LATE-BREAKING ABSTRACTS

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EXPRESSION OF CANCER TESTIS ANTIGENS IN HUMAN BRCA-ASSOCIATED BREAST CANCERS - POTENTIAL TARGETS FOR IMMUNOPREVENTION?

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Introduction: Novel breast cancer risk-reducing strategies for individuals with germline mutations of the BRCA1 and/or BRCA2 genes are urgently needed. Identification of antigenic targets which are expressed in early cancers, in situ or invasive, but absent in normal breast epithelium of these high-risk individuals could provide the basis for the development of effective immunoprohylactic strategies. Cancer Testes (CT) antigens are ideal vaccine targets due to their known immunogenicity and tumor-associated expression pattern. The objective of this study is therefore to examine the expression pattern of CT antigens in BRCA-associated breast cancers.

Methods: Archived breast cancer tissues (n=26) as well as morphologically normal breast tissues (n=7) from women carrying deleterious BRCA 1 and/or 2 mutations were obtained for antigen expression analysis by immunohistochemistry. Expression of the following CT antigens was examined: MAGE-A1, MAGE-A3, MAGE-A4, MAGE-C1.CT7, NY-ESO-1, MAGE-C2/CT10 and GAGE.

Results: CT antigens were expressed in 61.5% (16/26) of BRCA-associated in situ and invasive cancers. 13/26 (50%) tumors expressed 2 or more CT antigens. CT antigen expression was more frequent in cancers from BRCA1 than BRCA2 mutation carriers (69% and 54%, respectively). Poorly differentiated and/or hormone receptor negative tumors expressed CT antigens more frequently (69% versus 50% for hormone receptor negative and positive tumors, respectively and 65% versus 50% for poorly and well to moderately differentiated cancers, respectively). None of the CT antigens were expressed in adjacent or contralateral normal breast epithelium.

Conclusions: We report a high CT antigen expression rate in BRCA-associated breast cancer as well as the lack of expression in benign breast tissue of carriers, identifying CT antigens as ideal vaccine targets for breast cancer prevention in these high-risk individuals.

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ASSOCIATION OF CTLA-4 GENE POLYMORPHISMS WITH SURVIVAL IN MELANOMA PATIENTS TREATED WITH INTERFERON

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In patients with melanoma who have a high risk of recurrence and no evidence of residual disease interferon alpha (IFN-α) has been shown to improve both disease free survival (DFS) and overall survival (OS). However, there is significant toxicity associated with IFN-α therapy. A relationship between OS and interferon induced autoimmunity has previously been demonstrated. Thus the ability to predict which patients would be more likely to have interferon induced autoimmunity would be very valuable. CTLA-4 is a protein involved in blocking T-cell activation and thus limiting immune response. Different polymorphisms within the CTLA-4 gene have been associated with autoimmune diseases including Graves disease, type 1 diabetes, and others. Patients with a +49GG polymorphism have decreased expression of CTLA-4 and improved T-cell responsiveness. Conversely those patients with +49AA have higher expression of CTLA-4. Peripheral blood mononuclear cells (PBMCs) were isolated from 308 melanoma patients, DNA was extracted and PCR was performed to identify CTLA-4 polymorphisms. When all patients were analyzed regardless of stage or treatment there was no statistically significant difference in progression free survival or overall survival. Of the patients with stage IIb, IIc, and III disease 77 were treated with interferon, the remainder were observed or received biochemotherapy. In the interferon treated group those patients with the +49AA polymorphism had a trend towards improved overall survival ($p=0.06$, median OS for +49AA and +49AG groups have not been reached, median OS for +49GG group is 149 months with a 95% CI of [56, 149] months). However, there was no significant difference in DFS between the different polymorphisms observed ($p=0.15$, median DFS for +49AA is 139 months with a 95% CI of [77, -] months, for +49AG groups is 43 months with a 95% CI of [27, 187] months, and for +49GG group is 88 months with a 95% CI of [10, 138] months). This analysis showed that the CTLA-4 genotype, which correlates with higher levels of CTLA-4 expression, may be useful as a predictor for response to interferon.

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USING TIMING TO SYNCHRONISE IMMUNOTHERAPY THROUGH MICROMANIPULATION OF THE UNDERLYING TUMOR IMMUNE RESPONSE: FROM COLEY TO ROSENBERG, LOTZE, ALLISON, WOLCHOK AND BEYOND!

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Introduction: Immunotherapy studies in advanced cancers appear to show that the immune response can be driven in either an ‘effector’ or ‘regulatory’ direction or a ‘mixture’ of these, leading to the heterogeneous clinical responses (CR, PR, PD) seen. Using CRP as a marker, we have shown that a repeating cyclical, dependent, sequential, orchestrated, homeostatic physiologic process appears to be occurring in most, if not all, cancer patients, most likely as a result of chronic antigenic stimulation. The correct timing of immunotherapy or chemotherapy with this ‘immune cycle’ appears to be associated with better cancer control and improved clinical outcomes.

We are concerned that the timing of therapy with respect to the underlying tumor immune response fluctuations is the principal determinant of efficacy in cancer treatment. The observations and model may also extend to other chronic inflammatory states to determine therapeutic efficacy.

Methods: Using serial CRP measurements in late-stage cancer patients, we have recently been able to expose sequential and time dependent oscillations in the inflammatory/ immune response that may represent a homeostatic, repeating or cyclical process of tumour immune responsiveness then tolerance. The periodicity of these cycles appears to be reproducible at approximately 6-7 days.

By serially measuring CRP around the time of vaccination or chemotherapy, the position on the underlying immune curve can be established. Timing with respect to this cycle appears to be critical to modulating the immune system with each intervention, and pivotal to the clinical efficacy of therapy (1,2).

Results: Using these methods, we have been able to correlate the timing of vaccination/ chemotherapy with the induction of clinical responses. Induction of complete responses, stable disease, slowed growth even with persistent metastatic disease, appears possible using these principles, thereby improving overall survival.

Conclusions: The historical ‘random successes’ seen with vaccines, cytokines and receptor directed monoclonal antibodies may be due to their fortuitous accidental interference with the underlying persistent, homeostatically regulated tumor immune kinetics by their ‘random’ untimed administration. By accurately and appropriately synchronizing therapy to each patient’s immune system’s periodic oscillations - immune cycle - we predict that the current low random immunotherapy successes seen to date, can be made more predictable by accurately timing therapy better, and will achieve greater clinical efficacy.

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TLR8 AGONIST AND DOXIL CHEMOTHERAPY POTENTLY ACTIVATE HUMAN ANTITUMOR IMMUNE RESPONSE IN A HUMAN IMMUNE SYSTEM MOUSE MODEL

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Because of differences between mouse and human immune systems, many of the effects of immunomodulatory drugs cannot be fully studied in syngeneic mouse models. We thus generated a novel tumor-bearing mouse model with human immune system (HIS) to study interactions between chemotherapy and immune modulatory therapy. We tested the individual effects and the interactions between doxorubicin, a drug which induces immunogenic tumor cell death and activates antigen-presenting cells, and VTX-2337, a novel small-molecule TLR8 agonist, which induces potent activation and type 1 polarization of human myeloid DCs but shows, reduced activity on murine leukocytes. Nod/SCID/ILRgamma knock out (NSG) mice were inoculated with human CD34+ cord blood cells from HLA-A2+ human donors; transplanted s.c. with human HLA-A2+ OVCAR5 ovarian cancer tumors; and treated with pegylated liposomal doxorubicin (Doxil); VTX-2337; or the two agents in combination. NSG-HIS mice exhibited a full human hematopoietic system, including human monocytes, macrophages and plasmacytoid and myeloid DCs as well as T cell subsets. In NSG-HIS mice, VTX-2337 induced dose-dependent activation of human CD14+ and CD11c+ cells in vivo within 6 hrs. Transient, dose-dependent up-regulation of human Th1 cytokines but also IL-10 was observed in the plasma of mice treated with VTX-2337, reaching peaks within 6 hrs and subsiding within 24 hrs. Doxil alone also induced mild activation of CD11c+ DCs in vivo and mild up-regulation of Th1 cytokines. The combination of two drugs induced potent activation of CD11c+ DCs and monocytes, and markedly increased Th1 cytokines but not IL-10. HLA-A2+ OVCAR5 tumors were successfully engrafted, exhibiting infiltration by human leukocytes. VTX-2337 and Doxil treatment independently induced tumor-infiltrating human leukocytes and restricted growth of human ovarian tumor xenografts in a dose-dependent manner, while the combination of the two drugs induced the highest frequency of tumor-infiltrating human leukocytes and potently restricted growth of ovarian tumors. Combined activation of innate and adaptive immunity by VTX-2337 and Doxil, as well sensitization of tumor cells by Doxil to adaptive and innate immune effector mechanisms was at the basis of the observed interactions suppressing tumor growth. We conclude that the NSG-HIS provided a suitable tool to establish potent interactions during TLR8 agonist and Doxil chemotherapy combination, and the results warrant clinical testing.

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DYSFUNCTIONAL TUMOR ANTIGEN-SPECIFIC CD8+ T CELLS UPREGULATE Tim-3 AND PD-1 IN MELANOMA PATIENTS

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In patients with advanced melanoma, we have previously shown that the cancer-germline antigen NY-ESO-1 stimulates spontaneous NY-ESO-1-specific CD8+ T cells that upregulate PD-1 expression. We also observed that PD-1 regulates NY-ESO-1-specific CD8+ T cell expansion upon chronic antigen stimulation. In the present study, we show that a fraction of PD-1+ NY-ESO-1-specific CD8+ T cells in patients with advanced melanoma upregulates Tim-3 expression and that Tim-3+PD-1+ NY-ESO-1-specific CD8+ T cells are more dysfunctional than Tim-3-PD-1+ and Tim-3-PD-1- NY-ESO-1-specific CD8+ T cells, producing less IFN-γ, TNF and IL-2. Tim-3/Tim-3L blockade enhanced cytokine production by NY-ESO-1-specific CD8+ T cells upon short ex vivo stimulation with cognate peptide, thus enhancing their functional capacity. In addition, Tim-3/Tim-3L blockade enhanced cytokine production and proliferation of NY-ESO-1-specific CD8+ T cells upon prolonged antigen stimulation and acted in synergy with PD-1/PD-L1 blockade. Collectively, our findings support the use of Tim-3/Tim3-L blockade together with PD-1/PD-L1 blockade to reverse tumor-induced T cell exhaustion/dysfunction in patients with advanced melanoma.

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HELIOS POSITIVE THYMIC-DERIVED REGULATORY T CELLS CONTINUE TO DOMINATE IN PBMC OF PATIENTS FOLLOWING COMBINATION IMMUNOTHERAPY WITH NONMYELOABLATIVE CHEMOTHERAPY, ADOPTIVE TRANSFER AND VACCINATION

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Clinical trials therapeutic tumor vaccines have generally failed to provide evidence of efficacy. Preclinical tumor models suggest that vaccination during homeostasis-driven proliferation improves therapeutic efficacy. On this basis we have initiated phase I/II clinical trials, where patients receive nonmyeloablative chemotherapy and adoptive transfer of PBMC prior to vaccination. We hypothesized that this strategy reduces regulatory T cells (Treg) and improves the immune response to vaccination. However, analysis of PBMC over time document rapid recovery of Treg cells. Recently, Helios was reported to be a marker of natural thymic-derived Treg. Here we asked whether the Treg populations that develop following nonmyeloablative chemotherapy are natural or peripherally-induced Treg. PBMC from pre-treatment or week 11 aphereses were analyzed for CD3, CD4, CD25, FoxP3, Helios, ICOS and Ki67. Preliminary results suggest there was no change in the frequency of CD25+/FoxP3+/Helios+ T cells pre/post treatment. However, the ratio of ICOS+/ICOS- cells increased in the Helios+ Treg compartment post-treatment in all patients analyzed to date. Current studies are extending the phenotypic analyses of Treg in these patients.

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ARTIFICIAL ANTIGEN PRESENTING CELLS ENHANCE THE PRODUCTION OF TUMOR-INFILTRATING LYMPHOCYTES FOR ADOPTIVE CELL THERAPY

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Adoptive T cell therapy (ACT) is an effective strategy to treat patients with advanced melanoma. A unique tumor-infiltrating lymphocyte (TIL) culture for ACT is generated from a resected tumor sample for each patient. However, a portion (15-20%) of patient samples do not generate a TIL suitable for use in therapy. We examined the use of artificial antigen presenting cells (aAPC) comprised of K562 cells engineered to express 4-1BBL and CD64 (the high affinity FcR) to improve TIL generation from resected melanoma tumors. Tumors with a low frequency of infiltrating lymphocytes (average of 21% TIL) were selected for analysis. TIL cultures were successfully generated from 10 of 33 samples (30%) with conventional methods and 26 of 33 samples (79%) with the addition of aAPCs (p2=0.0002). TIL generated with aAPCs maintained in vitro anti-tumor recognition and contained a higher frequency of CD8 T cells compared to TIL generated in the absence of aAPCs. The addition of aAPCs to tumor cultures would permit treatment of patients that would not be possible with conventional methods and improve the speed of TIL generation from productive tumor biopsies. The subsequent expansion of TIL to large numbers for patient infusion also presents logistic and regulatory hurdles. Current technology uses a rapid expansion protocol (REP) that requires 1E10 irradiated peripheral blood mononuclear "feeder" cells for each patient treatment. We investigated the use of the aAPC to substitute for feeder cells in a REP. aAPCs loaded with OKT3 (anti-CD3) alone were inferior to feeder cells, but aAPC and CD4 feeder cells were sufficient to mediate potent expansion. When optimized for clinical use, aAPCs reduced the number of feeder cells required for TIL expansion by 75 percent. In the presence of aAPCs, 50:1 ratio of feeder cells to TIL was needed as opposed to the conventional 200-fold excess feeder cells. These data provide a foundation to conduct a clinical trial examining efficacy, toxicity, and the immunological parameters of patients treated with TIL generated and expanded in the presence of engineered K562 aAPCs.

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A NOVEL STAT3 INHIBITOR REVERSES TUMOR-ASSOCIATED IMMUNOSUPPRESSION AND ENHANCES THE EFFICACY OF ADOPTIVE T CELL THERAPY OF CANCER

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It is becoming increasingly evident that an oncogenic transcriptional factor STAT3 is a master regulator of the tumor-associated immunosuppression that inhibits effective immunotherapy of cancer. Activated STAT3 in tumor cells and immune cells promotes the production of immune-suppressive cytokines (such as IL-6, IL-10, VGEF or TGFβ), restrains dendritic cell activation and function, and increases the activity of immune-suppressive cells such as myeloid-derived suppressor cells (MDSCs) or regulatory T cells (Tregs).

Utilizing an adoptive T cell transfer therapy model with tumor specific TCR-transgenic mouse-derived CD8+ T cells, we have found that the multifunctionality (assessed by the secretion of IFNγ and TNFα and the expression of degranulation marker CD107a) of transferred T cells is a critical determinant of the successful tumor eradication. However, tumor progression was found to inhibit the induction of multifunctionality in transferred T cells via Tregs accumulation in tumor site resulting in unsuccessful tumor eradication.

In this study, we examined if STAT3 inhibition by a novel small-molecule inhibitor of STAT3 dimerization, STX0119 (Matsuno et al., ACS Med. Chem. Lett. 2010), reverses the immunosuppression and enhances the efficacy of adoptive T cell therapy. Administration of STX-0119 to BALB/c mice harboring syngeneic fibrosarcoma CMS5 markedly reduced the frequency of MDSCs and Tregs at the tumor site. Combination of STX-0119 with the adoptive transfer of CD8+ T cells derived from TCR-transgenic mice that recognize a CMS5-expressing tumor antigen inhibited tumor growth more effectively than each of monotherapy. These results suggest that our novel STAT3 inhibitor effectively counters negative immune regulation, and is a promising molecular adjuvant for immunotherapy of cancer.

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SYNERGISTIC ANTITUMOR EFFICACY OF AN INHIBITOR OF IDO1 AND CYTOTOXIC AGENTS WHICH INDUCE IDO ACTIVITY

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Multiple suppressive mechanisms exist in tumor microenvironments to limit the activity of antitumor immune responses. Among these is tumor expressed indoleamine-2,3-dioxygenase (IDO1), which catalyzes the conversion of tryptophan to kynurenine and other metabolites and creates an environment unfavorable to the expansion and activation of antitumor T cells. We have previously described the discovery of novel hydroxyamidine inhibitors of IDO1 which suppress tryptophan conversion in vitro and in vivo. Although these molecules inhibited the growth of IDO1-expressing tumors as single agents, we hypothesized that enhanced antitumor activity would be seen with treatment in combination with cytotoxic agents due to the release of antigens associated with tumor cell death. Synergistic activity was noted in two murine models of colon cancer when doxorubicin was coadministered with an IDO1 inhibitor. To establish the mechanism of action of this synergy, we measured the degree of tryptophan catabolism in treated mice and determined that doxorubicin alone increased the levels of Kyn in mouse plasma. Evaluation of other cytotoxic agents revealed that several agents, including cisplatin, oxaliplatin, and gemcitabine, cause increased Kyn levels after a single dose in wild type but not in IDO1-deficient Balb/c mice. To determine if the induction of IDO activity by cytotoxic agents would predict enhanced antitumor activity in combination with IDO1 inhibition, we tested several Kyn-inducing agents in the CT26 colon cancer model and observed synergistic or additive activity for all tested combinations. Alternatively, those cytotoxic agents which do not induce tryptophan catabolism would not be expected to synergize with an IDO1 inhibitor. Indeed, treatment with dacarbazine, an agent that did not induce Kyn levels, did not provide any additional benefit to B16 melanoma bearing mice receiving an IDO1 inhibitor. These data suggest that evidence of induction of tryptophan catabolism by a cytotoxic agent may predict enhanced antitumor activity in combination with potent IDO1 inhibition.

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SAFETY AND PHARMACODYNAMIC RESULTS OF A PHASE I/IIA TRIAL WITH A DIRECTLY INJECTABLE MESSENGER RNA-BASED VACCINE CV9103 ENCODING 4 DIFFERENT PROSTATE ANTIGENS IN PATIENTS WITH HORMONE-REFRACTORY PROSTATE CANCER

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CureVac, a privately held German biopharmaceutical company develops innovative and potent immunotherapeutic agents based upon modified mRNA using its proprietary RNActive®-technology. Four different prostate-specific antigens (PSA, PSMA, PSCA, STEAP-1) were chosen to be included with the mRNA-based vaccine CV9103 for the treatment of hormone-refractory prostate carcinoma. Twelve patients (Gleason Scores between 5 - 9, age 52 -84 years) in a Phase I dose-escalating trial did receive one of three different doses (256, 640, or 1280 µg total mRNA per vaccination) of CV9103 by intradermal injection in weeks 1, 3, 7, 15 and 23 for a total of five immunizations. One dose limiting toxicity (urinary retention) was reported at the highest dose. However, a maximum tolerated dose was not established. Most adverse events were transient and mild (grade I WHO toxicity scale) and mainly related to injection site reactions, which were observed in almost all patients shortly after administration. Only few adverse events grade III were observed, which were not drug-related.

After a safe recommended dose had been established in the phase I part of the study, 32 additional patients were enrolled receiving five consecutive vaccinations in weeks 1,3,7,15,and 23. Blood sampling for immunological analysis was done prior to first vaccination in week 1 and in weeks 5, 9 and 17, two weeks after each immunization, Antigen-specific T cell responses were analyzed ex vivo by ELISPOT (IFNγ), intracellular cytokine (TNFα, IFNγ) or tetramer staining. PSA -specific antibodies were monitored by ELISA. An immunological responder was defined as a patient with a positive immune reaction according to modified criteria by the Association for Immunotherapy CIMT or the Cancer Vaccine Consortium CVC in either ELISPOT, intracellular cytokine staining, tetramer analysis or ELISA. An immunological response was detected in more than two thirds of the patients. Importantly, the majority of immunological responders reacted against more than one antigen. Immune responses could be elicited against all antigens employed.

This first study of the mRNA-based vaccine CV9103 suggests that immunization with mRNA is safe, well-tolerated and biologically active. Further evaluation in controlled trials of the therapeutic potency of this novel approach for immunization against a multitude of cancer antigens appears warranted. Detailed results of the study will be reported at the annual ISBTC meeting.

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TUMOR-DRIVEN DEVIATION OF MONOCYTE COMMITMENT TO DENDRITIC CELL PATHWAY CAN BE COUNTERACTED BY INDUCING IMMUNOGENIC TUMOR CELL DEATH

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The idea behind cancer vaccine is that, for the tumor antigens (TA) to be recognized by the immune system and an appropriate response be mounted, signals must be provided to both break the tolerance and overcome the immunosuppressive pathways. Dendritic cells (DC) and their progenitors are major targets of tumor-driven immune subversion. We have observed that blood monocytes of patients with prostatic cancer (PCa) fail to generate mature (m) DCs ex-vivo, but recover their ability after the tumor is removed. The fact that the PCa inhibits in vivo the formation of competent DC precursors poses a strong limitation to preparation of autologous DC vaccines for tumor bearing PCa patients. Moreover, even the preparation of DC vaccine in a tumor-free, adjuvant setting may be impaired when the tumor itself is the source of tumor antigens (TAs). We have therefore addressed i) the inhibitory effect of PCa on DC-TA development from blood monocytes of healthy individuals and ii) the possibility of counteracting the putative DC suppression by driving the tumor to secondary necrosis, a cell death previously found to be highly immunogenic. As expected, the PCa LNCaP cell line strongly suppressed the uptake of the tumor by immature (i) DC. Similar inhibition was observed on progression of monocytes to mDC under cytokine maturation conditions. The highest inhibition was observed on the expression of the CD83 and CCR7 mDC markers. Crosspriming of tumor specific CTLs was also strongly inhibited. Administration to the tumor of the UVC-based VIVITM treatment completely restored both DC functions and CTL generation. Secondary necrotic death and adiuvancy/counteraction were associated with the release of DAMP molecules HSP70 and HMGB-1. Literature data showed that chemotherapy-induced death induces a protective immune response against further tumor challenges in vivo. We were then interested at confirming the immunogenic effect of secondary necrosis by using as anthracyclins as death agents. Indeed, treatment of the LNCaP cell line with doxorubicin induced massive AnnV+PI+ secondary necrosis and completely inhibited tumor immunosuppression. However, this kind of death and the associated immunogenicity were only observed with 20 ug/ml doxorubicin, whereas lower, pharmacological doses produced non-immunogenic apoptotic death and no reversal of the tumor immunosuppression. While still to be defined in an in vivo setting, the microenvironment induced by necrotic cell death is very likely to neutralize the immunosuppressive effect created by the tumor during ex-vivo generation of DC-TA vaccines.

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FIRST-IN-MAN PHASE I CLINICAL TRIAL OF VTX-2337 - A SELECTIVE TOLL-LIKE RECEPTOR 8 (TLR8) AGONIST - IN ONCOLOGY PATIENTS

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Background: VTX-2337 is a novel, potent and selective small molecule (<500 MW) agonist of TLR8 that was assessed for safety, tolerability, potential clinical benefit and evidence of pharmacodynamic (PD) activity in a Phase 1 clinical trial in late stage cancer patients. TLR8 activates myeloid derived dendritic cells and monocytes to produce chemokines and Th1 polarizing cytokines. TLR8 activation is also reported to increase the activity of NK cells and to reverse Treg cell function. Collectively, these TLR8 mediated effects serve to drive the induction of innate and adaptive immune responses directed towards tumor cells, which has been seen in a preclinical ovarian tumor model.

Methods: In this Phase I clinical study, VTX-2337 was administered by the subcutaneous (SC) route to 32 adult subjects with advanced solid tumors or lymphoma. The initial study cohort received a 0.1 mg/m2 dose of VTX-2337, while 7 addition cohorts (n = 3-6) received higher doses escalated a stepwise manner, and reaching a top dose of 3.9 mg/m2 based on tolerability. At each dose level the cohort was administered VTX-2337 on days 1, 8 and 15 of a 28-day treatment for 2 cycles. Subjects, who tolerated VTX-2337 and failed to show disease progression at 8 weeks received additional treatment cycles.

Results: VTX-2337 was generally safe and well tolerated. Fever and mild flu-like symptoms were seen in some subjects, particularly at higher dose levels. VTX 2337 was detected in the plasma at all dose levels, while mean Cmax and AUC values increased proportional to dose over the 8 dose levels. Plasma levels of multiple immune mediators, including both cytokines and chemokines, also increased in a dose-dependent manner. Generally these biomarkers of TLR8 activation were elevated within 4 hours of dosing and returned to baseline levels by 24 hours. There was no evidence of either desensitization or augmentation of the PD response over multiple VTX-2337 treatment cycles.

Conclusion: The SC administration of the TLR8 agonist VTX-2337 was safe, reasonably well tolerated, has a predictable pharmacokinetic profile and shows dose-dependent pharmacologic activity. A randomized Phase 2 clinical study of VXT-2337 in combination with an approved anticancer treatment will be initiated in the near future.

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MECHANISMS OF IFN-ALPHA RESISTANCE IN RENAL CELL CARCINOMA

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Renal cell carcinoma (RCC) remains one of the most resistant tumors to chemo-, radio-, and immunotherapy. Therapy with interleukin-2 (IL-2) and interferon (IFN-α) induces durable complete responses but only in 10%-20% of RCC patients. As seen, despite the relative beneficial effects of IFN-α in RCC, substantial proportion of patients with RCC fail to respond to IFN-α treatment and the mechanisms forming the basis for the resistance of RCC to IFN-α are still not completely clear. We believe that IFN-α response and resistance might be modulated by the regulation of the expression of arginase 2 and inducible nitric oxide synthase (iNOS) in association with defective components of the Jak-Stat pathway in a time dependant manner. In the present study, we investigated in vitro, the antiproliferative effect of IFN-α (10 U/ml) and the mechanisms of IFN-α resistance in three murine RCC cell lines. One RCC cell line Renca (spontaneous-RCC) was sensitive to IFN-α whereas other RCC cell lines, CL-2 and CL-19 (induced-RCC) where resistant to this cytokine. The results indicate that IFN-α resistance in CL-2 and CL-19 cells is associated with the lack of expression of IFN-α-receptor-1 in a time dependant manner. In addition a defective phosphorylation of Stat-1 was observed in these cells when compared to Renca cells. We did not observe alterations in the expression of Jak-1, Tyk2 and Stat-1 proteins in the 3 cell lines tested. The downregulation of IFN-α-receptor-1 appears to be regulated by L-arginine levels at late time points. The results suggest that a possible restoration of the receptor and Stat-1 might strikingly increase the susceptibility of RCC to IFN-α and may be a new strategy for the improvement of the response of RCC to IFN-α treatment. The role of L-arginine in this process it is currently investigated in detail.

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TARGETING THE MUC1-SEA MODULE WITH ANTIBODIES FOR ABLATING HUMAN MUC1 POSITIVE CANCER CELLS

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MUC1 is an oncoprotein heterodimer composed of two subunits, a large extracellular alpha chain that is noncovalently bound to a membrane-bound beta subunit.Because MUC1 is overexpressed by breast cancer it represents a highly effective molecule for targeted therapy. Almost all anti-MUC1 Abs reported to date recognize the highly-immunogenic tandem-repeat-array of the alpha subunit. Because the alpha subunit is shed into the peripheral circulation it sequesters such anti-alpha chain Abs, precluding effective targeting of MUC1+ cancer cells. We identified the MUC1 SEA module that comprises the alpha-beta junction, which because it is attached at all times to the cell surface represents a therapeutically-exploitable target. We have generated a series of anti-MUC1 junction monoclonal Abs with high affinity binding to MUC1+ breast cancer cells.The novel mAbs bind well to MUC1-expressing breast cancer cells.In addition to binding to mature tumor cells,our studies to date indicate binding activity of the DMB mAbs antibodies against human tumor stem cells, the sub-population of tumor cells capable of repopulating tumor by inducing new tumor growth.To see whether the antibodies we generated could bind to and kill MUC1-positive cancer cells, we reacted anti-MUC1 alpha-beta junction DMB antibody with a recombinant ZZ-PE38 toxin to form anti-MUC1/X:ZZ-PE38 immunoconjugates. ZZ-PE38 is a fusion protein comprising the PE38 pseudomonas exotoxin. Significantly the ZZ-domain derived from Protein A allows the fusion protein to bind to the Fc portion of an IgG.The anti-MUC1/X: ZZ-PE38 immunoconjugates were potently cytocidal for MUC1+ tumor cells. No cell killing was seen with the toxin alone, indicating that antibody targeting of the MUC1 alpha-beta junction is essential for cytocidal activity.

These results clearly demonstrate that the MUC1 alpha-beta junction is a feasible and realistic target for antibodies to kill tumor cells expressing the human-cancer-associated MUC1/TM protein.

The next step of our research is focused on generation of stable humanized immunotoxins composed of the DMB antibody and toxin covalently bound at its carboxyl terminus.To test biological effects of these antibodies against the MUC1 alpha-beta junction, we add the purified antibodies to in-vitro cell cultures of MUC1-expressing human cells and assess their effect on cell proliferation.To extend the above experiments to a human tumor model,we will establish a quantitatively measurable human tumor xenograft model. This will be done by assessing the effect of the monoclonal antibodies.on tumor growth in 6-8 week old SCID mice.Our current research into the MUC1 alpha-beta junction will likely provide innovative reagents in the form of novel immunoconjugates for human cancer therapy.

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FINAL EFFICACY RESULTS OF A3671009, A PHASE 3 STUDY OF TREMELIMUMAB VS CHEMOTHERAPY (DACARBAZINE OR TEMOZOLOMIDE) IN FIRST LINE PATIENTS WITH UNRESECTABLE MELANOMA

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Background: A3671009, a randomized phase 3 study comparing tremelimumab (anti-CTLA4 monoclonal antibody) to dacarbazine or temozolomide in pts with unresectable melanoma, was the first large randomized study comparing immunotherapy to chemotherapy. The final survival results, including population subset analyses, will be presented.

Methods: Pts with unresectable stage IIIc-IV melanoma without brain metastasis, with LDH below 2x ULN, and no prior systemic treatment for advanced melanoma were randomized 1:1 to either tremelimumab 15 mg/kg IV q90d, or physician's choice of temozolomide 200 mg/m2 p.o. d1-5 q28d or dacarbazine 1,000 mg/m2 IV q21d (chemotherapy arm). Primary endpoint was OS, secondary endpoints included response, durable tumor response, 6-mo PFS, and safety. Exploratory analyses were performed to evaluate the potential predictive value of baseline characteristics for benefit from immunotherapy with tremelimumab compared to chemotherapy.

Results: 655 pts were enrolled between March 2006 and July 2007, 328 pts randomized to tremelimumab (324 treated), 327 to chemotherapy (319 treated). There were no significant imbalances in age, sex, LDH, or stage (5% stage IIIc, 15% M1a, 22% M1b, 58% M1c). The log rank test statistic crossed the futility boundary at the second interim analysis, but survival follow up continued. At the final analysis, median OS by intent-to-treat was 12.6 mo in the tremelimumab arm and 10.7 mo in the chemotherapy arm, but this difference in OS was not statistically significant. Response rates were approximately 10% in each arm, but the duration of response was significantly longer for the tremelimumab arm. Tumor response to tremelimumab and hazard ratio for survival were not adversely affected by baseline characteristics such as advanced tumor stage, elevated serum LDH, poorer performance status (ECOG 1 vs ECOG 0) or by age > 65. Exploratory analysis of baseline labs revealed that low baseline CRP and adequate baseline lymphocyte count were each associated with better response rate in the tremelimumab arm and better HR for survival in the tremelimumab arm compared to the chemotherapy arm.

Conclusions: In this clinical trial tremelimumab did not improve survival over chemotherapy in the first line therapy of patients with metastatic melanoma. Selection of patients based on CRP may enrich for patients with higher likelihood of response. In addition, the long duration of responses with tremelimumab provide evidence of the impact of this mode of therapy in advanced melanoma.

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SALMONELLA-INDUCED CONNEXIN 43 IS REQUIRED FOR AN EFFECTIVE IMMUNOTHERAPY AGAINST THE MURINE B16 MELANOMA

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The general objective of this project is to investigate the potential of the bacterium Salmonella Typhimurium as a melanoma cancer treatment aid, studying its capacity to induce mechanism of action in the immunotherapeutic protocol previously developed in our lab.

Salmonella, directly injected in the murine melanoma B16, is able to induce the regression of this tumor and the retardation of the growth of a distal untreated lesion contralaterally located. We previously showed that the beneficial effect on the untreated tumor is due to the cross-presentation of the tumor antigens by dendritic cells (DCs) to CD8+ T cells. In the present study we investigated deeply the mechanism that leads to this antitumor systemic immune response. The results show that Salmonella or its products upregulate in several human and murine melanoma cells the expression of Connexin43 (Cx43), the main component of small molecular pores called gap junctions (GJs), therefore facilitating the formation of these channels between tumor cells and DCs. This approach allows the generation of a potent anti-tumor immunity that controls the growth of distant untreated tumors via a CD8-dependent mechanism. Tumor cells infected in vivo with Salmonella, when stably silenced for the expression of Cx43, fail to elicit the antitumor response.

This cross-presentation pathway can be employed in vitro for the generation of DC-based vaccines. In fact, the most striking result obtained in our preclinal studies consists in the induction of a potent antitumor vaccination, in both preventive and therapeutic setting, through the use of DCs loaded in vitro with Salmonella-treated melanoma cells. We were able to load DCs in vitro, by the means of the GJ-mediated transfer of tumor-associated peptides from melanoma cells, following the upregulation of Cx43 in tumor cells through Salmonella. We have compared different pathways of tumor antigen cross-presentation like direct loading with tumor peptides, tumor lysates and apoptotic bodies, but we found that the Cx43-dependent mechanism was the only one having a benefit in a therapeutic setting. In addition, when tested in a preventive setting that is the ideal situation in patients having undergone surgical resection of the tumor, vaccination with DCs loaded with infected tumor cells led to 100% of the mice free of tumor.

Therefore, we anticipate that this approach will become a powerful tool to allow DCs to present a repertoire of tumor peptides that reflects those presented by tumor cells.

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CHRYSOTILE-INDUCED GENE EXPRESSION PROFILE IN HUMAN BRONCHIAL EPITHELIAL CELLS

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Chrysotile exposure has been known to contribute several lung diseases named asbestosis, malignant mesothelioma and lung cancer, but the disease-related molecular and cellular mechanisms are still largely unclear. To address the effects of chrysotile exposure in human bronchial epithelial cells at molecular level, gene expression analysis was performed. Chrysotile-treated cells were recovered, and total RNA was isolated to generate cDNA probes. Microarray results revealed that 528 of the 45034 genes queried were significantly up-regulated and 411 of the 45034 genes were significantly down-regulated in response to 10 ug chrysotile treatment. The genes with chrysotile-induced changes belonged to the functional gene categories of transport, transcription, immune response, cell differentiation, cell cycle and cell proliferation. Differential gene expression profiles may provide clues that could be used to define mechanisms involved in chrysotile-induced lung disease. This work was supported by Korea Ministry of Environment as “SoonChunHyang Environmental Health Center for Asbestos Related Disease: The healthcare survey for residents who live near asbestos mines” The travel expense to attend the conference was supported by SoonChunHyang University.

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COMPARISON OF TUMORICIDIC PROPERTIES OF ANTI EGFR IgG AND IgE ANTIBODIES

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Background and Aims: Although five different antibody classes are expressed in humans, each of them conferring a distinct function to the immune system, all clinically used anti-cancer monoclonal antibodies comprise the IgG class. For instance, the chimeric anti-EGFR antibody cetuximab derived from the murine hybridoma cell line m225 which is applied for treatment of metastatic colorectal cancer is an IgG1. However, recent studies have indicated that also IgE antibodies have anti-tumor efficacy, suggesting that tumor specific monoclonal IgE antibodies could serve as novel therapeutic tools with modified effector functions. The aim of this work was to compare the tumoricidic effects of cetuximab with an in house produced cetuximab-like human IgE in a colon cancer model.

Methods and Results: Mouse-human chimeric 225-IgG1 and 225-IgE were recombinantly expressed in HEK293 cells and their specificity was demonstrated by flow cytometry and immunofluorescence staining of EGFR-overexpressing A431 cells, indicating similar binding properties as compared to cetuximab. Next we tested the ability of 225-IgE to trigger degranulation in vitro using the basophilic mast cell line RBL-SX38 transfected with human FcεRI subunits. This assay confirmed that the ε-Fc domains were fully functional and could clearly demonstrate that only membrane-bound EGFR on A431 cells triggered degranulation, whereas monomeric soluble EGFR could not. Moreover, both cetuximab-like antibodies, IgG and IgE, specifically inhibited tumor cell proliferation in an MTT cell viability assay.

To investigate the isotype-dependent effector functions of anti-EGFR IgG1 and IgE regarding antibody dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) of tumor cells, we co-cultured monocytic U937 as effector and the EGFR-overexpressing A431 as target cells. The results of this three-color flow cytometric assay indicated that that the IgG mediated more phagocytosis, whereas IgE tended to kill via ADCC.

Conclusion: This is the first study describing the tumoricidic properties of recombinant EGFR-specific human IgE. Compared to IgG1, IgE may harness alternate effector cells for tumor cell surveillance and killing. Our work points to the importance of further research to investigate the efficacy and elucidate the underlying mechanisms of action of tumor-antigen specific antibodies of different classes with a special focus on IgE.

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A POTENTIAL INSIGHT AGAINST BREAST CANCER CELLS: SHORT PEPTIDES SELECTED TO HER2

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HER2 is an epidermal growth factor receptor that is an essential mediator of cell proliferation and differentiation. In patients with breast cancer, HER2 overexpression is associated with poor prognosis, aggressive disease and resistance to chemotherapy and hormonal therapy. A monoclonal antibody against HER2, trastuzumab, is currently in use as a treatment for breast cancer; however, there are some reports of resistance to this treatment and it can develop a high rate of cardiac failure, despite the high cost. As an alternative to trastuzumab we have selected specific peptides to this receptor using a phage display technology. A cyclic 7 aminoacids random peptide library was panned using recombinant HER2. Specific peptides were dislodged and selected using trastuzumab. After each round of binding assays, peptides were selected, sequenced and analyzed by ClustalW program. The selected peptides were synthesized and assayed using different breast cancer cell lines in comparison with trastuzumab. It was observed that one of the selected peptides designate Tavelorb is able to induce apoptosis/ necrosis in 70% of SKBR3 cells and when associated with another peptide called Hercid, the effect increases to 90%. In addition, confocal microscopy confirmed co-localization of HER2 and selected peptides. These peptides also co-localize with acidic vesicles in 40-50%, suggesting that they might be able to induce endocytosis and destruction of HER2.The data propose a potential use of these peptides as an alternative for breast cancer treatment.

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	Annexin V (%)	Annexin V + PI(%)
Control	6.9	3.2
Trastuzumab	25.7	8.6
Hercid	23.5	4.0
Tavelorb	56.6	13.6
Hercid + Tavelorb	57.6	33.0

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HELPER ACTIVITY OF NATURAL KILLER CELLS DURING THE DENDRITIC CELL-MEDIATED INDUCTION OF MELANOMA-SPECIFIC CYTOTOXIC T CELLS

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NK cells have been shown to mediate important immunoregulatory “helper” functions in addition to their cytolytic activity. In particular, NK cells are capable of inducing DC maturation while preventing maturation-related DC “exhaustion,” resulting in mature DCs with an enhanced ability to produce IL-12p70, a factor essential for the development of type-1 immune responses and effective anti-cancer immunity, upon subsequent contact with T cells (type-1 polarized DC; DC1). Here we show that, directly in the context of patients with advanced cancer, the NK cell-mediated type-1 polarization of DCs (NKDC1) can be applied in clinically-relevant serum-free conditions to enhance the efficacy of DCs in inducing melanoma-specific CTLs. NK cells isolated from late-stage (stage III and IV) melanoma patients responded with high (average of 100-fold) IFN-γ production and the induction of type-1-polarized DCs upon exposure to several defined combinations of stimulatory agents, including IFN-α plus IL-18, IFN-α plus K562 cells (NK cell sensitive leukemia), or IFN-α plus opsonized melanoma cells (nominally NK cell non-sensitive). Such NKDC1 showed strongly-enhanced (average of 64-fold) capacity to produce IL-12p70 upon subsequent interaction with T cells, augmented by direct cell-to-cell contact and synergy with additional poly-I:C co-stimulation, compared to immature DCs and non-polarized IL-1β/TNF-α/IL-6/PGE2-matured “standard” (s)DCs. These NKDC1 demonstrated high levels of surface co-stimulatory and antigen-presentation molecule expression, including CD86, CD40, and HLA-DR, as well as enhanced CCR7 expression and migratory responsiveness to the lymph node chemokine CCL21. When compared in vitro to non-polarized sDC, NKDC1 were superior (average of 60-fold) in inducing high numbers of functional melanoma-specific CTLs capable of recognizing multiple melanoma-associated antigens and killing melanoma cells. These results indicate that the helper function of NK cells can be utilized in clinical settings to improve the effectiveness of DC-based cancer vaccines.

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EPITOPE ARRAYS FOR MULTIPLEX MONITORING OF AUTOANTIBODIES IN CANCER PATIENTS

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Circulating autoantibody (Ab) against clinically relevant tumor-associated antigens (TAA) in cancer patients provide useful information regarding the clinical status of cancer. However, current autoAb detection approaches require preparation of phage lysates or purification of TAA proteins. To circumvent these difficult and expensive procedures, we have been focused on identifying dominant B cell epitopes from clinically relevant prostate cancer-associated antigens (PCAA). Peptide epitopes from a targeted panel of PCAA were predicted and synthesized, followed by screening and validation as antigenic targets recognized by sera from prostate cancer patients. An epitope array was then compiled by individually conjugating each peptide epitope from cancer/testis antigen NY-ESO-1, XAGE-1b, SSX-2,4, as well as prostate cancer overexpressed antigen AMACR, p90 autoantigen, and LEDGF to internally colored xMAP microspheres. The resultant epitope array allowed multiplex measurement of autoAb present in serum samples of prostate cancer patients.

In contrast to classic ELISA, epitope arrays based on the xMAP platform performed entirely in the liquid phase, with significantly reduced background, shortened incubation time, and fewer washing steps. Epitope arrays thus provided high throughput analysis of biomarkers and the potential point-of-care diagnoses.

Furthermore, the multiplexing power of epitope array allowed quantifications of autoAb plus total PSA, a conventional biomarker for prostate cancer in one reaction (termed the A+PSA assay). In a retrospective study of 131 biopsy-confirmed prostate cancer patients and 121 benign prostatic hyperplasia/prostatitis patients, A+PSA improved the area under Receiver Operating Characteristic curve from 0.75 to 0.91 comparing to the PSA assay. Epitope array provides a novel and multiplex platform that can be combined with conventional biomarkers to potentially enhance the monitoring of cancer progress and recurrence.

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CHARACTERIZATION OF scFv78: AN ANTI-TEM1 scFv

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Tumor endothelium marker 1 (TEM1) is an important target in tumor vasculature. We have previously isolated from a human yeast display scFv library scFv78, a high affinity single chain antibody (scFv) recognizing human and mouse TEM1. To further characterize its potential for tumor targeted imaging and/or therapy, here we measured its affinity; mapped its epitope recognition and modeled its 3-D interaction with TEM1; evaluated its internalization; and tested its tumor targeting in vivo.

The calculated affinity of scFv for human TEM1 was 1.8 nM by Biacore. The epitope of scFv78 was measured by a combination of yeast display and competitive ELISA. A 390 amino acid N-terminal residue of TEM1, previously used to screen the scFv library, was divided into six adjacent fragments and displayed on yeast surface. FACS analysis indicated that scFv78 binds to the sixth fragment, containing the 324-390 amino acid residue. Further epitope mapping using competitive ELISA showed that none of the 15-amino acid peptides derived from this fragment could block the binding of scFv78 to TEM1. This indicated that scFv78 binds to a conformational epitope instead of a linearized one. A docking model of the scFv78-TEM324-390 complex was generated by Zdock software using the 3D structure of TEM324-390 built based on homology model using Threader and Rosetta programs and MOE software, and a 3D model of scFv78 built by BioInfobank server and the Modeller program. The docking model confirmed the possibility of this interaction and suggested that the epitope formed by the TEM1 329-346, 348-353 and 368-378 residues contribute to the interface recognizing scFv78. To evaluate the potential of scFv78 for targeted delivery of toxic drugs into tumor associated TEM1+ cells, we checked its ability to be internalized by TEM1- or TEM1+ cells following incubation for 2h, 6h and 15h at 37°C. scFv78 was strongly internalized only by TEM1+ cells but not TEM1- cells. Lastly, the ability of scFv78 to target tumor in vivo was evaluated by conjugating biotinylated scFv78 with streptavidin-IRDye and injecting this complex into mice bearing subcutaneous tumors expressing human TEM1 or upregulating endogenous murine tem1. The distribution of scFv78, as monitored by detecting the conjugated infrared dye, preliminarily suggested that scFv78 binds not only to human TEM1+ tumor but also mouse tem1+ tumor.

In summary, our study indicates that scFv78 holds potential for tumor targeted imaging and/or therapy. Its affinity is sufficient for direct clinical use without any further maturation. The docking model we developed is a useful tool to guide further studies in scFv78 characterization and application.

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CANCER STEM CELL VACCINATION CONFERS SIGNIFICANT ANTI-TUMOR IMMUNITY BY SELECTIVELY TARGETING CANCER STEM CELLS

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The isolation of human cancer stem cells (CSC) represents a new paradigm for the development of cancer treatments. So far, the majority of CSC studies have been confined to human tumors inoculated into severely immunosuppressed hosts (e.g. SCID mice), which are not suitable for immunologic evaluation. In this study, we used ALDEFLUOR/ALDH1 as a marker and identified CSC-enriched populations in two histologically distinct murine tumors (melanoma B16-D5 and squamous cell cancer SCC7). We found that a very small percentage (0.5-5%) of ALDEFLUOR+ cells exist in these tumors; as few as 500 sorted ALDEFLUOR+ cells could form tumors in syngeneic hosts. The stem cell nature of the ALDELFUOR+ cells was confirmed by their ability to form spheres in culture and to self-renew in vivo. We evaluated the protective anti-tumor responses induced by vaccination with dendritic cells (DC) pulsed with the lysate of ALDEFLUOR+ D5 cells (CSC-TPDC) in the syngeneic immunocompetent host (B6). Normal mice were vaccinated with CSC-TPDC or DC pulsed with lysate from unsorted heterogeneous D5 cells (H-TPDC), followed by challenge with D5 tumor cells intravenously. Lungs were harvested to enumerate metastases. Compared with non-vaccinated, PBS-injected controls, H-TPDC induced modest protective immunity against tumor challenge, which is consistent with our previous observations. More importantly, mice that received CSC-TPDC had significantly fewer lung metastases than both the control group and the H-TPDC treated group ($p < 0.02$). CSC-induced protective antitumor immunity was confirmed in a second tumor model (SCC7) syngeneic to a genetically different immunocompetent host (C3H) in a subcutaneous tumor setting. Immune sera collected from D5 or SCC7 CSC-TPDC vaccinated hosts contain high levels of IgG and IgG2b which bound specifically to D5 CSCs or SCC7 CSCs, respectively. This binding resulted in the CSC lysis in the presence of complement. In addition, CTLs generated from PBMCs harvested from D5 or SCC7 CSC-TPDC-vaccinated hosts selectively killed D5 or SCC7 CSCs efficiently. Together, these results suggest that enriched CSCs are immunogenic and are significantly more effective as an antigen source compared with unselected tumor cells in inducing protective anti-tumor immunity. We provide direct experimental evidence that targeting of cancer stem cells by CSC-primed antibodies and T cells may represent the mechanisms involved in CSC vaccine-conferred anti-tumor immunity. The findings from these studies may help develop novel immunological approaches for cancer treatment by specifically targeting cancer stem cells.

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ALTERED IMMUNE MODULATORY ACTIVITY AND DIFFERENTIAL PROTEIN EXPRESSION PATTERN OF TUMOR CELLS UPON TREATMENT WITH THE TYROSINE KINASE INHIBITOR AXITINIB

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Axitinib is an oral inhibitor of the vascular endothelial growth factor receptors (VEGFRs) thereby exerting potent anti-angiogenic and anti-proliferative activity. Axitinib is currently been investigated in clinical trials including renal cell carcinoma (RCC). It has been shown to affect downstream signalling pathways, such as the inhibition of the phosphorylation of the extracellular signal regulated kinase (ERK) and AKT. The efficacy of axitinib in tumor models has been correlated with a block of downstream signalling pathways, which caused a reduction in cell proliferation, in tumor vessel density as well as in induction of apoptosis. The activity of axitinib appears to be independent of the tumor model analysed and of the target tyrosine kinase expressed. The effect of axitinib on immune modulatory molecules has not yet been investigated. Therefore we analysed the effect of axitinib on 6 RCC and 4 melanoma cell lines cultured under standard conditions or as sphaeroids, respectively, using dose response)and time course analyses. These experiments showed (i) that RCC were more sensitive than melanoma cells to the growth inhibitory effects of axitinib and (ii) that sphaeroid cultures were more sensitive to axitinib than standard 2D cultures. The expression of immune modulating molecules, such as components of the antigen processing machinery, like the peptide transporter TAP, tapasin and/or the low molecular weight proteins (LMP), were upregulated upon axitinib treatment of RCC and melanoma cells resulting in an increased HLA class I surface expression. Furthermore, the expression of members of the B7 family, such as B7-H1 and B7-H3, were also altered in the presence of this substance suggesting a modulation of the immunogenicity of both RCC and melanoma cells.

2DE-based proteome analysis of untreated and axitinib-treated RCC cell lines resulted in the characterisation of a distinct protein expression pattern characterized by a number of upregulated and downregulated protein spots identified by mass spectrometry. The proteins were classified into different protein families involved in proliferation, cytoskeleton formation, signal transduction as well as in the cellular metabolism. Interestingly, some differentially expressed proteins identified could be directly linked to the activity of axitinib. Thus, axitinib exerts immune modulatory activity and affects the protein expression of tumor cells thereby suggesting the utility of these differentially expressed proteins as potential biomarkers of axitinib efficacy and to improve immunotherapy by using treatment combinations of TKIs with T cell-based immunotherapy.

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ON THE LONG TERM USE OF A THERAPEUTIC CANCER VACCINE

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Therapeutic vaccines continue to be one of the most active fields of cancer research. However, despite clear evidence of antitumor effect in laboratory animals, and despite the ability of current vaccine candidates to elicit tumor specific antibodies and T cells in humans, objective responses in the clinical trials are rare. The role of therapeutic vaccines in advanced cancer patients, if any, would be to decrease the rate of progression of the disease, and to increase survival and quality of life. Due to the redundant regulatory loops contracting the immune response to antigens that cannot be eliminated, such a role would require chronic vaccination, which is at first sight at odds with the classic experience of vaccinology. During the last decade our team has been developing a therapeutic vaccine for advanced lung cancer, which consists in human recombinant Epidermal Growth Factor (EGF) chemically conjugated to a carrier protein from Neisseria meningitides. Several clinical trials have been carried out, showing increase in anti-EGF antibody titters, decrease in plasma EGF concentration and survival advantage in vaccinated patients. In the present paper we review data from 58 patients who were vaccinated monthly for more than one year or two. Long term vaccination was feasible and safe, and there was no evidence of cumulative toxicity. Patients kept high anti-EGF antibody titters during all the time of vaccination, without evidence of exhaustion of the immune response. Continued vaccination increased the probability to get a high antibody response, which has been previously shown to be, in turn, associated with a better survival.

Observations done in this series of patients suggest that long term therapeutic vaccination is a feasible strategy, worth being further explored in the aim of transforming advanced cancer into a chronic disease.

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