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Epstein Barr Virus (EBV)- and Adeno-Associated Virus (AAV)-based Vectors as Novel Tools for CLL-targeted Gene Therapy

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Gene therapy for B-CLL remains a challenge due to the low transduction efficiency and lack of cellular targeting of currently available vectors. We explored the characteristics of recombinant EBV vectors for transducing B-CLL cells. A packaging cell line which contains the entire EBV genome but lacks the terminal repeats allows encapsidation of nontransforming rEBV vectors in the absence of EBV helpervirus. Samples derived from 14 B-CLL patients were transduced with rEBV virions (MOI<1) encoding EGFP resulting in transgene expression up to 85% of cells. Transduction could be specifically abrogated by the mAb 72A1 blocking the viral envelope glycoprotein gp350/220. Due to its high packaging capacity (150 kb), this EBV-based vector system holds promise for the development of cellular vaccines for B-CLL patients.

Furthermore, recombinant AAV was explored for CLL-targeted gene transfer. Using an optimized adenovirus-free packaging system, rAAV vectors coding for EGFP and CD40 ligand were packaged (titers up to $3x10^{11}$). Cells obtained from 15 patients with B-CLL were infected (MOI of 100) resulting in transgene expression in up to 48% of cells. Transduction with AAV/CD40L induced upregulation of the costimulatory molecule CD80 not only on directly transduced cells, but also on noninfected bystander leukemia B cells, while both effects could be specifically blocked by an anti-CD40L mAb. Vaccination strategies using B-CLL cells infected *ex vivo* by rAAV vectors seem now possible.

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Adenovirus-Mediated Inhibition of NF-6B Activation Promotes Chemosensitization and Apoptosis in Prostate Cancer Cells.

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Prostate cancer (PCa) patients with hormone refractory disease do not only have higher serum level of TNF-∀, but also exhibit resistance to conventional chemotherapy. Recent evidence suggest that the constitutive activation of nuclear transcription factor NF-6B may counteract the apoptotic stimuli of TNF-∀ and cancer chemotherapeutic agents. The objective of this study was to examine whether inactivating NF-6B would enhance sensitivity of PCa cells to TNF-∀- and chemotherapy-induced cytotoxicity. Androgen-dependent (LNCaP) and independent (DU-145) cell lines were transduced (20 MOI) with a dominant-negative super-repressor I6B mutant (pAxCAmI6B-M) or wild-type (pAxCA) adenoviral constructs prior to addition of Taxol (0, 1 or 10 :M) or rhTNF-∀ (0, 1 or 10 ng/ml). NF-6B activation was evaluated by shift assay and apoptosis was monitored by DNA fragmentation assay and acridine orange staining *in situ*. MDR1 gene expression was monitored using RT-PCR. Treatment of cells with either Taxol or TNF-∀ had resulted in a dose-dependent induction of apoptosis in cells transduced with the dominant negative I6B construct as opposed to controls. Induction of apoptosis was associated with a decrease in the MDR-1 gene transcripts. The results suggest a role for NF-6B in enhancing the expression of anti-apoptotic and MDR1 gene products in the chemoresistent cells, thereby decreasing the intracellular concentration and efficacy of TNF-∀ and anticancer drugs. We conclude that inhibition of NF-6B activation may have therapeutic implications for advanced prostate cancer.

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DISC-GMCSF for Immunotherapy of Metastatic Melanoma – Preclinical and Clinical use of HSV for Gene Delivery

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We have commenced a phase I clinical trial using an HSV2-based vector to deliver the GM-CSF gene to subjects with metastatic melanoma. The DISC vector was derived by deletion of the essential gH gene and insertion of GM-CSF. In human tumour cells the vector completes a single cycle of replication which lyses cells and expresses GM-CSF. DISC-GMCSF thus combines the 'oncolytic' effects of the HSV vector with expression of GM-CSF to boost anti-tumour immune responses. Preclinical studies in mice have shown that injection of DISC-mGMCSF into tumours results in tumour regression and complete cure in a proportion of animals. Animals with regressing tumours were shown to have novel anti-tumour CTL responses. Preclinical safety testing has shown that DISC-GMCSF can infect a wide range of human tumour cells with high efficiency and express GM-CSF, but cannot spread from cell to cell. In vivo studies of toxicity, latency, reactivation and biodistribution provide an assurance of safety. Following approval from the UK Medicines Control Agency and Gene Therapy Advisory Committee, DISC-GMCSF is now being tested in a phase I clinical trial by direct intra-tumoural injection in metastatic melanoma patients. This represents the first clinical use of HSV to deliver a potentially therapeutic gene.

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Proteom analysis of mantle cell lymphomas – standardization and initial analysis of differential effector patterns of cell cycle regulators

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Secondary genetic alterations are detectable in the vast majority of Mantle cell lymphoma (MCL). The *p53* and the *p16* gene cluster have been shown to be frequently involved in the secondary transformation to aggressive MCL variants. So far, little is known on the differential effect of the $p16^{INK4A}$-CyclinD1 and $p16^{ARF}$-MDM2-p53 signal pathway.
Only expression profiling expression profiling of proteins allows the overall analysis of molecular alterations on the posttranslational level as well as those which may have occurred on the DNA or RNA level.We have established a two-dimensional protein electrophoresis which allows the reproducible analysis of protein patterns applying the computer assisted registration of landmarks (CAROL) program (Kriegel et al., Electrophoresis 2000). A p53-negative, p16-negative hematological cell line (HL-60) was transfected with either p53 wild type or p53 mutant constructs. Comparative two-dimensional proteom analysis of both derivatives and the wildtype cell line (transfected with an empty vector construct) has been performed and differentially expressed protein spots were identified which will be further characterized by MALDI-TOF. Results will be confirmed by Western Blot and RNA array analysis.