cells(APC). SV-BR1 cells had been previously incubated in 100 u/ml interferon-gamma to upregulate cell-surface antigens and stored frozen over liquid nitrogen. Immediately prior to use the cells are thawed, washed and irradiated to 200cGy. Inoculation was repeated q2weeks × 3, then monthly until progression. Patients received low-dose cyclophosphamide, 300 mg/m^2, 2–3days prior to inoculation and also subcutaneous GMCSF, 125 mcg, immediately prior to inoculation and then daily × 8 to enhance APC function. Fourteen patients have been entered on study. In 44 treatments, the median dose was 14×10^6 cells (range $9–26 \times 10^6$), median viability 93% (range 73–97%). The median age was 53.5 years (range 39–70), HER2/neu positive 6 of 11, median previous chemotherapy regimens: 3 (range 1–5). Of 13 patients treated two are still alive at 48 and 129 weeks; overall median survival 48.7 weeks (range 5.4–139 weeks); Five patients survived >52 weeks. There were no objective responses in 11 evaluable patients but 4 of 6 patients developed significant increases in anti-SV-BR1 antibody by ELISA., and DTH responses to SV-BR1 developed in 3 of 7. Toxicity was mild and consisted principally of erythema and pruritus at injection sites. One patient developed transient atrial fibrillation and one patient, after 3 inoculations, declined further treatment for psychological reasons. While selection bias cannot be excluded, the survival data compares favorably with other third-line studies. Given the safety and feasibility of this preliminary trial, SV-BR1 has been transfected with the pcDNA3.1/GS/GM-CSF plasmid (Invitrogen) using Zeocin selection. GMCSF production by ELISA in 3 representative lots was 115, 129, and 110 ng/10^6 cells/24 hours. Biological activity was validated by culture with the GMCSF-dependent MUTZ-2 leukemic cell line. A clinical trial is planned pending final regulatory review.

Dendritic Cell Vaccines in Patients with Non Small Cell Lung Cancer

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Non small cell lung cancer (NSCLC) is a leading killer of men and women throughout the world. We have developed an immunotherapy for this disease using dendritic cells (DCs) pulsed with apoptotic bodies derived from an allogeneic NSCLC cell line called 1650TC. The 1650TC line, (HLA-A2; B15; C7), expressed the NSCLC associated antigens Her-2neu, CEA, WT1, Survivin and Mage-2. The DCs were generated from leukapheresis products obtained from 16 patients with disease stages ranging from 1A to 3B. CD14+ monocytes were purified from the leukapheresis products using Miltenyi magnetic bead separation technique. The CD14+ cells which were greater that 95% pure were cultured in XVIVO 15 serum free medium containing 10% human allogeneic AB serum in the presence of 20 ng/ml GMCSF and IL-4 for 7 days. The CD14+ cells were cultured in either 6 well cell culture plates or T175cm^2 flasks at 1.0×10^6 CD14+ cells/ml. At day 7, 1650 TC was subjected to uvB light and irradiation. The resultant % of apoptotic cells after this treatment ranged from 35–40%. The apoptotic cells were added to the DCs at a ratio of 1:1. After 18hr culture, the DCs were harvested for intradermal injection in the patients. All 16 patients received an average of 100 million antigen pulsed DCs in both a prime and a boost dose separated by 30 days. The DCs used for injection appeared to be immature based on the low levels of expression of CD80 and low levels of secretion of IL-12p70. There were no adverse events associated with the DC injections. Peripheral blood was obtained from each patient post injection and lymphocytes monitored for anti-tumor reactivity by ELISPOT technique. Results of ELISPOT analysis examining gamma interferon release showed that 5 of 16 patients had no clear anti-tumor immunologic response (no response); 5 of 16 patients had lymphocytes which showed a tumor antigen independent response(recognized autologous DCs alone) and 6 of 16 patients had lymphocytes showing an anti tumor immunologic response (recognized DCs following ingestion of 1650-apoptotic bodies). Immunologic responses were independent of prior therapy or stage of the disease. Both favorable and unfavorable clinical outcomes were independent of measured immunologic outcomes. In conclusion, vaccines were well tolerated and induced biologic activity in a number of patients. Studies are continuing in our lab to determine the antigens recognized by the anti-tumor reactive lymphocytes and also examine the nature of the reactivity against DCs alone. The clinical trial is also continuing and includes a randomized trial using DCs and the COX-2 inhibitor Celebrex in NSCLC patients.

HEMATOLOGIC MALIGNANCIES

A Phase I/II Study of the Oral mTOR Inhibitor RAD001 in Patients with Advanced Hematologic Malignancies

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RAD001 (everolimus, Novartis) is an orally bioavailable derivative of rapamycin with demonstrated anti-proliferative activity against a broad panel of tumor cell lines and antitumor activity in experimental animal models of human cancer. RAD001 inhibits the mammalian target of rapamycin (mTOR) signaling pathway, which is involved in regulating many aspects of cell growth and cell cycle progression. Several lines of evidence implicate the importance of the PI3K/Akt/mTOR pathway in hematological malignancies. As there has been extensive experience with this agent in the solid organ transplantation setting, two dose levels (5 and 10 mg/day) were evaluated in the Phase I portion of this study to determine the maximum tolerated dose (MTD) to be used in the Phase II portion. RAD001 was administered orally once a day at the starting dose level of 5 mg. Seventeen patients have been enrolled, of which 16 (4 B-CLL, 4 MDS, 1 MF, 1 NK/T cell lymphoma/leukemia, 2 MCL, 3 AML, 1 T-PLL) were evaluable for safety and toxicity. Median age was 65 years (range, 51 to 76 years). Fourteen patients (87.5%) had received prior therapy (median, 2 regimens; range, 0 to 6 regimens). Median time on study was 39 days (range, 5 to 119 days). No dose-limiting toxicities were observed in the 6 patients enrolled in the Phase I portion of the study (cohorts of 3 patients per dose level). The most common adverse events were grade ≤ 2 and consisted of hyperglycemia, hyperlipidemia, hypocalcemia, hypomagnesemia, hypophosphatemia and hypokalemia, transaminitis, diarrhea or constipation, dermatitis, mucositis, anorexia, and asthenia. Grade 3 toxicities consisted of asymptomatic hypophosphatemia (2), hyperglycemia (4), asthenia (1), anorexia (1), and bone pain (1). No patient experienced grade 4 toxicities or death from RAD001. Of the 16 evaluable patients, 2 patients with B-CLL had a 33% and 65% reduction in the size of lymph nodes, respectively, and 1 patient with MDS had a minor increase in the platelet count. At the time of analysis, 2 patients had discontinued therapy due to disease progression and 1 patient had died from bleeding secondary to AV malformations in the gastrointestinal tract (unrelated to RAD001 therapy). These preliminary findings indicate that RAD001 is well tolerated at a daily dose of 10 mg/day and may have clinical activity in patients with hematologic malignancies. Enrollment is ongoing.

CPG Oligodeoxynucleotides Enhance Immunogenicity *In Vitro* in All Cytogenetic Subgroups of B-Cell Chronic Lymphocytic Leukemia (B-CLL), but Preferentially Augment Apoptosis in B-CLL with Good Prognosis Cytogenetics

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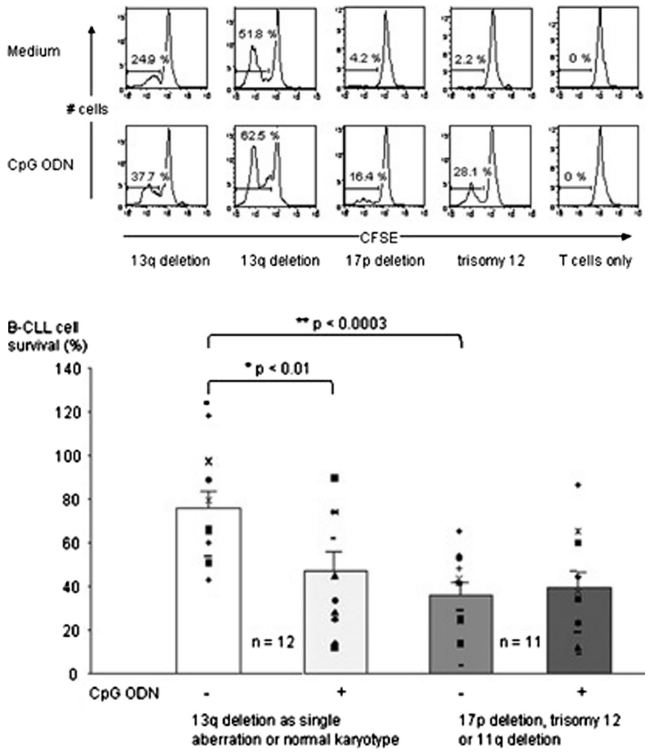
Immunostimulatory CpG oligodeoxynucleotides (CpG ODN) are TLR9 agonists that mediate a number of immunologic effects in normal and malignant B cells including upregulation of immunogenic molecules. They are therefore felt to be attractive as potential components of immunotherapy for B cell chronic lymphocytic leukemia (B-CLL). The cytogenetic status of B-CLL is known to be predictive of clinical prognosis, but little is known about how treatment of B-CLL cells correlates with cytogenetic status. The present study was designed to explore the impact cytogenetic status has on *in vitro* response to CpG ODN. B-CLL cytogenetic status was determined by interphase FISH. Immunophenotype and cell survival in the absence or presence of CpG ODN was determined in 23 samples. CpG ODN decreased *in vitro* survival of B-CLL cells with good prognosis cytogenetics, but had little effect on cells with poor prognosis cytogenetics. In contrast, CpG ODN upregulated costimulatory and antigen-presenting molecules and enhanced allogeneic T cell response in samples with either good or poor prognosis cytogenetics (Figure 1). We conclude CpG ODN induce changes in B-CLL consistent with enhanced immunogenicity in all samples studied, but induce apoptosis most effectively in the subset of B-CLL cells with good prognosis

cytogenetics. These studies suggest CpG ODN may be useful as immunotherapeutic agents for B-CLL irrespective of cytogenetic status because of their potential effects on immunogenicity.



Notable Phenomena of the 1960s Recapitulated. I. Graft-Versus-Leukemia Reaction and Natural Hybridoma Formation in Mice

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In 1962–3 at MD Anderson Hospital viral leukemia-susceptible Balb/c mice were rendered to be chronically runted with allogeneic disease due to cellular chimerism by injections of lymphoid cells from leukemia virus-resistant C57Bl mice. In these Balb/c mice leukemia viruses replicated to very low titers and induced no or low incidence of leukemia (AACR 1963; TX Rep Biol Med 1965; 7th Internat Symposium: Autologous Marrow and Blood Transplantation, Arlington, TX 1994). Later 20% of Balb/c mice were cured of virally induced leukemias by lethal irradiation followed by inoculations of Balb/c lympho- and hematopoietic cells deriving from donors that were actively immunized with a photodynamically inactivated leukemia virus vaccine (CR 1965; MD Anderson 20th Symposium: Carcinogenesis: A Broad Critique, WW 1967). A virally induced but cell-passaged murine lymphoma (#620) presented with the starry sky histological picture (J Inf Dis 1968; 1969), induced cell- and antibody-mediated immune reactions in its host and it could be rejected. Immune modulators either accelerated or decelerated its course (Science 1967; J Retic Soc 1970). The diploid lymphoma cells expressed budding retroviral particles and could fuse with immune plasma cells secreting leukemia virus-neutralizing antibodies. The fusion product tetraploid cells (#818) contained both leukemia virus antigens and IgG2b immunoglobulins; grew in suspension cultures and as lethal ascites tumors, while continuously secreting the specific antibody for over 10 years (1968–1980) (Lancet 1970; MD Anderson 14th Clinical Conference: Leukemia-Lymphoma, Yearbook, Chicago 1970; CR 1970; 1981). Antibody-coated lymphoma cells can be phagocytized by macrophages or dendritic cells (hence the starry sky feature of Burkitt lymphoma as the EBV-positive tumor cells are antibody-coated) (Critic Rev Immun 1991) to present tumor antigens to immune T cells. In human lymphomas two-directional FasL to Fas reactions occur between lymphoma cells and immune T cells (Inter J Oncol 2001). A human lymphoma cell line (T1 or #778) established in 1968 (JM Trujillo and the author) replicated unidentified retroviral particles. Lymphoma cells fused with plasma cells of the patient. During the RAG-induced V(D)J somatic hypermutations silent resident retrotransposons may acquire *env* sequences and emerge as endogenous retroviruses (AACR 2001; ASCO 2002). When these lymphoid cells generate autoantibody production, they may fuse with the antibody-producer plasma cells. Natural hybridoma formation in human lymphomas may be of decisive prognostic significance (Wainwright: Persp Biol Med 1992). This presentation will be illustrated with genuine original data and microphotographs from the 1960s to 1970s.

IMAGING

Homing of Tumor-Specific T Cells in the B16-OVA/OT-I Model System - towards T Cells as Carriers of Cytotoxic Substances in Therapy of Cancer

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Background: The use of adoptively transferred, tumor-specific T cells as carriers of cytotoxic substances to tumor tissue represents a possible new therapeutic modality for the treatment of cancer. In this approach, optimizing homing of adoptively transferred T cells to tumor tissue is critical. We have studied the ability of radioactively labeled, adoptively transferred tumor-specific T cells in the B16-OVA/OT-I model system to home to tumor sites using combined PET and MR imaging.

Methods: OVA-specific CD8+ T lymphocytes labeled with 124-Iodine-conjugated deoxyuridine (124-IdU) were injected into C57BL/6J mice carrying subcutaneous tumors of the ovalbumin (OVA)-expressing malignant melanoma cell line B16-OVA

Five days after adoptive transfer of the labeled cells, the mice were killed and subjected to PET- and MR imaging. Using a newly developed method for co-registration of the two image modalities, the anatomical localization of the transferred cells could be visualized and the amount of radioactivity in various anatomical locations accurately determined.

Results: The experiments showed a clear accumulation of the transferred cells in the tumors. In two independent experiments comprising 12 and 13 mice, respectively, we found a statistically significant difference in the mean activity between the tumor regions and the control regions ($p = 0.002$ and $p = 0.011$, respectively) in the corresponding contra-lateral control volumes).

Conclusions: These studies show that tumor-specific T lymphocytes home to subcutaneous tumors in substantial numbers, and that the cells can act as carriers for radioactive imaging substances. We suggest that such migrating T cells could be employed in a future therapy of cancer as carriers of toxic substances to tumors. This form of therapy would rely solely on the ability of the adoptively transferred cells to home effectively to tumor sites, and would not depend on any inherent capacity of the T cells to elicit an anti-tumor response.

Quantification and Visualization of Peptide/Major Histocompatability Complex Antigens on Live Cells Using High-Affinity Soluble T Cell Receptors

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Like monoclonal antibodies, soluble T-cell receptors (TCRs), have evident uses in the study and treatment of cancer, autoimmunity, and infection. However, the use of soluble TCRs for analytical and therapeutical applications is hampered by both the difficulty of producing stable, soluble TCRs and the natural low affinity of TCRs for their ligands.

We have overcome these limitations by introducing an inter-chain disulphide bond to stabilize the abTCR, and subsequent phage-display based affinity