The project entitled “Tumor suppressor function of FOXO1 in diffuse large B-cell lymphomas: mechanisms of regulation and personalized rational targeting strategies” is now completed.

FOXO proteins have emerged as important regulators of wide spectrum of cellular functions, including cell cycle, apoptosis, DNA damage response, glucose metabolism, and oxidative stress resistance. In humans, FOXO proteins are converging points integrating signaling pathways triggered by growth factors, cytokines, nutrients, oxidative stress and B-cell receptor activity. In normal B lymphocytes, nuclear exclusion of FOXO1 is a critical effector of pro-survival signaling mediated by B-cell receptor (BCR). Diffuse large B-cell lymphomas (DLBCLs), the most common type of lymphoma in adults, are clinically and genetically heterogeneous tumours. In line with the clinical heterogeneity of DLBCLs, multiple signalling pathways have been identified that mediate survival benefit in different subsets of tumors. For example, a large subset of DLBCLs has surface expression of immunoglobulins and a constitutive B-cell receptor activity. Disruption of this signal with small molecule SYK or BTK inhibitors are capable of inducing apoptosis in DLBCL cells, the effect at least in part mediated by decreased AKT activity. Since decreased AKT activity leads to decrease in FOXO1 phosphorylation and its subsequent activation, we hypothesized that FOXO1 might be an important effector of BCR inhibitor-mediated toxicity in human BCR-dependent lymphomas. For this reason, we have first examined the role of AKT-FOXO1 pathway in BCR-dependent DLBCL cells treated with SYK inhibitor. In BCR-dependent DLBCL cell lines (DHL4,DHL6, Ly7, Ly1), AKT and FOXO1 phosphorylations were sensitive to SYK inhibition and decreased after incubation with a SYK inhibitor fostamatinib. Diminished FOXO1 phosphorylation resulted in its nuclear relocalization and induction of FOXO1-dependent gene expression. Complementation of AKT activity by its constitutively active form (myrAKT) disrupted the effect of SYK inhibitor on FOXO1 phosphorylations and rescued DLBCL cells from fostamatinib toxicity. To assess whether the increased activity of FOXO1 is sufficient to induce apoptosis of DLBCL cells, we transduced DHL4 cells with a constitutively nuclear and transcriptionally active FOXO1 mutant. The mutant induced G1/S cell cycle arrest and triggered apoptosis, demonstrating that FOXO1 activation is sufficient to induce apoptosis of DLBCL cells. Next, we assessed the toxicity of SYK inhibitor in cells lacking FOXO1. Cells with silenced FOXO1 exhibited 70% lower sensitivity to SYK inhibitor, as measured with proliferation and apoptosis assays (p