xenografts by tumor targeted T cells. In immunodeficient mice we observed regression of disseminated tumors without evidence of toxicity. To address a more clinically relevant model, we have now used tumor specific T cells to deliver retroviral vectors to tumors in immunocompetent mice. We used T cells from OT-1 TCR transgenic mice, whose T cell receptor recognizes the SIINFEKL peptide epitope from chicken ovalbumin, ova, presented in the context of H2Db by B16 cells that stably express ova (B16ova). We have previously shown that the adoptive transfer of OT-1 T cells *in vivo* results in the partial regression of B16ova tumors. We have developed two methods to engineer OT-1 T cells to deliver retroviral vectors. In the first method, OT-1 T cells were generated to produce retrovirus by transducing them with an HSV-derived amplicon vector encoding the *gag, pol* and *env* genes, and a retroviral vector encoding the beta-galactosidase gene. In the second method, OT-1 T cells were incubated with retroviruses that can adsorb to the cell surface whilst entering the cell at very low efficiency. We have demonstrated that OT-1 T cells that have retrovirus adsorbed to their surface will hand this virus off to target cells in culture. For example, 5 x 105 OT-1 T cells loaded with retroviral stocks at an MOI of about 100, washed and then co-cultured with B16 cells resulted in transduction of target B16 cells at a level of 2.7 x103 c.f.u. T cell hand off-mediated target cell infection is strictly dependent upon the presence of a functional envelope and is not the result of carry over of virus in the media. Handoff is more efficient when the viral envelope utilizes a receptor that is poorly, or not, expressed on the T cell itself. OT-1 T cells coated with retrovirus can both kill and transfer virus into B16ova cells. This transfer does not entirely depend upon the presence of an intact envelope on the viral particle surface.

To confirm that hand off of virus could occur *in vivo*, we coated OT-1 T cells with a retrovirus encoding the HSVtk gene under the control of a melanoma specific promoter. These cells were injected intravenously into mice bearing B16ova lung metastases and ganciclovir was given. OT-1 T cells coated with retrovirus increased the survival of mice bearing B16ova lung metastases compared to OT-1 T cells alone or intravenous administration of cell free retrovirus stocks.

In summary, we have shown that it is possible to combine the natural homing and effector functions of T cells with the delivery and transfer of gene therapy vectors to tumors.

24. Antibody-Targeted Fusion Provides a Versatile Platform for Novel Targeted Therapies

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Cell fusion is a basis for stem cell plasticity and has therapeutic applications in cancer gene therapy, virotherapy and the generation of novel cancer vaccines. We recently engineered the fusogenic viral membrane glycoprotein complex of measles virus to restrict and retarget membrane fusion through various antibody-receptor interactions. To ablate its natural tropism for CD46 and SLAM, mutations of positions 481 and 533 were introduced in the coding sequence of the virus attachment protein H, and three different single chain antibodies were then fused to its C-terminus. To demonstrate the potential of targeted cell fusion for cytoreductive gene therapy of human cancer, we generated bicistronic adenovirus vectors expressing measles F with EGFR-targeted, CD38-targeted or untargeted H proteins, and compared their specificity and potency against human ovarian SKOV3ip.1 tumor cells as a treatment model. The SKOV3ip.1 cells express abundant CD46 and abundant EGFR, but not CD38. Surprisingly, compared to adenoviruses expressing untargeted measles H, the EGFR-targeted adenoviruses could mediate very efficient targeted fusion and killing of SKOV3ip.1 cells. In contrast, control vectors expressing the CD38-targeted H protein showed no fusion activity in SKOV3ip.1 cells. We next evaluated the *in vivo* effects of these adenoviral vectors against established SKOV3ip.1 xenografts implanted subcutaneously in athymic mice. Adenoviral vectors mediating EGFR-targeted fusion showed greatly increased therapeutic potency in this intratumoral therapy model compared to control vectors mediating fusion through CD46 (untargeted) or CD38 ($P=0.0013$ compared to PBS, or $P\leq 0.05$ compared to Ad H/F and Ad $H_{481A,\ 533A}$-CD38/F with comparison on day 32). Histological analysis of explanted tumors three days after they were injected with the different adenoviral vectors showed that cell fusion was considerably more prominent in tumors inoculated with vector expressing the EGFR targeted H protein. Taken together, these data demonstrate the superior specificity and potency of vectors mediating antibody-targeted cell fusion. Increasingly, cells are exploited as therapeutic agents and antibody-targeted fusion has considerable potential to enhance the therapeutic outcome; stem cells are used for tissue repair, immune effector cells for tumor therapy, and vector-modified cells for delivery of diverse genetic payloads. To demonstrate that heterologous cell fusion between an immune effector cell and an epithelial tumor could be accurately targeted we infected K562 cells with adenoviral vectors expressing nontargeted, EGFR-targeted or CD38-targeted H proteins. K562 cells transduced with the EGFR-targeted or CD38-targeted vector did not fuse with each other but underwent heterologous fusion with EGFR-positive epithelial tumor cells (A431) or with CD38-expressing suspension Jurkat T cells, respectively. Thus, our data suggest that receptor choice is not a significant limitation for cell fusion and that it should be possible to target the process through a broad array of cell surface antigens. Antibody-targeted cell fusion offers a versatile new technology with many potential applications.

25. Molecular Imaging-Guided Gene Therapy of Experimental Gliomas

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Background: The combination of transgenes encoding prodrug-activating enzymes serves synergistic anti-tumor activity (e.g. E. coli cytosine deaminase (*cd*) and HSV-1 thymidine kinase (*tk*); Aghi et al. *J Nat Cancer Inst* 1998). We used positron emission tomography (PET) and magnetic resonance imaging (MRI) for imaging-guided targeted application of universal HSV-1 amplicon vectors (Jacobs et al. *Human Gene Therapy* 2003) and assessment of therapeutic efficiency.

Methods: Human Gli36dEGFR glioma cells were grown as s.c. tumors in 22 nude mice, and they were transduced *in vivo* with HSV-1 amplicons carrying *cd*, enhanced HSV-1-*tk* (*tk39*, Black et al. *PNAS* 1996) and *gfp* (HSV-*cd*IRES*tk39gfp;* $2x10^7$ t.u.). Non-transduced tumors served as negative controls, stabily *cd*IRES*tk39gfp*-expressing Gli36dEGFR cells as positive controls. Prodrug application was performed daily with 5-fluorocytosine (500mg/kg bw) and ganciclovir (25mg/kg bw). Tumor sizes were measured (calipers), and growth slopes were calculated. MRI and PET-imaging was performed for (i) localization of tumors (MRI); (ii) identification of viable target tissue ([^{18}F]FLT-PET); (iii)

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assessment of tissue-dose of vector-mediated gene expression ([^{18}F]FHBG-PET); and (iv) induced therapeutic response ([^{18}F]FLT-PET). Therapeutic efficiency was quantified by differences in (i) tumor volume and (ii) [^{18}F]FLT-accumulation.

Results: All positive control tumors disappeared within 10 days of treatment. 15/22 *in vivo* transduced tumors responded to prodrug therapy (n= 4 complete responders; n=11 partial responders). Growth slopes of tumors responding to gene therapy differed significantly from negative control tumors (t-test; $p<0.05$). The complete set of imaging data was obtained in 11/22 animals. Transduction efficiency as measured by [^{18}F]FHBG-PET was 1.22±0.83 %ID/g (p.c.) and 0.37±0.30 %ID/g (*in vivo* transduced tumors). Therapeutic effects could be monitored by PET with significant differences in [^{18}F]FLT-accumulation in 11/11 p.c. tumors (3.38±3.65 %ID/g before and 0.06±0.19 %ID/g after therapy; paired t-test; p=0.01) and 8/11 *in vivo* transduced tumors (1.91±1.12 vs. 0.42±1.31 %ID/g; $p<0.01$). For all stably transfected and *in vivo* transduced tumors, the level of TK39GFP expression as measured by [^{18}F]FHBG-PET correlated to the therapeutic efficiency as measured by [^{18}F]FLT-PET (r=0.73; $p<0.01$). Volumetric data did not correspond directly to [^{18}F]FLT- and [^{18}F]FHBG-PET as some tumors showed a partial response as indicated by [^{18}F]FLT-PET with no reduction in overall tumor volume.

Conclusion: These data indicate that (i) imaging-guided vector application, (ii) determination of the tissue-dose of vector-mediated gene expression, and (iii) correlation to the induced therapeutic effect is feasible using molecular imaging technology. Transduction with HSV-1 amplicon vectors *in vivo* causes distinct levels of therapeutic gene expression correlating to the effect of gene therapy, and volumetry revealing complementary information on the success of gene therapy. This type of imaging-guided gene therapy protocol will greatly facilitate the development of safe and efficient protocols for clinical application.

26. Tropism Illustrated: How a Morbillivirus Takes over the Immune System

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In the search for novel cancer therapies that can be used in conjunction with existing treatments, one promising area of research is the use of replicating vectors based on viruses. Viruses have the ability of selectively entering certain cells and disseminate before being cleared by the immune system. Similarly, the ideal oncolytic vector will enter and replicate preferentially in malignant cells, have the ability to treat disseminated metastases, and ultimately be cleared. To redirect viral tropism to cancer cells, a profound understanding of its determinants is necessary. We are studying the tropism of Morbilliviruses, a family of immunosuppressive negative strand RNA viruses including measles and canine distemper (CDV). Immunosuppression was discovered in Morbilliviruses by von Pirquet, who observed in 1908 that the tuberculin skin test response is transiently depressed during the course of acute measles. We have established a ferret model of CDV virulence and immunosuppression (von Messling et al., 2003, J.Virol. 77; 12579-91).

RESULTS. To facilitate the study of Morbillivirus tropism we introduced an additional transcription unit expressing green fluorescent protein (GFP) in a cDNA copy of the genome of the CDV wild type strain 5804P. We rescued a 5804Pgreen virus and showed that it retains wild type virulence and pathogenesis in ferrets. Macroscopic examination of the skin of an infected animal in daylight or after fluorescence excitation indicated that GFP expression reporting virus replication colocalizes with the rash. Whole body examination after sacrifice of a moribund animal and dissection revealed the sites of preferential virus replication including the lymph nodes, the Peyers patches in the intestine, and the thymus. Fresh sections through the spleen documented selective replication in white pulp areas, and similar analysis of organs with large epithelial surfaces like oesophagus and bladder documented high levels of replication in epithelial cells. Using FACS analysis, we found that the number of GFP-expressing peripheral blood mononuclear cells was strikingly high: up to 30% of PBMC were infected, the most heavily infected cells being T lymphocytes, followed by B lymphocytes, whereas no infected monocytes were detected. We conclude that CDV replication is very pronounced in lymphatic tissue throughout the body. Moreover, epithelial cells of certain organs also strongly support viral replication.

PERSPECTIVES. Morbillivirus tropism is determined both by cell entry through specific receptors and by post-entry host control evasion mechanisms. We have produced genetically modified CDVs defective in their interactions with their receptor SLAM, an immune cell-specific protein, or in the V and C proteins, two non-structural proteins that interfere with STAT protein phosphorylation and interferon activation. As for the parental virulent 5804P strain we will follow in ferrets the time course of disease symptoms, viremia, suppression of the DTH response, leukocyte number, antibody titers, and in vitro lymphocyte proliferation induced by the mutant viruses. These studies will extend the framework of knowledge sustaining current and future efforts to retarget Morbillivirus tropism.

27. Differential Phage Display for Discovery of Nuclear Localization Peptides: Application to Mammary Adenocarcinoma

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This study reports the discovery of nuclear targeting ligands for normal and tumorigenic mammary epithelium via combinatorial phage display techniques. Molecular targeting to the nuclei of neoplastic cells using these new ligands represents a new and important tool in improving the therapeutic index of anticancer therapies.

Novel peptides that target MCF10A normal mammary epithelial cells and their nuclei and MCF7 mammary adenocarcinoma cells and their nuclei were identified using a random linear 7-mer peptide phage display library. Phage that bound to nuclei were eluted subsequent to inactivation of extracellular phage and isolation and lysis of nuclei. Phage that bound to cells were eluted using a low pH buffer. After elution, phage were titrated and amplified for further rounds of selection.

After two rounds of selection, ten sequenced clones recovered from MCF10A nuclei revealed only one peptide sequence, QSPSPSPT. Eight sequenced clones recovered from MCF7 nuclei revealed five different peptide sequences. The QSPSPSPT peptide found in all MCF10A clones was present in four of the MCF7 clones. The four remaining peptide sequences were unique to MCF7 nuclei. These four peptides are the candidates for cell-specific nuclear delivery and were generally characterized by the presence of both an aromatic residue and a basic residue within the peptide sequence.

Each of the fifteen peptides recovered from extracellular binding was unique. Eight of the sequenced clones were recovered from MCF10A cells with an additional seven sequenced clones recovered from MCF7 cells.

Sequence alignment analysis revealed no homology between the extracellular and nuclei-specific peptides discovered here and proteins with known cellular or nuclear targeting capabilities. Experimental results of this study combined with peptide sequence analysis are consistent with the discovery of novel, cell-specific nuclear targeting molecules.

Practical applications of nonviral gene therapy are currently limited by transfection efficiency. Nuclear translocation is generally

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