POINTS TO CONSIDER

TECHNICAL ABSTRACT

Improvements in radiochemotherapy have correspondingly improved the prognosis of patients with EBV-positive Hodgkin and non-Hodgkin lymphoma. However, patients resistant to the standard therapeutic approaches have a poor outcome. Moreover, life expectancy and quality of life of patients cured of lymphoma are both significantly reduced by treatment related mortality and morbidity. These limitations of current treatment protocols illustrate the need for more effective and less toxic therapeutic approaches. In Hodgkin's and non-Hodgkin's lymphomas up to 40% of specimens have been shown to carry EBV-DNA and express EBV-genes. Our group has successfully generated EBV-specific CTL (EBV-CTL) in patients with EBV-positive Hodgkin's lymphoma.[1] After infusion these CTL home to the tumor sites and persist in the circulation for up to 9 months. However although transient clinical benefits were seen, only two complete responses were achieved with CTL therapy alone.

At present the anti-lymphoma activity of EBV-CTL is limited by several factors, including 1) EBV-CTL lines generated by standards methods are dominated by T-cell clones not reactive to the subdominant EBV proteins LMP1 and LMP2 expressed in Hodgkin's disease and non-Hodgkin's lymphoma, and 2) infused EBV-CTL did not significantly expand *in vivo* after adoptive transfer. We are currently conducting 2 RAC and FDA approved Phase I clinical trials as an initial step to overcome both limitations. The majority of EBV-positive lymphomas in immunocompetent patients express a limited subset of EBV antigens, EBNA1, LMP1, and LMP2. In the first clinical trial we are generating CTL against the subdominant LMP2 (LMP2-CTL) in an effort to increase the tumor-specificity of the infused CTL lines. One strategy to enhance the *in vivo* expansion of T cells is to reduce the lymphocyte pool prior to T-cell infusion. This should result in homeostatic proliferation of the infused T cells to restore the lymphoid compartment. In the second clinical trial we are therefore evaluating a pair of monoclonal antibodies, targeting the CD45 antigen (CD45MAbs), to lymphodeplete patients prior to adoptive transfer of LMP2-CTL.

The preliminary results of both studies are encouraging. Infusion of LMP2-CTL was safe and resulted in 4 complete responses in 8 patients treated with active disease. The use of CD45 monoclonal antibodies was safe, resulted in transient lymphodepletion, and enhanced CTL expansion in comparison to giving CTL alone. *Although these results are encouraging we hypothesize that broadening the tumor-specificity of CTL will further increase anti-tumor effect of infused CTL.* Since CTL escape mutants have been described for tumors as well as viruses and the expression of LMP1 and LMP2 in tumors is heterogeneous, we propose the infusion of LMP1- and LMP2-specific CTL after lymphodepletion to 1) ensure that good CTL epitopes are available regardless of the patient's HLA type and 2) generate the broadest CTL response possible against the malignant lymphoma cells.

LMP1-CTL have been detected in EBV-seropositive donors.[2-4] We and others have shown that LMP1-CTL can be activated and expanded from EBV-seropositive donors with antigen presenting cells expressing a non-toxic form of LMP1. LMP1-CTL generated by this method can recognize and kill LMP1 expressing lymphoma cells.[5-7] In addition, *in vivo* activation of LMP1-CTL in an animal model resulted in regression of LMP1 positive tumors.[8] In the phase I clinical trial proposed here, patients will receive a fixed dose of CD45 MAbs followed by escalating doses of autologous LMP1- and LMP2-CTL that will be administered to lymphoma patients with refractory or relapsed disease.

Of note, the pair of monoclonal antibodies and the adenoviral vector used for CTL generation are the same as in an earlier RAC reviewed FDA approved protocol except for the addition of the LMP1gene to the vector.