POINTS TO CONSIDER

TECHNICAL ABSTRACT

This protocol is an extension of our promising earlier study of allogeneic neuroblastoma vaccines for children with this disease (RAC 9712-223). We will use the identical gene modified allogeneic neuroblastoma cell line as in our previous study, but we will add a second, genetically unmodified, cell line to broaden the range of tumor antigens presented to the patients' immune systems. We will administer these vaccines following autologous transplantation, when tumor burden is at its lowest.

For patients with high-risk neuroblastoma, the prognosis remains poor with >80% of patients with disseminated disease relapsing within 3 years. For these patients, immunotherapy is an appealing strategy. Animal models have shown that gene modified tumor cells expressing a variety of cytokines are often highly immunogenic delaying tumor growth and even producing outright rejection. A Phase 1 trial of vaccination with genetically modified Interleukin-2 secreting allogeneic neuroblastoma cells demonstrated both the safety and potential therapeutic utility for this strategy. Experimental data suggested that combinations of a cytokine and a chemokine may act synergistically to produce a stronger immune response. In murine models, transgenic chemokine-cytokine tumor vaccines overcome many of the limitations of single-agent immunotherapy by producing the sequence of T-cell attraction followed by proliferation of tumor antigen-activated clones. In a previous study the safety and immunologic effects of this approach in humans were tested in patients with relapsed or refractory neuroblastoma. (RAC 9712-223). The aim of this study was to determine the safety of up to four subcutaneous (SC) injections of allogeneic neuroblastoma cells which have been genetically modified by a retroviral vector to secrete Interleukin-2 and by a plasmid to secrete lymphotactin. This combination vaccine enhanced the anti-tumor immunity induced by IL-2 transduced cells alone. 28 pediatric patients with relapsed/refractory neuroblastoma received the IL2/LPTN combination expressed in the SJNB-JF tumor cell line. Toxicity was limited to grade I/II injection site reactions. Skin biopsies of injection sites showed infiltration of CD4+ and CD8+ cells, eosinophils and Langerhans cells. Expansion of peripheral blood NK and CD4+ T-cells occurred as well and cytokine profiles of in-vitro vaccine restimulated peripheral blood mononuclear cells indicated a predominance of T-helper (TH2) response. Measurable tumor responses included complete remission in 4 patients that were sustained long term in 2, and partial responses in 2 patients. Hence, allogeneic tumor cell vaccines combining transgenic lymphotactin with IL-2 seem to have little toxicity in humans and can induce an antitumor immune response. In this earlier study, the immune response was insufficient to overcome active recurrent neuroblastoma. In the current proposal we will extend the study by adding a second, genetically unmodified neuroblastoma tumor cell line (SKNLP) to the current vaccine product. The rationale for this modification is the heterogeneity of neuroblastoma. SKNLP, unlike SJNB-JF is N-MYC amplified and has a 17q gain which alters gene expression by neuroblasts and markedly changes the surface proteins expressed. Increasing the number of antigens to which an immune response can be mounted not only increases the probability of matching the immune response made to the antigens expressed by the host's own tumor, it also increases the probability that a multiplicity of antigenic epitopes will be recognized. We therefore intend to test the safety, and immunologic and clinical efficacy of a combination of 2 allogeneic neuroblastoma tumor cell line vaccines, one of which has been genetically modified to secrete the cytokine/chemokine combination of IL-2 and lymphotactin, in patients who have received chemotherapy for newly diagnosed, high risk neuroblastoma and will be

treated by a single autologous stem cell rescue as consolidation therapy. Primary end points will be safety, development of an immune response and effects on residual tumor, and the secondary endpoints will be survival and disease free survival at 3 years