In conclusion, IL-2 and TNF-a coding adenoviruses can break tumor-associated immunotolerance and significantly increase the levels of active, tumor-reactive T-cells both in injected cutaneous lesions and in non-injected metastatic lymph nodes. Importantly, this triple modality may represent an efficient approach to achieve "CD19-like" clinical responses in the treatment of solid, metastatic cancers currently incurable by standard therapies.

406. Development and Optimization of PSCA-Specific CAR T Cells for the Treatment of Bone Metastatic Prostate Cancer

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Prostate Cancer (PCa) is the third most common cancer type in the United States, with over 200,000 new cases projected to be diagnosed this year. In approximately 80% of PCa patients, tumor phenotype includes overexpression of prostate stem cell antigen, or PSCA. Furthermore, PSCA is expressed on nearly 100% of bone metastatic prostate cancers, making it an attractive immunotherapeutic target. We have genetically engineered T cells to express chimeric antigen receptors (CARs) which specifically target PSCA. Recent clinical trials with CARs targeting CD19 for B-cell malignancies have demonstrated impressive results, yet replicating this success with other antigen targets remains elusive. Immunotherapy against solid tumors poses a more difficult tumor challenge because of the immunosuppressive microenvironment that can significantly hinder CAR efficacy. Additionally, there have been instances of on-target, off-tumor toxicity due to low levels of antigen expression on normal tissue.

In the current project we have modified various components of our CAR constructs to improve specificity and overall therapeutic efficacy. Through various *in vitro* functional assays and *in vivo* xenograft models, we have evaluated and optimized a PSCA-targeting CAR. We first compared two single-chain variable fragments with different paratopes. While both show comparable potency, one of the scFvs showed nonspecific activity against PSCA-negative tumor lines. Similarly, our data suggest that the 28ζ-costimulatory domain, regardless of linker length, also shows non-specific activation and killing of PSCA-negative tumor lines as compared to the 4-1BB costimulatory domain. Finally, we have demonstrated differences between long, middle, and short linker lengths in intracellular cytokine production, activation, and killing capacities *in vitro* and *in vivo*. By modifying both the ectodomain and intracellular region, we are able to improve the specificity and functionality of our PSCA-CARs, which is essential to developing effective immunotherapies for this advanced disease.

407. A Novel Rabbit Antibody-Derived, Anti-CD123 LV/CAR Construct for AML Immunotherapy

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Chimeric antigen receptors (CARs) have emerged in the immunotherapy field as an exciting new option for cancer treatment, with clinical trials of CD19-directed CARs having already demonstrated long-lasting responses in patients with ALL and CLL. As a hematological malignancy, acute myeloid leukemia (AML) may be another viable target for CAR-mediated therapy. Furthermore, with a 5-year survival rate of just 5.5% for patients over 65, new treatments for AML are very much needed.

CD123 (the IL-3 receptor α-chain) is upregulated on AML blasts/stem cells and plays a role in proliferation and apoptotic resistance. This antigen demonstrates much lower expression levels on normal hematopoietic cells, where its expression is restricted to the myeloid progenitor subpopulation. Previous attempts to target CD123 in AML through several forms of immunotherapy have had variable success. Specifically, results of previous CD123 murine antibody-derived CARs have been mixed, with some results showing CAR-mediated eradication of normal myelopoiesis via targeting of HSCs with low CD123 expression.

We have developed a CD123 CAR, derived from a novel rabbit anti-CD123 mAb that we generated. Rabbit antibodies are reported to have a broader avidity and higher range of affinities than mouse mAbs; this may lead to a CAR with a unique binding profile. We will determine whether such a rabbit-derived CD123 CAR will lead to more specific binding, resulting in optimized killing of AML cells, while minimizing cytotoxic effects on HSCs.

To generate our CAR, human CD123 was purified as a GST-tagged protein and used for immunization of rabbits. Hybridoma cell lines were developed from the spleen cells of rabbits with positive immune responses, and novel antibodies were purified and screened for specificity to CD123 using a combination of ELISA, flow cytometry, and ADCC. A candidate antibody was selected, and the VL and VH chains were subcloned, sequenced, and assembled into an scFv. A second generation CAR was then designed that includes a CD8 hinge and transmembrane region, a 4-1BB costimulatory domain, and a CD3ζ signaling domain. This construct was then subcloned into a lentiviral backbone to facilitate expression in immune effector cells.

Our CD123 CAR has been transduced into primary T cells and the NK-92 cell line for in vitro testing. Flow cytometry demonstrates that our CARs are expressed at the cell surface. Furthermore, the expression of the CD123 CAR has been shown to be stable in the NK-92 cell line. Cytotoxicity assays are being performed in vitro in order to confirm binding specificity and cytotoxic potential of the CD123 CARs in both NK-92 and T cells. Future work will compare CAR T and CAR NK killing in vivo using NSG mouse models of AML.

AML may be an ideal target for CAR therapy, and we will exploit our novel CD123 CARs as therapeutic entities. We will examine whether the use of a novel rabbit anti-CD123 scFv in our LV/CAR construct will optimize the killing of AML cells while minimizing HSC eradication. This novel second-generation CAR has the potential to greatly impact the treatment of AML patients in the future.

408. Oncolytic Vaccines in Combination with PD-L1 Blockade for the Treatment of Melanoma

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The Immunological escape of tumors represents one of the main obstacles to the treatment of malignancies. The approval of drugs able to disrupt the immune suppressive pathways through anti-CTLA-4 monoclonal antibodies represented a milestone in the history of immunotherapy. However, treatment with these immune checkpoint inhibitors (ICIs) seems to be effective only in small cohorts of patients. It has been proposed that the efficacy of ICIs relies on the presence of an undergoing immunological response. For this reason, we hypothesized that oncolytic vaccines, able to elicit a tumor specific response, would synergize with anti-PD-L1 therapy. B16 murine melanomas were established in immunocompetent C57 mice. Then mice were treated with anti-PD-L1 monotherapy,

PeptiCRAd (oncolytic vaccine) monotherapy or a combination of the two. The growth of the tumors was analyzed. At the end of the experiment, all the mice mice were euthanized and organs collected for immunological analysis. We investigated antigen-specific T-cell responses and immune suppressive background by flow cytometry and ELISPOT assays.

Cancer-Oncolytic Viruses I

409. Novel Recombinant Coxsackievirus B3 Infection Elicits Robust Oncolytic Activity Against Human Non-Small Lung Cancer and Triple-Negative Breast Cancer

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Oncolytic virotherapy using enteroviruses emerges as a promising anticancer strategy. As therapeutic advantages, enteroviruses immediately induce robust oncolytic activity and do not have oncogenes that may lead to tumorigenesis. We recently showed coxsackievirus B3 wild type (CVB3-WT) infection elicited remarkably oncolytic activity against human non-small cell lung cancer cells (NSCLC). However, CVB3-WT infection caused adverse events of weight loss, pancreatitis, and myocarditis in mice. To overcome these pathogenicity, we engineered CVB3-WT genome for the development of microRNA (miRNA)-regulated oncolytic virus. We focused on two miRNAs (miR-1 and miR-217) expressed mainly normal muscle or pancreas. We successfully genetically constructed a novel recombinant CVB3-miR-1&217T (CVB3-miRT) by inserting 4 tandem target sequences complementary to two miR-1 and two miR-217 into the 3' UTR of CVB3-WT genome. Recently, we investigated whether an infection with CVB3-miRT displays oncolytic activities against NSCLC. We found that CVB3-miRT infection induced potent oncolytic activity comparable to CVB3-WT in human NSCLC *in vitro* and *in vivo*. Here, we attempted to explore the oncolysis to triple-negative breast cancer (TNBC) because TNBC are highly aggressive and intractable tumors with dismal prognosis. We performed in vitro crystal violet staining to examine the effect of CVB3-miRT on TNBC. These results showed that CVB3-miRT had potent oncolytic activity against TNBC cell lines in a MOI-dependent manner. Furthermore, consecutive administrations of CVB3-miRT into subcutaneous xenografts of human TNBC pre-established in athymic nude mice significantly suppressed the tumor growth with a prolonged survival rate. The intratumoral CVB3-miRT administrations into human TNBC xenograft tumor mice model displayed dramatically decreased side effects of CVB3-WT-induced pathogenicity. Collectively, we showed that CVB3-miRT infection indicated marked oncolytic activity against human NSCLC and TNBC cells *in vitro* and *in vivo* as well as CVB3-WT. This approach could be a promising new therapeutic modality to improve survival in patients suffering from NSCLC and TNBC in advanced stage.

410. Oncolytic Adenoviruses Armed with TNFa and IL-2 Induce Antitumor Immune Responses and Protection from Tumor Rechallenge

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During the past few years, immune system-stimulating factors have emerged as promising means to treat malignant tumors. We aim to use cytokine-bearing oncolytic adenoviruses to improve and to extend the usage of adoptive T cell therapy, a treatment known to benefit a portion of melanoma and leukemia patients. Administered systemically or intratumorally the virus induces immune responses against the tumor by revealing tumor antigens, but immunostimulatory cytokines augment the effect further. We have shown that the most promising cytokines in this regard are interleukin (IL) -2 and Tumor Necrosis Factor alpha (TNFa). IL-2 is a common treatment for malignant melanoma and renal cell carcinoma, but systemic administration may lead to severe side effects. While IL-2 has a key role in recruiting and activating T cells, TNFa has prominent anti-immunosuppressive actions. Further, it directly promotes tumor cell death by apoptosis and necrosis. Armed oncolytic viruses accomplish local, long-lasting, high-level cytokine expression while systemic level remains low. Moreover, we have proven that adenoviruses enhance adoptive T cell therapy. In this study, we treated Syrian hamsters (*Mesocricetus auratus*) with Ad5/3-E2F-d24 virus bearing human IL-2, TNFa, or both in its *E3* region. Hamster cell lines are semipermissive for 5/3-chimeric adenoviruses and produce active transgenes from these viruses. In addition, human IL-2 and TNFa are evidently active in hamsters. Hamster pancreatic cancer (HapT1) was implanted subcutaneously and treated once with TILs extracted from syngenic tumors, and with five viral injections. We saw synergy between unarmed virus and TILs, while the armed viruses turned out to be even more effective. When the cured animals were rechallenged with the same cancer cells, previous treatment with cytokine-armed viruses protected the animals against new tumors. Also, splenocytes derived from animals treated with cytokine-bearing viruses proliferated more actively *ex vivo* than the controls. To conclude, our data demonstrates the immunological benefit gained with viruses bearing IL-2 and TNFa, and further supports combining oncolytic viruses with adoptive cell transfer.

411. Predicting Tumor Response to Oncolytic Virotherapy Using Dual Isotope SPECT/CT Imaging with NIS Reporter Gene and Duramycin

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Oncolytic virotherapy is a promising modality for cancer therapy and diverse viruses from various families have been genetically engineered to be tumor selective anticancer agents. To obtain an early readout on tumor susceptibility to the oncolytic virus, and potential toxicity from off target viral infection, we have engineered our oncolytic viruses to encode the thyroidal sodium iodide symporter gene (NIS) to enable noninvasive longitudinal imaging of the pharmacokinetics and sites of virus spread. The high resolution images obtained from the new generation of small animal SPECT/CT or PET/CT machines enable us to visualize increasing numbers of infectious foci daily within the subcutaneous tumors