**Title : Genetically modified blood stem cells reverses autoimmune diabetes**

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**Summary**

Type 1 diabetes mellitus (T1D), begins with the immune-mediated destruction of insulin producing pancreatic β-cells. Recombinant insulin is the cornerstone of T1D therapy; despite improvements in the regimen of insulin administration precise glucose regulation is not possible. T1D patients, while surviving to the acute events associated with hyperglycemia, experience chronic complications, such as chronic kidney disease, diabetic retinopathy and cardiomyopathy. Most immunotherapies tested thus far failed to cure T1D or halt the T cell insult exerted on β cells, mainly because they were not specific/tailored for T1D. Only autologous hematopoietic stem cell transplantation in combination with non-myeloablative immunosuppressive regimen has achieved complete independence from exogenous insulin in T1D patients in 60% of treated individuals. Interestingly, hematopoietic stem cells (HSPC), blood cells progenitors located in the bone marrow, are endowed with immunoregulatory properties, which have been shown to be linked to the expression of the immune checkpoint PD-L1 (or CD274). Indeed, mice deficient in PD-L1/PD-1 develop accelerated diabetes and defect in PD-L1 expression in HSPCs impair their immunoregulatory ability.

In our investigation, we used NOD mouse, a model that develops spontaneous autoimmune diabetes, characterized by lymphocyte infiltration of pancreatic islets, mimicking the human T1D disease. We assessed the gene expression profiling of HSPC in NOD mice which showed a markedly downregulation of PD-L1 as compared to control mice. This defect in PD-L1 in HSPCs from NOD mice was also confirmed by multiple techniques such as RT-PCR, western blot and confocal microscopy. In order to understand the mechanism behind PD-L1 downregulation in HSPCs from NOD mice, a genome wide analysis comparing HSPCs from NOD to those from C57BL/6 mice depicted a network of small RNA (microRNAs), 14 of which controlled PD-L1 expression. In fact, silencing one those miRNA key player/controller of PD-L1 expression, miR-1905 restored the defect PD-L1 as stated/confirmed by RT-PCR and Western blot analysis. Based on this, we sought to overcome this defect in PD-L1 on HSPCs by genetic engineering and pharmacologic approaches. Our genetically engineered HSPCs successfully reversed of hyperglycemia in 100% of the treated mice, which showed reduced lymphocyte infiltration and preserved insulin staining. When tested in vitro, our genetically engineered HSPCs markedly abrogated CD4 and CD8 specific autoimmune response and exerted robust immunoregulatory properties almost comparable or higher to those obtained with CD4+CD25+ regulatory T cells. Paralleling the gene therapy approach, we generated by pharmacological modulation method pre-culturing HSPCs with a cocktail of small molecules to upregulate PD-L1 expression. Our newly generated HSPCs, achieved comparable results to those obtained with the genetically engineered HSPCs as they markedly abrogated the autoimmune response in vitro and in vivo by reversing diabetes in 40% of hyperglycemic NOD mice. Similarly to our murine studies, PD-L1 expression in HSPCs from T1D patients was reduced as compared to healthy controls. The defect in human HSPCs from T1D was confirmed by multiple techniques: Flow cytometry, RT-PCR, western blot and confocal microscopy. In a similar way to the murine assays, we generated by pharmacological approach, human PD-L1+ HSPCs which markedly upregulate PD-L1 expression. When tested in an in vitro autoimmune setting, our pharmacologically modulated PD-L1 HSPCs exerted a robust immunomodulatory effect. Overcoming the defect in PD-L1 by genetic or pharmacologic restoration halt the autoimmunity and eventually cure the disease. This study opens new venues for a possible therapeutic intervention in the future and could be the first step toward establishing a tailored clinical trial in T1D.