGvHD samples (n = 17; p <05) were higher compared to non-GvHD GI inflammation biopsies (n = 10) after HSCT indicating the potential use of BCL2 expression for diagnosing GIT aGvHD
- To evaluate the association between BCL2 mRNA levels at diagnosis and patient outcomes, levels were correlated to patient characteristics, response to steroid treatment and overall outcome of Cohorts 2 (aGvHD) and Cohort 3 (cGvHD)
    - Elevated BCL2 mRNA levels in GvHD skin at diagnosis coincided with SR-GvHD (p <05)
    - In the blood of patients with SR-GvHD higher frequencies of BCL2+ leukocytes (p <01) and BCL2+ CD8+ T cells (p < 0.01) were observed than in

patients with non-SR-GvHD

    - BCL2 expression was negatively associated to survival post-HSCT ($p = 0.042$)
    - High expression level of BCL2 at the onset of GvHD was predictive of poor prognosis

- An increase in BCL2+ total leukocytes was observed in PB samples from the aGvHD cohort (n = 10) and the cGvHD cohort (n = 16; $p < 05$) *vs* no GvHD controls (n = 4). The upregulation of BCL2 was due to an increase in BCL2+ CD4+ in cGvHD patient samples, whereas in the aGvHD samples it was caused by BCL2+ CD8+ T cells and NK cells
- An analysis of skin lesions of aGvHD (n = 18) and cGvHD samples (n = 13) and healthy controls (n = 12) revealed an increase in BCL2-expressing T-, B-, and NK cells in GvHD skin lesions in comparison to controls
- The levels of BCL2 in regulatory T cells (Treg) and conventional T cells were compared between GvHD-affected skin (aGvHD and cGvHD; n = 5 each) and healthy controls (n = 5). Both Treg and conventional T cells expressed higher levels of BCL2 compared to healthy controls

Susceptibility to BCL-2 inhibition

Further analysis revealed an increase in BCL2/BCL2L1 ratio in aGVHD skin samples (n = 11) compared to controls (n = 13), indicating high sensitivity to venetoclax (a BCL2 inhibitor), further supporting the inhibition of BCL2 for the treatment GvHD. To test the effects of venetoclax on cytotoxic T cells in vitro, mixed leukocyte reactions from PBMC of unrelated donors (n = 7) were established and venetoclax was added after five days of culture:

- The effect of venetoclax on T cell apoptosis was dose-dependent
- After 24 hours of treatment, there was a significant decrease in the proportion of CD8+ T cell (of which 79% expressed BCL2) compared to CD4+ T cells (of which only 20% expressed BCL20.

- *In vitro*, BCL2 inhibition reduces numbers of cytotoxic effector cells by the induction of apoptosis in cells expressing high levels of BCL2

The effect of BCL2 inhibition was also compared between T cells of patients with SR-GvHD and non-SR-GvHD (Cohort 4). After BCL2-inhibition, apoptosis was observed in both CD4+ and CD8+ T cells of patients with SR-GvHD but not in healthy non-SR-GvHD T cells. Also, BCL2-inhibition lead to a favorable increase in the Treg/conventional T ratio.

Conclusions

- The results of this study showed that BCL2 is upregulated in T cells early in GvHD development as well as in cGvHD and SR-GvHD. Over-expression of BCL2 in GvHD-affected organ samples and immune cell subsets was associated with poor outcomes
- There was increased BCL2 expression in GI samples from patients compared to control samples with non-GvHD GI inflammation suggesting the potential use of BCL-2 expression for diagnosing GI GvHD
- The inhibition of BCL2 induced selective apoptosis in allo-reactive CD4+ cells from patients with aGvHD and in both, allo-reactive CD4+ and CD8+ T cells of patients with SR-GvHD. In contrast, there was no induction of apoptosis in T cells from healthy controls
- In summary, BCL2 can be relevant in diagnosis of aGvHD and cGVHD and as a predictive marker of outcome after allo-HSCT
- The authors suggest BCL2 inhibition should be tested as a target in the treatment of patients with SR-GvHD

References:

1. Strobl J, Pandey RV, Kausgruber T et al. Anti-apoptotic molecule BCL2 is a therapeutic target in steroid-refractory graft-versus-host disease. *J Invest Dermatol*. 2020. DOI: 1016/j.jid.2020.02.029