

**S13****HSV/AAV HYBRID AMPLICON VECTORS: IS AAV *REP* EXPRESSION COMPATIBLE WITH EFFICIENT HSV-1 DNA REPLICATION?**Cornel Fraefel<sup>1</sup>, Thomas Baechli<sup>2</sup>, Anne Greet Bittermann<sup>2</sup>, Christa Meyer<sup>1</sup>, Thomas Heister<sup>1</sup>, and Mathias Ackermann<sup>1</sup><sup>1</sup>Institute of Virology and <sup>2</sup>Center for Microscopy, University of Zurich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland

HSV-1 amplicons can accommodate foreign DNA of any size between 1 kbp and 150 kbp and, therefore, give room for genomic sequences as well as cDNA, large transcriptional regulatory sequences, multiple transgenes, and genetic elements from other viruses to create hybrid vectors. Hybrid amplicons use genetic elements from HSV-1 that allow replication and packaging of the vector DNA into HSV-1 virions, and genetic elements from other viruses that either direct integration of transgene sequences into the host genome or allow the vector to replicate autonomously as an episome. For example, adeno-associated virus (AAV) has the unique capability of integrating its genome into a specific site, designated AAVS1, on human chromosome 19. The viral *rep* gene and the inverted terminal repeats (*ITR*) that flank the AAV genome are sufficient for this process. We and others have previously designed HSV-1 amplicons that incorporate the AAV *rep* gene and a transgene cassette that is flanked by AAV *ITRs*. We have reported that these HSV/AAV hybrid vectors direct integration of transgene sequences into AAVS1 and support long-term gene expression. However, we also observed that *rep* expression inhibits replication from the HSV-1 origin of DNA replication, which drastically reduces the titers of vector stocks. We have now identified strategies to (i) analyze the interactions between AAV and HSV-1 and (ii) prevent inhibition of HSV-1 replication by AAV *rep*.

**S15****A NOVEL TISSUE-SPECIFIC ADENOVIRAL VECTOR WITH DUAL FEEDBACK REGULATIONS FOR CANCER GENE THERAPY**

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It has been difficult to fully realize the potentials of cancer gene therapy because the majority of currently available regulated vectors either deliver only modest levels of cytotoxic proteins in target cells, or lack sufficient cell type specificity to be considered safe. To overcome these limitations, we have developed a regulated and tissue-specific adenovirus vector that expresses transgene at very high levels in permissive cells but at a non-detectable level in non-permissive cells. In addition, the levels of transgene expression can be regulated by administering a small molecule (tetracycline or its derivative). We have achieved this high level of tissue specificity and regulation by incorporating two tissue-specific feedback mechanisms into a single complex adenovirus. In non-tumor cells, a negative feedback loop is activated to block basal level of gene expression; while in permissive cells, a positive feedback loop becomes activated so that the transgene expression is autonomously amplified. At the same time, the negative suppression is alleviated. Combinations of these effects result in high levels of transgene expression. In addition, because we incorporated tetracycline-response elements into the positive feedback loop, the levels of gene expression in permissive cells can also be regulated by concentrations of tetracycline or its derivatives. Using animal models, we have demonstrated that the high level of tissue specificity further enhanced safety and efficacy of the cancer-apoptosis therapy. We believe that this novel vector strategy may provide a new tool in cancer therapy.

**S14****OVERCOMING TUMOR IMMUNE EVASION STRATEGIES TO IMPROVE CTLs FOR THE ADOPTIVE IMMUNOTHERAPY OF EBV-RELATED TUMORS**

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Adoptively-transferred EBV-specific cytotoxic T lymphocytes (CTL) have been safe and effective in restoring EBV-specific immunity after T cell-depleted stem cell transplantation. EBV-specific CTL expanded 3-4 logs after infusion, restored EBV specific immunity, reduced the high virus load seen in ~20% of patients, persisted for up to 6 years and prevented the development of post-transplant lymphoproliferative disorder (PTLD) in all patients. Complete remissions were observed in 3 of 4 patients. Other EBV-associated malignancies, including Hodgkin lymphoma (HL) and undifferentiated nasopharyngeal carcinoma (NPC), are good candidates for immunotherapy with CTL, however, CTL while producing tumor responses have been less successful. Unlike PTLD, these tumor must survive in immunocompetent hosts and use multiple immune evasion strategies. These are particularly well-defined in HL, which expresses only viral proteins that are non-immunogenic or subdominant to CTL, as well as chemokines and cytokines that are inhibitory to CTL. For adoptively transferred CTL to be effective they must survive and function in a hostile tumor environment. We have generated CTL specific for subdominant viral proteins and rendered them genetically resistant to HL-derived inhibitory molecules. Modification of the host environment using specific immunomodulation may also facilitate CTL expansion, persistence and anti-tumor activity. Success in the models of HL and NPC will translate to many other human tumors that use similar immune evasion strategies.

**S16****IDENTIFICATION OF TARGETING AND THERAPEUTIC MOLECULES FOR CANCER GENE THERAPY**Ivy Ho, S.M. Wang, Paula Lam and Kam M. Hui  
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The field of cancer gene therapy has made promising progress through the identification of novel therapeutic genes and improved gene delivery systems. With the current progress especially in the context that recent advances in molecular genetics have introduced new tools to target and sustain gene expression and improved understanding of the molecular basis of disease would make available new targets for therapy. The highly parallel analysis of gene expression made possible by the development of cDNA array technology provides a powerful tool for the molecular dissection of cancer. A better understanding of the molecular changes associated with tumour formation and progression could improve the classification of cancer and provide clues to the development of specific therapies for pathogenetically distinct tumour types. Results on the identification of genes that are differentially expressed in human could therefore potentially serve as "signatures" for diagnosis and therapy of human cancers will be presented. In addition, to enhance tumor-specific gene delivery, we have also employed the 12-mer phage display peptide library to isolate phages that bind specifically to human cancer cell lines. One of the isolated peptides, tentatively denoted as MG11, was demonstrated to be glioma-specific and compared to non-glioma cells. Phages bearing the MG11 peptide binding motif enables the phages to home specifically to glioma xenografts.