37. Engineering CD20-Specific Chimeric Receptor Redirected T Cells with Inducible Co-Expression of a Caspase-9 Based Suicide Switch for Adoptive Immunotherapy of Mantle Cell Lymphoma

Lihua Pan,[1] Carolina Berger,[1] Yukang Lin,[1] Jinjuan Wang,[1] Stanley R. Riddell,[1] Oliver W. Press.[1]
[1]Clincial Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Mantle Cell Lymphoma (MCL) is a distinct clinicopathologic subtype of Non-Hodgkin's Lymphoma (NHL) that afflicts around 5000 North Americans each year and is considered incurable by conventional treatment, with a median survival of 2-3 years. Surface expression of the CD20 molecule is an invariant feature of MCL cells. Work from our laboratory using T cells bearing a transfected CD20-specific chimeric T cell receptor (cTCR) has demonstrated promise in a murine model and a phase I clinical trial. However, limitations including low transfection efficiency, low surface expression of cTCR, and risk of insertional mutagenesis hinder the further exploitation of this approach. Here we describe a new immunotherapeutic approach for treatment of MCL using autologous T lymphocytes that have been genetically modified with a bicistronic IRES retroviral vector to express both a cTCR recognizing the human CD20 antigen and a suicide gene using inducible activation of caspase 9. The cTCR gene was designed to encode a SP163 translational enhancer, a 1F5scFvFc anti-CD20 recognition domain, CD28 and CD137 co-stimulatory domains, and a CD3ζ signaling region for maximal expression, activation and cytolytic activity. Transduced Jurkat T cells display robust and sustained surface expression of the chimeric T cell receptor for more than 6 months. When exposed to chemical inducers of dimerization (CID), only Jurkat T cells transduced with both cTCR and iCas-9 genes but not cTCR alone underwent CID-induced caspase-mediated apoptosis. The same constructs were also tested in primary human T cells in vitro. We have been able to achieve transduction efficiency ranging from 15% to 70%. All transduced primary T cells expressed the cTCR at a level 10 to 100 fold higher than cells transfected with naked DNA plasmids encoding a similar cTCR. These cTCR+ T cells are able to execute highly effective cytolytic functions when cultured together with ^{51}Cr-labeled CD20+ lymphoma cell lines including EL4-CD20, Daudi and Granta, a MCL cell line. They had no effect on CD20-negative cell lines. This demonstrates the high specificity of the modified T cells. We detected CID induced activation of caspase activity and elimination of T cells transduced with both cTCR and iCas-9 genes via flow cytometric-based analysis, whereas CID had no effect on control T cells transduced with cTCR alone. In vivo testing of these T cells will be carried out in a murine MCL model as well as in a non-human primate *Macaca nemestrina* model in the near future. Our work demonstrates the feasibility and promise of this approach in treating relapsed MCL and other CD20 bearing B cell malignancies in a safer and more efficient manner.

38. A Phase I Trial for the Treatment of Chemo-Refractory Chronic Lymphocytic Leukemia with CD19-Targeted Autologous T Cells

Renier J. Brentjens,[1] Daniel R. Hollyman,[3] Mark Weiss,[1] Jolanta Stefanski,[3] Mark Przybylowski,[3] Shirley Bartido,[3] Oriana Borquez-Ojeda,[3] Clare Taylor,[3] James Hosey,[3] Mark Heaney,[1] Michel Sadelain,[1,2,3] Isabelle Riviere.[1,2,3]
[1]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Molecular Pharmacology & Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Gene Transfer & Somatic Cell Engineering Facility, Memorial Sloan-Kettering Cancer Center, New York, NY.

Building on our earlier demonstration that human peripheral blood T cells genetically targeted to CD19 can eradicate established, systemic B cell tumors in mice, we have developed a novel immunotherapy for the treatment of chronic lymphocytic leukemia (CLL). This strategy is based on the genetic modification of patient T cells to recognize the B cell-specific cellular antigen CD19, expressed on B cell tumors, through the retroviral expression of a chimeric antigen receptor (CAR) specific for CD19 (19-28z). We have initiated a clinical trial utilizing 19-28z$^+$ autologous T cells in patients with purine analog-refractory chronic lymphocytic leukemia (CLL) (BB-IND 13266). Enrolled patients initially undergo a leukopheresis procedure in order to obtain T cells. Following activation with Dynabeads® ClinExVivo CD3/CD28 magnetic beads, the T cells are transduced with the CD19 specific 19-28z CAR using cGMP gammaretroviral vector stocks generated in our facility, and expanded utilizing a Wave™ bioreactor platform-based rapid expansion protocol. To assess safety, patients enrolled in the first cohort of this trial received an infusion of the lowest planned dose of modified T cells alone. Subsequent cohorts will receive infusions of 19-28z$^+$ T cells following escalating doses of cyclophosphamide chemotherapy. Patients treated in the first cohort with the lowest modified T cell dose alone experienced grade 2 fevers and rigors during infusion but no dose limiting toxicities. Treated patients variably experienced decrease in lymph node size, decreased CD19$^+$ B cell numbers in the peripheral blood, and a decreased dependence on red blood cell transfusions. We conclude so far that infusion of CD19-targeted T cells alone is well tolerated in patients with refractory CLL, with objective evidence of transient anti-tumor responses. Patients on the second cohort, who will receive prior lymphodepleting chemotherapy with cyclophosphamide, are being enrolled. The trial presented here is the first to utilize gene modified autologous T cells for the treatment of CLL, as well as the first to target CD19$^+$ tumors utilizing a rapid T cell expansion protocol, which represents a promising approach for patients with B cell malignancies.

39. Cross-Talk between Tumor Cells and Endothelium Triggers a Strong Chemotactic Signal Recruiting T Lymphocytes to Distant Tumor Deposits

Nabil Ahmed,[1] Vita Salsman,[1] Kwong-Hon Chow,[1] Huseyin Kadikoy,[1] Xia-Nan Li,[1] Laszlo Perlaky,[1] Meenakshi Bhattacharjee,[2] Cliona Rooney,[1] Helen Heslop,[1] Stephen Gottschalk.[1]
[1]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX; [2]Pathology, Texas Children's Hospital, Houston, TX.

Background: Failure of local control of medulloblastoma (MB) is a poor prognostic factor that heralds incurable disease recurrence that is multi-focal in up to 60% of patients, adding to the dismal prognosis of these patients. We have shown that genetically modified T cells expressing HER2-specific chimeric antigen receptors (*HER2*-T cells) induce regression of *HER2*+ human MB growing in the brains of mice after intratumoral injection. The objective of this project