

gene therapy alone. This combination strategy provided similar therapeutic effects in CAR-negative and -positive bladder cancer utilizing the Ad5F35 chimeric vector.

CONCLUSIONS: In both CAR-negative and -positive bladder cancer xenografts, combination of radiation and suicide gene therapy using the CAR-independent Ad5F35 vector yielded significantly greater anti-tumor effects than gene therapy alone. These data provide the potential for enhanced local-regional control which would be especially valuable in the setting of a bladder-sparing approach for advanced bladder cancers.

287. *mda-7*: A Remedy for Oncogene Addiction in Breast Cancer?

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The concept of "oncogene addiction" has been proposed to support the rationale for development of molecularly targeted therapies. It is clear that acquisition of genetic mutations results in dysregulation of oncogenic signaling pathways in tumor cells, and recent studies have indicated that persistence of these dysregulated oncogenes is essential for maintaining the tumorigenic phenotype. We evaluated the role of *mda-7* gene transfer in oncogene-addicted breast cancer cells. Current therapies used in the treatment of breast cancer are limited by systemic toxicity, rapid drug metabolism and intrinsic and acquired drug resistance.

We previously showed that adenoviral mediated transfer of the melanoma differentiation-associated gene-7 (*mda-7*) elicits growth inhibition and apoptosis in various tumor types. Here, we evaluate the effects of Ad-*mda7*, alone and in combination with other therapies, against a panel of nine breast tumor cell lines and their normal counterparts; we report tumor-selective p53-independent growth inhibition, G2/M cell cycle arrest, and apoptosis induction by Ad-*mda7*. *In vivo*, Ad-*mda7* induced p53-independent tumor growth inhibition ($p < 0.004$) in multiple xenograft models. We then evaluated the combination of Ad-*mda7* with agents commonly used to treat breast cancer: radiotherapy (XRT), Tamoxifen, Taxotere, Adriamycin, and Herceptin. These agents exhibit diverse modes of action, including formation of bulky adducts, inhibition of DNA replication (Adriamycin, XRT), damage to microtubules (Taxotere), non-steroidal estrogen antagonists (Tamoxifen), or Her2/neu receptor blockade (Herceptin). Treated with conventional anti-cancer drugs or radiation, MDA-7-expressing cells display additive or synergistic cytotoxicity and apoptosis that correlates with decreased BCL-2 expression and BAX up-regulation. *In vivo*, animals that received Ad-*mda7* and XRT underwent significant reduction of tumor growth ($p < 0.002$). This is the first report of the synergistic effects of Ad-*mda7* combined with chemotherapy or radiotherapy on human breast carcinoma cells, and suggests that *mda-7* gene transfer can overcome the oncogene addiction observed in breast cancer.

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288. A System for GMP Expansion and Transduction of Human T Cells with High Functionality Proven by Consistent Induction of GvHD in a Mouse Xenotransplant Model

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Here we demonstrate the GMP scale-up process for a human gene therapy clinical trial utilizing retroviral vector transduced allogeneic T cells eliciting a Graft vs. Leukemia effect in a Donor Lymphocyte Infusion (DLI) setting. After elimination of leukemic cells by DLI, a fusion CD34-TK suicide gene conferring sensitivity to ganciclovir will allow killing of transduced T cells and abrogation of Graft vs. Host Disease (GvHD), a serious side effect of DLI. The truncated CD34 receptor, normally not present on T cells, allows for purification of transduced cells to >95% in a magnetic cell sorter (CliniMacs, Miltenyi, Auburn, CA). PBMCs from 8 donors were stimulated in a closed bag system using the new GMP grade Stemline serumfree T cell medium (Sigma, St. Louis, MO). This medium allowed for elimination of donor variability, which we experienced in T cell expansions using other serumfree media. T cell stimulation was done using clinical grade magnetic beads coated with CD3 and CD28 antibodies (Xcyte Therapies, Inc., Seattle, WA). At least a 2 fold T cell expansion is needed, since transduction and expression of the transgene using a Moloney leukemia virus based retroviral vector depends on cell division and activation. Maintenance and transduction of CD4 and CD8 cells in a physiological ratio is imperative for normal function of infused T cells. Requirements stipulated by regulatory agencies demanded not to introduce more than 1-2 vector copies per cell to keep the risk for insertional mutagenesis to a minimum. A transduction frequency of 20-30% obtained at an MOI of 1-2 was required to generate this copy number (Rettig et al., 2003). In the past we showed that high concentrations of IL-2 (500 U/ml, as previously used in clinical T cell expansions) impair the *in vivo* functionality of T cells, using our NOD SCID/B2 M deficient mouse model of T cell expansion. We therefore lowered the IL-2 concentration to 50 U/ml. After 48 hours of pre-stimulation, T cells were transduced twice, medium was replaced by bag spinning, removing 2/3 of the supernatant and replacing it with fresh vector containing medium. Cells were harvested on day 4 by disrupting bead/cell clumps and removing the magnetic beads by application of a strong magnetic field. Although a low IL-2 concentration was used, a 3 fold expansion of T cells with 30% transduction efficiency, equally well distributed in the CD4 and CD8 compartment, was observed. The CD4 and CD8 ratios were maintained at input ratio, cell viability was greater than 95%. The most remarkable result was the outstanding activation and *in vivo* functionality of the expanded T cells. All NOD SCID/B2 M deficient mice (n=12) injected at a cell dose of 10^7 developed lethal GvHD at day 15 post injection. Activated cells clearly outperformed the GvHD potential of naïve T cells (Nervi et al., 2005). For the first time, these results demonstrate consistent GvHD elicited in a mouse xenotransplant model by 10^7 expanded and activated human T cells produced in a closed system, serumfree GMP manufacturing process.

289. Highly Inducible and Prostate Specific Oncolytic Adenovirus with E1A-AR Chimera

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Previously, we demonstrated prostate-specific replication and passive cell lysis using E1A driven by a prostate-specific antigen promoter (PSA) and enhancer (PSE). However, the expression of