vaccines further. IL-12 has shown to drive cellular immune responses by aiding the priming and expansion of CD8 T cells. We have previously demonstrated that a highly optimized engineered DNA vaccine targeting HPV 16 and 18 E6/E7 (pGX3001 and pGX3002) elicited potent TH1 and cytotoxic cellular immune responses in a Phase I clinical trial. Here, we sought to determine whether the HPV vaccine-induced antitumor immunity could be further enhanced by the addition of IL12 as an adjuvant.

We first demonstrated that IL-12 could enhance HPV16 E6 and E7-specific cellular responses measured by ELISpot in mice (average of 1037 vs 504 SFU/106 splenocytes). Then we performed an in vivo tumor therapy study to determine whether IL-12 could enhance the therapeutic efficacy of pGX3001 in TC-1 tumor bearing C57BL/6 mice. The results induced that mice immunized with pGX3001+IL-12 exhibited smaller tumors compared to those in the no adjuvant group. Thirty days post tumor transplantation, 9 out of 10 mice in the pGX3100+IL-12 group showed complete tumor regression, while there were only 4 out of 10 mice in the pGX3001 group that showed complete tumor regression, indicating that the addition of plasmid-based mouse IL-12 enhanced the vaccine-induced antitumor immunity in a murine model. Furthermore, an additional study in a more relevant, non-human primate model was performed to ensure that the vaccine-induced cellular immune responses could also be augmented by using a rhesus macaque IL-12 plasmid. The data indicated that the addition of IL-12 increased the magnitude of vaccine-induced cellular immune responses about two fold post third immunization (average of 2711 SFU vs 1400 SFU/106 PBMCs as measured by ELISpot). The HPV antigen-specific memory responses in the HPV+IL-12 group were increased more than four-fold compared to the responses in the unadjuvanted group (average of 2570 vs 577 SFU/106 PBMCs). Flow cytometric analysis revealed the induction of both HPV-specific CD4 and CD8 T cells that efficiently increased IFN-ɣ responses. Both adjuvant and unadjuvanted groups showed an increase in effector and central memory CD8 T cells after immunization, while the IL-12 group exhibited stronger effector memory CD8 T cell responses. Taken together, these data support the further development of this HPV DNA vaccine in combination with IL12 as a cancer immunotherapy candidate.

219. Assessment of IL-15/sIL-15Rα Gene Therapy on Lewis Lung Carcinoma Growing in Mouse Lung, Liver and Kidneys

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Interleukin 15 (IL-15) is a multi-functional cytokine and plays an important role in stimulating proliferation and activation of NK and T cells. It binds to cells and signals through a complex formed between IL-15 and IL-15 receptor α (IL-15Rα). The objective of this study is to evaluate therapeutic activity of transferring IL-15 and soluble form of IL-15Rα gene (sIL-15Rα) against lung tumor growing in mouse lung, liver and kidneys. Antitumor activity of IL-15/sIL-15Rα gene transfer was compared to that of chemotherapy using gemcitabine, and the combination of IL-15/sIL-15Rα gene therapy and gemcitabine chemotherapy. Multi-organ tumor growth was established by hydrodynamic tail vein injection of 10^6 LL/2-Luc cells per mouse and tumor growth was monitored by luciferase-based bioluminescent imaging. Gene therapy was performed by hydrodynamic delivery of 2 mg/mouse of AG209 plasmid carrying IL-15/sIL-15Rα genes 3 days after tumor cell injection. Chemotherapy was conducted every 4 days by intra-peritoneal injection of gemcitabine (120 mg/kg) starting on day 3 after tumor cell injection. Luciferase assay on collected organs from tumor-bearing mice showed that hydrodynamic tail vein injection of LL/2-Luc cells resulted in a distribution of tumor cells in the lung (46%), liver (52%), and kidneys (6%). Compared to control animals with an average survival time of 10 days after hydrodynamic tail vein injection of tumor cells, animals with IL-15/sIL-15Rα gene transfer lived an average of 17 days, 1.7 folds longer than the control. Similar antitumor activity was seen in the gemcitabine treated animals, an increase in survival time of 1.75 folds. Combination therapy with IL-15/sIL-15Rα gene transfer and gemcitabine chemotherapy resulted in the best outcome with an average survival of animals for 33 days. Anatomic examination and bioluminescent imaging of tumor-bearing mice revealed that treatment with IL-15/sIL-15Rα gene transfer alone, or in combination with gemcitabine was effective in blocking tumor growth in the liver and kidneys, but failed to block tumor growth in the lung. These results demonstrate that same tumor growing in different organs responds to the same treatment differently. IL-15/sIL-15Rα gene transfer is effective in eliminating tumor growth in the liver and kidney. This work also suggests that mice with multi-organ tumor growth are valuable model for development of treatment strategy in dealing with late stage tumor with metastasis.

220. Evaluation of the Efficacy of a New Oncolytic Vaccine Platform in Humanized Mice

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Cancer immunotherapy represents a promising approach for the treatment of malignancies. However, breaking the immunosuppressive micro-environment is still a difficult but necessary condition to improve modern therapies. Oncolytic Adenoviruses (OAds) are able to elicit some degree of anti-tumor response and we are now investigating how OAds can be turned in novel cancer-vaccine platforms by exploiting their natural immunogenicity. The key feature of our peptide-coated conditionally-replicating adenoviruses (PeptiCRAd) is the physical conjugation of the adjuvant (i.e. OAds) to the tumor epitopes, in order to achieve a better co-delivery to antigen presenting cells (APCs).

We coated the negatively charged OAds with a positive lysine-extended version of the major histocompatibility complex (MHC) class-I model epitope SIINFEKL (polyK-SIIN), and we observed no significant changes in the oncolytic activity compared to naked viruses. Then, we confirmed the cross presentation on MHC-I of the modified polyK-SIIN in absence or presence of the virus. Afterwards, using B16-OVA tumor-bearing immunecompetent mice, we compared the administration of polyK-SIIN-coated OAds (PeptiCRAd) to the administration of an OAds-SIINFEKL mix solution. Mice treated with PeptiCRAd showed smaller tumor volumes and higher levels of OVA-specific CD8+ T-lymphocytes compared to all control groups. Interestingly, we observed a strong (negative) correlation between the size of the tumors and the anti-OVA immune response. Moreover, we evaluated whether or not the different activation of dendritic cells (DCs) could explain the advantage of PeptiCRAd over the OAds-SIINFEKL mix: consistent with our hypothesis, PeptiCRAd promoted the expansion of mature (CD86+) and SIINFEKL-cross presenting CD11c+ DCs. Then we tested our PeptiCRAd technology using a multipeptide vaccine approach to evaluate if a broader targeting could improve the anti-tumor immune response. In addition, by monitoring the effect of the treatment on uninjected tumors we were also able to assess the effect of PeptiCRAd on metastasis.

Finally, to collect human-relevant data, PeptiCrad efficacy, specificity and immunogenicity were assessed in humanized mice bearing implanted human melanomas.

221. The Utilization of Capsid-Optimized Adeno-Associated Virus (AAV) Vectors for Cancer Immunotherapy

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Cancer, a leading cause of death in the human population today, is generally being treated with chemotherapeutic methods, which have adverse toxic side effects. Immunotherapy represents an attractive alternative to common treatment since it is based on activation of host immune system.

In current studies we assessed the ability the of AAV vectors expressing antigen to activate a specific T-cell response in vivo. The ovarian albumin (OVA), commonly used in mouse models as an antigen for immunization studies was used to evaluate immune response. AAV6-WT-OVA and capsid-optimized AAV6-S662V+T492V-OVA vectors were injected intramuscularly in C57BL/6 mice with a dose of 5x10e11 vgs/mouse. Enchased green fluorescent protein (AAV6-EGFP) was used as negative control. In two weeks after injection, blood was collected and a number of OVA-specific T-cells were analyzed by stain with iTAg MHC Class I Murine Tetramer. The data shown suggest that the administration of AAV vectors expressing OVA led to a robust activation (approximately 9%) of specific T-cells compared to the mock and AAV-WT treated animals (less than 1%).

Next, we evaluated the killing ability of these OVA specific T-cell. Splenocytes from C57BL/6 mice i.m. injected with AAV6-WT and AAV6-mutant vectors were isolated in 14 days after injection. OVA-CD8 cells were expanded in vitro in the presence of a predominant for C57BL/6 mice, SIINFEKL peptide. Stimulated T-cells were used for killing assay against mouse prostate cell line RM1 stably expressing OVA. Two-color fluorescence assay of cell-mediated cytotoxicity was used as described above to estimate percentage of dead/alive target cells and generate a killing curve with different effectors to target cell ratio. Results of these experiments suggest that OVA-CD8 cells isolated from mice injected with AAV-S662V+T492V-OVA vectors have higher killing activity compared with OVA-CD8 cells from AAV-WT-OVA injected mice. Control T-cell do not show significant cytotoxicity which eliminates the possibility of autoreactive response.

Finally, we evaluated the ability of capsid-optimized AAV6 vectors to initiate a protective anti-cancer immune response. Prostatic acid phosphatase (PAP), a gene up regulated in both human and mouse prostate cancer, was used as a specific target. C57BL/6 mouse subcutaneously injected prostate cancer cell line, RM1, was used as an animal model. We genetically modified this cell line for a stable expression of firefly luciferase (FLuc) to monitor progression of tumor growth in live animals. First, animals were immunized with AAV6-S663V+T492-PAP or AAV6-WT-PAP vectors. Two weeks later mice were challenged with RM1-FLuc cells by subcutaneous injection. The effect of treatment on tumor growth was evaluated by whole body life imaging. Results indicate suppression of tumor growth by AAV6-S663V+T492-PAP for approximately four weeks in comparison to one week and two weeks for negative control AAV6-GFP and AAV6-WT-PAP treated mice, respectively.

In conclusion, successful inhibition of tumor growth in this artificial animal model would set the stage for potential clinical application.

222. AST-VAC2: An Embryonic Stem Cell-Derived Dendritic Cell Cancer Immunotherapy

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AST-VAC2 is a cancer immunotherapy product comprising embryonic stem cell-derived dendritic cells electroporated with an mRNA encoding a telomerase/lysosome-associated membrane protein 1 (LAMP hTERT) chimeric tumor antigen. Previous studies have shown that AST-VAC2 can elicit a telomerase-specific T-cell response from partially HLA-matched donor peripheral blood mononuclear cells. A related product (AST-VAC1), an autologous dendritic cell/LAMP hTERT cancer immunotherapy, has been tested in clinical trials on patients with prostate cancer or acute myeloid leukemia. The AST-VAC1 treatment was well tolerated and generated anti-telomerase immune responses. AST-VAC2 is being developed as an off-the-shelf allogeneic cancer vaccine for clinical testing in partially HLA matched patients with non-small cell lung carcinoma. The AST-VAC2 product is derived from the H1 human embryonic stem cell line. The stem cells are differentiated into mature dendritic cells in a multi-step process via embryoid bodies, prepared growth surfaces and specific growth factors. The differentiation process requires 34 days in culture. The cells are then electroporated with the LAMP hTERT mRNA and irradiated prior to cryopreservation. In preparation for clinical testing the manufacturing process has been scaled up from T-flasks to large surface area cell stacks. This has required a number of novel process modifications including large scale embryoid body filtration and flow-through electroporation. The final product will be rendered incapable of replication by gamma irradiation. The radiation dose required for halting replication while still preserving antigen presentation was determined using a combination colony formation/potency assay. It is anticipated that the proposed Phase 1/2a clinical trial will enroll approximately 30 patients and will include both a dose escalation and a broadening of inclusion criteria to permit an assessment of safety, toxicity and immunogenicity in patients with advanced disease. Asterias has recently partnered with Cancer Research United Kingdom (CRUK) and the University of Southampton for the GMP manufacturing and initial clinical testing of AST-VAC2 in the UK.

223. Consideration for Quality Control of NKG Cell Line Used for Adoptive Cellular Therapy

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NK cells play the important role in the adoptive cell therapy. As we know, NK-92 cell, a NK cell line, has been used for the preparation of cell therapy product for clinical trials in some laboratory and had been showed the potent effect in cancer therapy. Except NK-92, another NK cell line named NKG was established by Dr Tian Zhigang in University of Science and Technology of China. NKG cell line was derived from a male patient with non-Hodgkin's lymphoma. It was isolated from patient peripheral blood samples and cultivated through long-lasting period in the medium containing horse and fatal bovine serum and IL-2. Finally it was gradually adopted to grow in serum-free medium suitable for clinical use. The surface market of NKG showed CD56brightCD16- phenotype by FACS and showed the cytotoxicity to several kinds of caner cells by in vitro and in vivo assays. Then it will be potential for applying for clinical trial in the near future. So, it is the first step to qualify the master cell bank of NKG thoroughly before it is into clinical trial.