

## **Scientific Abstract**

The purpose of this Phase I study is to explore the safety and immunologic efficacy of combining depletion of regulatory T cells (Treg cells) in patients with B-Chronic Lymphocytic Leukemia (B-CLL) using the interleukin-2 immunotoxin directed to the CD25 antigen (denileukin diftitox, ONTAK) and vaccination with autologous tumor cells expressing human CD40 Ligand (hCD40L) and human IL-2 (hIL-2). Although the malignant B-CLL cells express tumor-specific peptides (including those derived from immunoglobulin molecules) and major histocompatibility complex class I antigens, they lack the capacity for co-stimulatory signaling to T cells. This contributes to their protection from host anti-tumor immunity. We demonstrated that transgene expression of the hCD40L and hIL-2 genes in B-CLL cells increases their capacity to stimulate an immune recognition both *ex-vivo* and *in vivo* in a recently completed a Phase I clinical trial. However, the immunological activation generated in B-CLL patients by this vaccine was transient and did not produce significant and sustained clinical benefits. Treg cells represent a subpopulation of T lymphocytes characterized by immunological markers (high expression of the hIL-2 receptor CD25 and Foxp3 molecule) and the functional capacity to inhibit the immune response. This subpopulation is particularly abundant in the peripheral blood of cancer patients including B-CLL patients and might contribute impairing the potency of the tumor vaccine. Animal experiments and preliminary experiences in human indicate that the elimination of these Treg cells might increase the immune response to anti-tumor vaccination. In this study we propose to combine depletion of Treg cells in B-CLL patients using a hIL-2 immunotoxin (ONTAK) already used in Phase 1/11 clinical trials and vaccination with the same vaccine preparation that we have already proved to induce an immune response in these patients. The end points of this study will be evaluation of the safety and immunologic efficacy of this combination.