POINTS TO CONSIDER

Technical Abstract

The purpose of this study is to determine if prolonged vaccination with autologous tumor cells expressing human CD40 Ligand (hCD40L) and human IL-2 (hIL-2) will produce prolonged suppression of putative tumor stem cell (SP) populations leading to subsequent diminution in the effects on the bulk of non-SP tumor cells. Using flow cytometric analysis, we found that normal murine and human bone marrow contains a "Side Population" (SP) of cells, defined by a high capacity to expel Hoechst dyes, a function attributed to overexpression of ABC transporter genes, most notably ABCG2.[1-3] Extensive study of this SP cell fraction has revealed that even a single SP cell from normal marrow can fully repopulate experimental animals with all hemopoietic lineages in serial transplants, suggesting that these cells are closely related to true hemopoietic stem cells (HSC). Subsequently, we found an SP population amongst the blast population in patients with acute myeloid leukemia[4] and in solid tumors.[5] More recently, we identified SP cells in primary tumor cells from patients with lymphoma and B-cell chronic lymphocytic leukemia (B-CLL). The tumor SP cells we previously described have a number of characteristics compatible with a tumor stem cell. They have a more primitive phenotype than the bulk population of tumor cells, they undergo asymmetric division (SP cells form new SP cells and non-SP tumor cells while non-SP cells form only other non-SP cells) and the SP population is transplantable in xenograft models.

Although 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 have previously 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 we immunized B-CLL patients by subcutaneously injecting an autologous vaccine consisting of malignant cells expressing CD40L, which activates the B-CLL cells in the vaccine through their CD40 receptor and increases their expression of co-stimulatory molecules. The vaccines are also engineered to secrete interleukin-2 (IL-2), which augments any resulting T cell immune responses.[6] Because only a relatively small number of cells are administered and because the route of administration is subcutaneous, the vaccine cannot directly affect the vast majority of endogenous B-CLL cells in peripheral blood and lymphoid organs. However, the vaccine was able to induce a significant, but transient, T cell response to autologous B-CLL cells, and 3/8 patients showed a transient diminution in lymph node size[6]. Surprisingly, since the vaccine was composed predominantly (>99%) of non-SP cells, immunization strikingly diminished the circulating SP B-CLL population. In all but one patient this effect was again seen only during and immediately after immunization. SP numbers returned to pre-treatment levels within 4 months, and no effect on circulating (non-SP) tumor cells was seen. In one patient, however, the SP population disappeared entirely during immunization, and did not return. This patient had no change in peripheral blood B-CLL counts for 6 months. At 9 months, however, he was found to have shrinkage of lymph nodes and spleen, and his cell counts were diminishing without additional therapy. By 12 months his B-CLL counts had progressively fallen from >35,000/mm^3 at the time of immunization to <1000/mm^3. B-CLL cells are believed to be long-lived in vivo, so that the kinetics of the decline in his malignant cells is compatible with the loss of a stem cell population and gradual failure to resupply the circulating pool from a dwindling reserve of "mature" non-SP tumor cells in lymph nodes and spleen.

Clearly, the observations outlined above are individually limited and preliminary, but taken together, they are nonetheless consistent with the concept that B-CLL SP cells are an important precursor population for the bulk of the (non-SP) tumor and could be targeted by immune cells following antitumor vaccination. Given these in vitro data, and their apparent in vivo correlates, we plan to extend our earlier Phase I/II clinical study of an autologous CD40L- and IL-2-expressing B-CLL vaccine, by administering subcutaneous doses of cells, already shown to have short term safety and immunological effectiveness, over a more prolonged (12-month) period in an effort to sustain the anti-SP cell immune response. We will determine whether prolongation of immunization also prolongs the antitumor immune response, whether SP populations are diminished long term and whether prolonged suppression of the tumor SP population is associated with a progressive but delayed tumor response.