Lack of Glucocorticoid-induced Leucine Zipper (GILZ) results in B cell lymphocytes in mice

M. Biagioli[1], D. Sorcini[1], S. Bruscoli[1], O. Bereshchenko[1], T. Frammartino[1], S. D'Angelo[1], M. Cimino[1], C. Riccardi[1]

[1]Dept. of Medicine, Section of Pharmacology, University of Perugia, Italy

Glucocorticoids (GC) are widely used as immunosuppressive drugs and antitumor agents in some acute leukemias and multiple myeloma. Therapeutic doses of GC induce growth suppressive and cytotoxic effects on various leukocyte types including B cells. Glucocorticoid-induced leucine zipper (GILZ) is a rapidly, potently and invariably GC-induced gene. It mediates a number of GC effects, such as control of cell proliferation, differentiation and apoptosis. It belongs to TSC22d family, sharing conserved TSCbox domain, important for the regulation of Ras/MAPK/Erk pathway; one of the TSC22d members were recently found mutated in diffuse large B cell lymphoma patients (6), suggesting that also TSC22d family members may play a role in lymphoma development. Therefore, new studies are needed to characterize the role of GILZ in normal and pathological hematopoiesis and to evaluate its role as a mediator of the effects of GC in leukocytes. Studies carried out by us are based on the use of mouse models of knock-out mice (KO) for GILZ, that determine GILZ deletion in total body (crossing with transgenic CMV-CRE mice) or specifically in B lymphocytes (crossing with transgenic CD19 Cre mice).

Our data shows, in young *gilz KO* mice normal body and lymphoid tissues weight; cell counts in peripheral blood (PB), thymus, spleen, peripheral lymph nodes. However, we observed in bone marrow (BM) an increase in cell number and flow cytometry analysis reveals that the increasing number of BM's cells is due to an increase of B220+ population in KO mice. Moreover, the preliminary data of old mice shows, an accumulation of B220 + cells in KO mice also in peripheral organs not only in the BM.

The analysis of different B subpopulation in young mice by flow cytometry using surface markers able to discriminate all the subpopulation among B cells of BM and spleen, revealed an increase in frequency and number of all B subsets only in BM but not in spleen of *gilz* KO mice compared to wt. In old mice, lack of *gilz* leads to a 1.5-2 fold increase in white blood cell counts in PB compared to the wt. The B220+ cell compartment revealed a strong increase in frequency and number in *gilz* KO mice compared to wt, but not of CD3+ cells, where frequency and number are the same in KO and WT mice, indicating that the increased lymphocytes number in *gilz* KO PB is due to an increased number of B lymphocytes.

We analyzed the expression of pro- and anti-apoptotic genes in B cells like Bcl-2, bim and bax. Data shown an increased levels of expression of anti-apoptotic gene Bcl2 and a moderate decrease of pro-apoptotic genes in the B cell of KO mice, while no differences in proliferation were found, as revealed by KI67 staining, a marker of proliferation, in B cell of KO and WT mice.

Together our data indicate that GILZ is important for B cell development and maintenance. Lack of GILZ leads to a B lymphocytosis disorder overtime due to a decrease in B cell apoptosis, and it may represent a new potential therapeutic target in the treatment of hematological malignancies with less adverse side effects than GC.