

S21**DEVELOPMENT OF RETROVIRAL VECTORS WITH IMPROVED SAFETY AND GENE EXPRESSION AND THEIR APPLICATION TO GENE THERAPY**

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Almost all currently available retroviral vectors based on murine leukemia virus (MLV) contain one or more viral coding sequences. Because these sequences are also present in the packaging genome, it has been suggested that homologous recombination may occur between the same nucleotide sequence in the packaging genome and the vector, resulting in the production of replication competent retrovirus (RCR). Up until now, it has been difficult to completely remove viral coding sequences since some were thought to be involved in the optimum function of the retroviral vector. We have now developed a series of retroviral vectors that are absent of any retroviral coding sequences but produce even higher levels of gene expression without compromising viral titer. In these vectors, the intron and exon sequences from heterologous cellular or viral genes are present. When compared to the well known MLV-based vectors, some of these newly developed vectors have been shown to produce significantly higher levels of gene expression for a longer period. In an experimental system that can maximize the production of RCR, our newly constructed vectors produced no RCR, while MFG generated a large number of RCR. Some of these newly developed vectors were manipulated to express gene coding for iduronate-2-sulfatase (IDS), glucocerebrosidase (GC), and MDR and were confirmed to produce high viral titer and high enzyme levels.

S23**CELLULAR IMMUNOTHERAPY FOR CANCER WITH ALLOGENEIC BLOOD STEM CELLS**

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It is now established that a clinical alloresponse from the donor graft can contribute a potential anti-tumor effect following allogeneic blood stem cell transplantation in many haematologic and some solid malignancies. This graft-versus-tumour (GVT) effect is contributed by tumour-specific and allospecific donor T cells in the HLA-matched setting. Reduced intensity blood stem cell transplant (RIST) can facilitate the successful engraftment of donor allogeneic blood stem cells and allow deliberate mixed lymphohaematopoietic chimerism which can either convert spontaneously or via donor lymphocyte infusions (DLI) to full donor chimerism. This potentially less toxic approach may allow for shorter hospital stay, the treatment of older patients and the potential reduction of graft versus host disease (GVHD). GVHD after RIST may be reduced due to the milder conditioning resulting in less early pro-inflammatory component, the potential role of host regulatory T cells in suppressing GVHD-inducing donor T cell and the hypothesis that a mixed haematopoietic chimera may result in immune tolerance. An update of RIST studies in cancer will be presented. Also, we report our preliminary single institution experience with a cyclophosphamide and anti-thymocyte globulin based RIST conditioning regimen to induce mixed chimerism as a platform for donor lymphocyte infusion in solid tumours. Lymphohaematopoietic and T cell kinetics data following RIST will be presented. Unusual clinical pattern of GVHD in this series of patients will also be discussed. In conclusion, RIST is an evolving strategy to induce a potential alloimmune reaction against even chemorefractory cancers.

S22**ADENOVIRAL VECTORS FOR TARGETING OF CANCER CELLS.** Albert Deisseroth, Yucheng Tang, Yanzheng Liu, Hakan Akbulut, Jonathan Maynard, Lixin Zhang and Phyllis-Jean Linton. Sidney Kimmel Cancer Center, San Diego, CA, USA

Our laboratory has developed adenoviral vectors (Adv) which target chemotherapy and the immune response to tumor associated antigens (TAA) for cancer treatment. For immunological targeting, we found that the subcutaneous (sc) injection of an Adv (Ad-sig-TAA/ecdCD40L) encoding a TAA linked to the extracellular domain (ecd) of the CD40 ligand (CD40L) can overcome the anergy which exists in tumor hosts against TAA. The binding of the TAA/ecdCD40L protein released from infected cells near the vector injection site to DCs increases in DCs expression of the CCR-7 chemokine receptor and cytokine release, followed by migration of the DCs to regional lymph nodes. Tetramer staining, ELISPOT assay, and cytotoxicity assays all showed that sc injection of the Ad-sig-TAA/ecdCD40L vector increased the levels of splenic CD8⁺ T cells which were specific for the two TAA (human MUC1 and HPV E7) tested. Vaccination of mice transgenic for the hMUC1 antigen with the Ad-sig-hMUC1/ecdCD40L vector suppressed the growth of human MUC1 (hMUC1) antigen positive tumor cells in 100% of the test mice which were previously anergic to the hMUC1 antigen. The immune resistance to E7 positive tumor cells induced by the Ad-sig-E7/ecdCD40L vector lasted up to a year and was shown to be antigen specific and HLA restricted. For targeting of chemotherapy, we replaced 5-Fluorouracil (5FU) in 5FU based combination chemotherapy regimens by an Adv (Ad-Lp-CDIRESE1A) carrying the L-plastin tumor specific promoter regulating a bicistronic cytosine deaminase-E1A transcription unit in combination with 5-Fluorocytosine. This reduced the toxicity and increased the efficacy of these 5FU based chemotherapy regimens. Pre-clinical studies are being carried out to prepare these vectors for cancer treatment programs in the future.

S24**HYBRID CELL VACCINATION (HCV): BASICS, IMMUNOLOGICAL EFFECTS AND CLINICAL RESULTS**

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HCV for cancer immunotherapy attempts to render antigenic tumors immunogenic by fusing tumor cells of the patients with autologous or allogenic dendritic cells. Based on *in vitro* and *in vivo* animal experiments clinical studies were done to test the clinical effects of this therapy. A clinical HCV phase I/II study with malignant melanoma patients mostly at stage IV yielded evidence for clinical anti-tumor effects. While complete responses were rare, many patients experienced stable disease. The median survival of the responders was 28 months compared to a life expectancy of 8 months for melanoma stage IV patients. The analysis of the patients' peripheral white blood cells demonstrated in all cases increased frequencies upon vaccination of CD8⁺ T cells with specificity for melanoma-associated T cell epitopes. These results prove that HCV induces immune responses against a complex variety of tumor-associated antigens. The responding T cells were CD45RA^{low}, CD45RO⁺ suggesting a post-memory effector type. By double staining with MHC-peptide tetramers and, after stimulation with specific peptides, of intracellular IFN- γ , active and anergic T cells with specificity for the same epitope were differentiated to reveal that only a fraction of the TAA-specific cells can mount effector responses. Depending on the particular epitope, a varying fraction of the cells was anergic. In conclusion, HCV can induced anti-tumor immune and clinical responses. While there were only few cases of complete remission, long-term stable disease was often achieved suggesting that HCV may be suited for maintenance therapy where cure is not possible.