Late Breaking Abstracts From the 26th Annual Scientific Meeting of the Society for Immunotherapy of Cancer (SITC)

(Presenting Authors are Italicized)

HIGH THROUGHPUT TECHNOLOGIES FOR IMMUNE MONITORING

A Novel Colorectal Cancer Vaccine Consisting of Multiple Naturally Presented Peptides

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To effectively treat cancer patients with T cell-based immunotherapy, T cells have to recognize peptides restricted by HLA molecules on tumors. For most of the published peptides it is unknown whether they are naturally presented or not. IMA910 is a novel peptide-based vaccine consisting of 10 HLA-A*02 binding and 3 HLA-DR binding tumor associated peptides (TUMAP), which were presented on colorectal tumors and were overexpressed in comparison to healthy tissues. 8 of the 10A*02-binding peptides had been confirmed to be naturally presented by peptide elution from surgically resected tumors and analysis by high-sensitivity mass spectrometry (XPRESIDENT approach). The two other A*02-binding peptides were chosen due to their characteristics described in literature. The multi-center clinical trial IMA910-101 enrolled 92 HLA-A*02+ advanced colorectal cancer (CRC) patients being at least clinically stable after 12 weeks of first-line oxaliplatin-based therapy. Patients were infused with a single low dose of cyclophosphamide (300 mg/m^2) and repeatedly immunized intradermally (up to 16 vaccinations) with IMA910 in combination with GM-CSF (cohort 1; n = 66) or IMA910 with GM-CSF plus topically applied imiquimod (cohort 2; n = 26) as immunomodulators. Before and post vaccination patients were analyzed for T-cell responses to IMA910 HLA-A*02 and HLA-DR restricted peptides by HLA-multimer assay and intra-cellular cytokine (ICS) assay for CD8 T-cell responses and by ICS assay for CD4 T-cell responses. Tumor status of patients was monitored repeatedly by CT/MRI according to RECIST, corresponding tumor scans were reviewed centrally for assessment of disease control rate (DCR), progression-free survival (PFS) and overall survival (OS). IMA910 overall was immunogenic in 75/80 (94%) evaluable patients. A moderate but significant effect of imiquimod treatment on the number of immune responses to IMA910 peptides as detected by the ICS assay was observed. Finally, we demonstrate significantly increased PFS and a trend for increased OS in patients with class I immune responses to multiple TUMAPs. Most interestingly, only immune responses to peptides confirmed to be naturally presented were significantly associated with OS. Responses to the two immunogenic peptides included from literature with unclear status of natural presentation did not correlate and actually diluted the overall correlation of immune response with clinical benefit. This latter finding suggests that peptide antigens confirmed to be naturally presented may be preferable for vaccination and immunomonitoring.

Key Words: Active immunotherapy, cancer vaccine, colorectal cancer.

Frequency of Strong Antibody Responses Following Combination Immunotherapy Correlates With Increased PSA Doubling Time in Men With Androgen-independent Prostate Cancer

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Development of a strong immune response results in B cells and T cells being primed against the same antigens. For a B cell to switch from producing IgM to IgG requires CD4 T cell help. Therefore, we postulate that identification of IgG antibodies against possible tumor/tumor-associated proteins, identified by ProtoArray, will be a surrogate for a T cell response against that same antigen. This strategy is strongly supported by reports in both human and murine settings.[1,2] We used 8217 protein spotted arrays (ProtoArrays, Invitrogen) to analyze antibody responses in sera from 10 evaluable patients on an investigator-initiated combination immunotherapy trial for men with advanced prostate cancer. While a small group, 3 men exhibited a 3 fold or greater increase in PSA-DT. The PSA-DT in the remaining patients was stable or decreased. Based on our analysis of these 10 evaluable patients, we found that men with an increase in PSA-DT (n = 3) had a significantly ($P < 0.05$) higher number of strong Ab responses (> 15 fold increase) than men whose PSA-DT remained stable or decreased. This finding, in the cohorts of men receiving combination immunotherapy and who were hypothesized to respond better to vaccination, has encouraged us that our immunological monitoring strategy is reasonable. Characterization of the T cell responses to the identified antigens as well as the development of a high-throughput screening system using custom protein bead arrays are ongoing.

Key Words: Antibody response, Biomarker.

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IMMUNOLOGY OF CANCER STEM CELLS AND EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT)

Naturally Occurring Immune Responses Against Sox2 And Bcl-2 in Patients with Advanced and Early-stage Cancer

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It has been documented by numerous reports that epitopes derived from human tumor-associated antigens can be recognized by the immune system of cancer patients. However, the majority of these antigens are not clinically relevant or important for cancer cell survival. Moreover, in several animal and clinical immunotherapeutic studies it has been demonstrated that there is immune selection and immune escape against most of these antigens. Deregulation of apoptosis proteins and pathways and death resistance of cancer "stem" cells have been observed in human cancer and often are due to over-expression of several proteins such as Bcl-2 and Sox2.
Sox2 and Bcl-2 are implicated in tumor progression, resistance and proliferation. The over-expression of these proteins in several tumors, acting as broad- spectrum antigens and the lack of relevant mutations makes them reliable targets for clinical immunotherapy trials. Our research group designed several peptides representing potential immunogenic epitopes for both proteins. We identified spontaneous humoral and cellular immune responses against these Sox2 and Bcl-2-derived peptides in early-stage and advanced cancer patients suffering from ovarian, breast, pancreatic, colorectal, melanoma and sarcoma. In this study, we demonstrated that Sox2 and Bcl-2 could be targets for humoral and cellular immune recognition in cancer patients.
Furthermore, we describe naturally occurring immune responses against specific peptides derived from Sox2 and Bcl-2 in cancer patients by ELISA and ELISPOT, whereas no detectable responses were found in healthy patients. Thus, cellular and humoral immune responses against important proteins related to tumor survival like Bcl-2 and Sox2 seem to be common in cancer patients and could serve as broad-spectrum tumor antigens to achieve better clinical responses in cancer immunotherapy trials.

Key Words: Sox2 immune response, advanced cancer immune response, advanced cancer.

IMMUNOTHERAPY COMBINATIONS

Autologous Vaccine AHICE, Therapy Results

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AHICE immunotherapy is distinguished by its unique selectivity and specificity against recognized tumors. The peculiarity of AHICE is the demasking of the tumor cells biochemically. Following that the autologous immune system is being able to detect them spontaneously and eliminates them apoptotically.
We report here the AHICE therapy outcomes of different carcinomata. AHICE was either sub cutan as a long term therapy of ninety days or i.v. administered as three up to ten infusions.
Before and after AHICE, every three weeks following were examined: A differential blood count, a lymphocytes immune-phenotyping, the related tumor markers, TNF-α-, IFN-γ-concentrations. At the end of AHICE the tumor situation was examined (MRI, CT or PET). We found a confluence with good therapy outcome relatively (steady-state, or melting down of tumor or remission) at a level of minimum 1700 lymphocytes/μL in peripheral blood, a rising T4 in relation to T8 lymphocytes-concentration, an index T4/T8 of better than 1,5. The related tumor markers were at first rising up in respect of the strength of the immune response—this is for increasing cell-death apoptotically of the respective tumor cells and doesn't have to be interpreted as a progress of the tumor.
A colon-ca. is still living without neoplasies at the best quality of life relatively, overcomes six years.
A pancreas ca. after surgery treated first AHICE at 2000, lived without metas at the best quality of life until December 2009, that is rest life prolongation of nine years.
A peritoneal ca. patient, with multifocal metas has after the first AHICE cycle a remission (CT) in June 2004 without neoplasies until December 2006.
A breast cancer patient with Parkinson, treated first AHICE-cycle at 2005, is still living without neoplasies over six years at best quality of life.
A small-cell lung-ca. with two brain metas and condition after a radiation treatment of that brain area and surgery of the lung tumors. Thereafter in 2009 start on the AHICE and during the over two years observation duration were no neoplasies noticed in the lung, liver as also the one brain-meta was melted down. The second brain-meta showed central a not agent incorporating area (CT, MRI, PET) but only in a small peripheral region of 1/4th. Because of the oedema an excision of the tumor was carried out. The immuno-histochemically examination showed multiple necrotic cells and increased CD56+ marker on cells (NKCs?). Therefore this is the proof of the in vivo effectiveness of AHICE. In conclusion we can refer that after a previous demasking of tumor-cells, the so activated autologous immune system is the significant point of reference for successful cancer therapy.

Key Words: Autologous Vaccine AHICE, Immunotherapy, Cancer, Auto-Immune Diseases.

Use of Complementary and Alternative Medicine in Patients With HAV/HBV/HCV Infections: Results from a Cross-sectional Study in the Sherpur District of Bangladesh

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Hepatitis A, Hepatitis B, and Hepatitis C are viruses (HAV/HBV/HCV), which causes HAV/HBV/HCV infections. HAV/HBV/HCV is one of the world's most common infectious diseases. Infections can lead to severe liver diseases, which may last throughout a patient's life. Around 25% of carriers will develop serious liver diseases, including chronic hepatitis, liver cirrhosis, and primary liver cancer. More than one million deaths per year are recorded due to HAV/HBV/HCV infections. HAV/HBV/HCV is the most common diseases in Bangladesh. The objective of this study was to conduct a survey amongst the local specialists in the Sherpur district of Bangladesh, to collect information on plants used to treat HAV/HBV/HCV infections. Local specialists of the study area were selected randomly and interviewed with the help of translators to gather information on the knowledge and use of plants used as a remedy for HAV/HBV/HCV infections. In-depth information regarding plants type, preparation of medicines, ailments for which they are used, dosages, and side effects if any, were obtained from the local specialists. All plants were photographed, collected, identified, and vouchers were stored at the Bangladesh National Herbarium. Information on thirty-six plants was obtained. The collected information indicates that the following plants are used to treat HAV/HBV/HCV infections: Lawsonia inermis L., Terminalia arjuna (Roxb. ex DC.) Wight & Arn., Terminalia bellirica (Gaertn.) Roxb., Sesamum indicum L., Terminalia chebula Retz., Cicer arietinum L., Swertia chirata Buch.-Ham. ex Wall., Abrus precatorius L., Daucus carota L., Citrus aurantiifolia (Christm.) Swingle, Aloe vera (L.) Burm.f., Phoenix sylvestris (L.) Roxb., Dillenia indica L., Ocimum gratissimum L., Cocos nucifera L., Saccharum officinarum L., Piper nigrum L., Andrographis paniculata (Burm.f.) Nees, Aegle marmelos (L.) Corrêa, Diospyros malabarica (Desr.) Kostel., Vitis vinifera L., Curcuma amada Roxb., Limonia acidissima L., Carica papaya L., Scoparia dulcis L., Azadirachta indica A.Juss., Boerhavia diffusa L., Lepidagathis hyalina Nees, Nigella sativa L., Aconitum napellus L., Agaricus campestris L., Achyranthes aspera L., Plantago major L., Santalum album L., Grewia asiatica

L., and Coccinia grandis (L.) Voigt. Information on indigenous use of plants has led to discovery of many medicines in use today. Scientific studies conducted on the above plants may lead to discovery of more effective drugs than in use at present.

Key Words: HAV/HBV/HCV infections, Plants, Bangladesh.

Radiation and Endoplasmic Reticulum Stress-inducer Promote Calreticulin Translocation, Contributing to Immunogenic Cell Death of Cancer Cells

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We hypothesize that cell damage and death from the effect of ionizing radiation (IR) and endoplasmic reticulum (ER) stress-inducing agents could: (1) be monitored in vitro; and (2) contribute to an anti-tumor immune response via the induction of mediators of immunogenic cell death (ICD) of cancer cells. ICD promotes the cross-presentation of tumor-derived antigens by dendritic cells (DCs) to T cells (*Semin Immunol*. 2010;22:113–124). Calreticulin (CRT, an ER chaperone protein) redistribution to the surface of tumor cells acts as a potent "eat me" signal for DCs involved in tumor associated antigen processing, thereby serving as a key step in ICD. In the clinical setting, IR or ER stress alone may not quantitatively and/or qualitatively achieve tumor cell death in a manner that specifically triggers immune-mediated tumor rejection. Thus, we hypothesized that clinically relevant doses of IR, when combined with thapsigargin (Tg, an ER stress-inducer via sarcoplasmic/ER calcium ATPase inhibition), may intensify CRT translocation to the cell surface. To test this, we employed the poorly immunogenic 4T1 mouse breast cancer cells. 4T1 cells were treated with IR (0, 6, or 20 Gy) followed by 24 hours. culture in the presence or absence of Tg (1 mM). Thereafter, the cells were assayed either via Western blot (WB) or immunofluorescence (IF). Cytotoxicity was determined via MTT assay at 12, 24, and 48 hours. Relative amounts of protein were determined via WB analysis with specific antibodies to phospho-EIF2-a, caspase-8, BAP-31, and PARP. Actin was used as a loading control. CRT redistribution was determined by IF analysis. When combined, IR (6 Gy) + Tg (1 mM) triggered elevated phosphorylation of EIF2-a (a marker for ER stress and protein translation inhibition) in 4T1 cells. In addition, IR (6 and 20 Gy) + Tg (1 μM) increased the cleavage of the apoptotic markers caspase-8, BAP-31, and PARP. Finally, we observed that cell death by IR (6 and 10 Gy, single dose) in the presence of Tg (1 μM) was preceded by enhanced CRT translocation to the cell surface. In this in vitro model, IR (6 Gy and 10 Gy) alone was unable to incite CRT redistribution. However, in the presence of Tg (1 μM), IR (6 Gy) CRT redistribution occurred and was superior to controls. Taken together, these findings suggest that IR combined with an ER stress-inducing agent is a novel application of radiotherapy that can potently trigger ICD and serve as a strategy to promote immune-mediated tumor rejection in cancer patients.

Key Words: radiotherapy, ER-stressors, calreticulin.

Immunomodulatory Effects of Dasatinib Synergize With CTLA-4 Blockade Resulting in Enhanced Antitumor Activity

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Dasatinib is a highly potent BCR-ABL inhibitor used for the treatment of patients with chronic myeloid leukemia (CML). Exposure of immune cells to Dasatinib in vitro has been associated with immunomodulatory effects on T-cells and CD11b+ myeloid cells. We hypothesize that activity of Dasatinib in vivo is in part related to its immunomodulatory effects. The purpose of these studies was to determine the antitumor activity and immunomodulatory effects of Dasatinib as monotherapy and in combination with CTLA-4 blockade in vivo. Efficacy studies were conducted in five solid tumor models: P815 mastocytoma, SA1N fibrosarcoma, CT-26 colon carcinoma, M109 lung carcinoma, and B16/F10 melanoma. Dasatinib was administered at 30 mg/kg BID (twice a day) on a 5-day on/2-day off schedule for a total of three dosing cycles or daily for 15 consecutive days; CTLA-4 mAb was dosed at 20 mg/kg every fourth day for three doses. CTLA-4 mAb was active in the P815, SA1N, and CT-26 models, with minimal activity against the M109 model and no activity observed against B16/F10. Dasatinib dosed intermittently (5 d on/2 d off) showed minimal activity against P815 and SA1N models and was not active against CT-26, M109 or B16/F10. Concurrent treatment with Dasatinib + CTLA-4 mAb resulted in additive or synergistic effects in the SA1N, P815 and CT-26 tumor models. In the M109 and B16/F10 models where both Dasatinib and CTLA-4 blockade are inactive, no antitumor effect was observed. Additional studies were conducted in the CT-26 tumor model to determine whether the enhanced antitumor activity was due to an expansion of cytotoxic T cells and whether the treatments were altering the composition of the immune cells in the tumor-draining lymph nodes and tumors. Increased in vivo cytotoxic activity against a CT-26 peptide was observed in animals treated with the combination treatment compared to animals treated with single treatments ($P < 0.05$), which correlated with an increase in the ratio of activated CD8+ effector cells over CD4+FoxP3+ T cells. Dasatinib therapy also induced changes in the composition of T cells promoting a more favorable ratio of T effectors/T regulatory cells compared to controls. In addition, microarray gene analyses of tumors treated with the combinatorial approach demonstrated marked changes on pathways involved with immune function. Thus, Dasatinib modulates the composition of immune cells in the tumor-draining lymph nodes and tumor microenvironment to promote enhancement of antitumor immune responses in combination with CTLA-4 blockade.

Key Words: CTLA-4, Dasatinib, Immunomodulation.

IFN-Alpha Increases the Cytotoxic Effect of CIK Cells on B-ALL

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Haematopoietic stem cell transplantation (HSCT) is required in about 20% to 30% of children with B-lineage acute lymphoblastic leukemia (B-ALL). Relapses after HSCT are usually refractory to further therapy and in these cases, the development of an optimized immunotherapeutic strategy would be of great clinical interest. In this setting, the Cytokine-Induced Killer (CIK) cells could represent an interesting tool for immunotherapy. Indeed, they were showed to be highly cytotoxic against many cancer types. Nevertheless, their cytotoxicity against ALL cells is not consistent. Therefore, we have investigated the possibility of combining adoptive immunotherapy with CIK cells and interferon alpha (IFNα), to optimise the cytotoxicity of CIK cells against B-ALL cells. CIK cells were differentiated from cord blood mononuclear cells or peripheral blood mononuclear cells for 21 days. At the end of the culture, there were around 45% CIK cells (CD3+CD56+). The other cells were 1% natural killer (NK) cells and 54% T cells. The bulk CIK (CIK cells, NK cells and T cells) showed a mild cytotoxic activity against B-ALL cell lines. However, when the bulk CIK was purified with CD56 human microbeads there was significant cytotoxic activity against B-ALL cell lines. In addition, we have showed that sorted CIK cells removed from NK and T cells, always showed a cytotoxic activity against B-ALL cells lines. Also, after pre-incubation of sorted CIK cells with IFNα overnight, we have observed an increase of cytotoxicity by more than 20% to 40%. CIK cells displayed a phosphorylation of STAT-1 after stimulation by IFNα. In addition, we have tested in vivo CIK cells in NOD/SCID/γc- (NSG) mice injected with human B-ALL cell lines and we could show that CIK cells (Target on effector ratio of 1:80) could significantly delay mice mortality. Also, we showed that CIK cells treated by IFNα did not

the induce of xeno-Graft-versus-Host Disease (GvHD) in NSG mice. In conclusion, we showed that CIK cells are cytotoxic against B-ALL when they are purified and also their effect is increased by the IFNalpha via STAT-1. Finally, the CIK cells have a GvL effect (graft versus leukemia) in the NOD/SCID/γc- mouse model.

Key Words: CIK cells, IFN-alpha, B-ALL.

Partial CD4-depletion Enhances the Efficacy of Multiple Vaccinations

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Few immunotherapists would accept the concept of a single vaccination inducing a therapeutic anti-cancer immune response in a patient with cancer. But what is the evidence to support the "more-is-better" approach of multiple vaccinations? Our group reported that T cells from mice thrice vaccinated with a GM-CSF secreting B16 tumor vaccine (D5-G6) were significantly less effective in adoptive transfer studies than T cells from mice receiving a single vaccination.[1] A striking difference observed in multiply vaccinated animals was an increase in the number of Tregs, that when partially depleted with anti-CD4 mAb restored therapeutic efficacy. However, we questioned whether tissue-resident tumor-specific T cells might have been missed in our adoptive transfer studies. To address this issue we used a protective vaccine model to test if thrice-vaccinated mice would reject a large tumor challenge ($20\times$ TD_{100}). This was not the case with 100% of mice (8 of 8) immunized 3-times growing tumor. Again, Tregs increased with more vaccinations. Partial depletion of CD4 T cells 1-day prior to the 2nd and 3rd vaccination increased survival to 33% (3 of 9) ($P < 0.006$). This CD4-depletion correlated with an increased ratio of CD8 to CD4FOXP3+ cells with each subsequent vaccination, compared to non-depleted mice. Fourteen days after the second vaccination CD4-depleted mice had a larger proportion of proliferating (Ki67+) FOXP3-negative CD4 T cells and lower frequency of Ki67+ "induced" HELIOS-negative Tregs compared to non-depleted mice. Suggesting a skewing of the T cell repertoire from immunosuppressive to activated. We also examined whether location of immunization altered vaccine efficacy. We compared two strategies: in one the total vaccine dose (5×10^6 D5-G6) was administered at 1-site, which rotated to a different limb for each vaccination. The second split the dose into 4 aliquots (1.25×10^6), administered to each limb for each vaccine. Fourteen days after the 3rd vaccination mice were challenged. The frequencies of B cells, macrophages and DCs were increased in the dLN and spleen 14 days after the initial immunization in mice vaccinated at 4-sites versus 1-site. However, there were no significant differences between protection [41% (5 of 12) versus 36% (4 of 11) survival] or the frequency of Tregs or MDSCs. Overall this data suggests that partial depletion of CD4 T cells early during immunization improves vaccine efficacy and provides support for the use of partial CD4-depletion as a potential strategy for combination therapy of patients with cancer.

Key Words: multiple vaccinations, immunization, regulatory T cells.

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STATE OF THE ART ANIMAL MODELS & VETERINARY APPLICATIONS FOR CANCER IMMUNOLOGY

NOD/scid IL2Rgnull Mice: A Model for Human Dendritic Cell-based Immunotherapies

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Ex vivo-generated dendritic cell (DC)-based vaccines are a powerful tool to induce tumor-specific immune responses. Although several clinical trials have demonstrated the in vivo capacity of DC to induce antigen-specific T cell responses in cancer patients, an in vivo model has been missing to compare different protocols of human DC generation and application as a prelude to clinical studies. We compared different human-derived DC for vaccination in a newly developed xenograft mouse model. In this model, NOD/scid/IL2Rgnull (NSG) mice were reconstituted with human peripheral blood mononuclear cells (PBMC) and vaccinated with autologous human-derived mature DC expressing the MART-1 antigen and prepared using different protocols. As a first step, two regimens for reconstitution were evaluated for engraftment rates and activation status of human T cells, leading to the selection of a 4-week engraftment protocol for vaccine evaluation. Xenografted NSG mice were vaccinated twice with human-derived mature DC, comparing a newly developed 3-day protocol versus a more conventional 7-day protocol. The 3-day mature DC which were clearly superior at inducing antigen-specific immune responses in vitro[1] also resulted in increased immune responses in vivo. In previous studies we investigated the use of Toll-like receptor (TLR) agonists to generate DC capable of polarizing Th1/CTL responses in vitro. Use of a maturation cocktail containing TLR agonists yielded DC secreting high levels of bioactive IL-12(p70), accompanied by tumor-reactive Th1 and CTL responses in vitro.[2] Consistent with these observations, vaccination using DC matured with a cocktail containing a TLR7/8 agonist (R848) resulted in enhanced immune responses in the NSG mouse model. Based on these results comparing different DC vaccine variations, we conclude that this new humanized mouse model enables investigation of human therapeutic cell reagents in an in vivo setting. In particular, this model allows in vivo comparisons of different vaccine strategies, different DC variants, as well as immunogenicity of different immunizing antigens prior to use in clinical studies.

Key Words: DC-based vaccine, humanized mouse model.

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TARGETED THERAPIES AND ANTI-TUMOR IMMUNITY

Forced NF-κB in T Cells Leads to Tumor Rejection

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T cells play an important role in the elimination of tumors. Tumor-specific T cells can be found in cancer patients despite tumor growth. However, in tumor-bearing hosts, tumor-specific T cells can have reduced viability, be intrinsically anergized, extrinsically suppressed, or lack sufficient effector function to successfully reject tumors. Therapeutic strategies aimed at promoting T cell survival and amplifying T cell differentiation/effector function would be extremely desirable as novel cancer therapies. NF-κB activity has been reported to be reduced in T cells from tumor-bearing hosts. Our previous results indicate that reduced NF-κB activation results in impaired survival of T cells, decreased Th1 and Th17 differentiation and increased iTreg differentiation. Mice with reduced T cell-NF-κB activity fail to reject cardiac and pancreatic islet allografts in the absence of any pharmacological treatment. We hypothesize that forced activation of NF-κB in T cells should

have the opposite effect and promote T cell survival, facilitate Th1/Th17 differentiation and prevent iTreg differentiation, which would be beneficial to reject tumors. We generated mice expressing a constitutively active form of IKKβ (CA-IKKβ) in T cells. Ectopic expression of CA-IKKβ resulted in phosphorylation of NF-κB. Transgene expression was limited to CD4+, CD8+ and NKT cells and T cells showed increased NF-κB activation and nuclear translocation. T cell numbers were comparable to littermate controls, but CA-IKKβ mice had fewer Tregs and increased frequency of activated T cells that produced IFNγ upon re-stimulation. When B16-SIY melanoma cells were injected subcutaneously, tumors grew progressively in control littermates, whereas they were rejected by mice expressing CA-IKKβ in T cells. CA-IKKβ expressing T cells were necessary for tumor control, as shown by antibody-mediated depletion of CD4+ and CD8+ T cells. Furthermore, adoptive transfer of CA-IKKβ-expressing, but not wild-type, T cells into immune-compromised (RAG-deficient) hosts prior to inoculation of tumor cells was sufficient for tumor control. Finally, enhanced tumor control was observed in immune-competent mice when fewer than 5% of T cells expressed CA-IKKβ. Our results may potentially be translatable to the clinic and demonstrate NF-κB to be at the cross-roads of major T cell fate decisions that uniquely synergize for control of tumor growth.

Key Words: Tumor immunity, T cells.

IDO1 Activity Correlates With Hepatocyte Growth Factor Levels and Immune System Impairment in Multiple Myeloma

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Purpose: Indoleamine 2,3-dioxygenase 1 (IDO1) degrades tryptophan into immune-suppressive kynurenines (KYN), thus inducing immune dysfunction through T-cell proliferative arrest, T-cell apoptosis and regulatory T-cell (Treg) differentiation. It is presently unknown whether plasma cells in multiple myeloma (MM) foster the differentiation of Treg cells through an IDO1-dependent mechanism and whether IDO1 activity correlates with over-production of hepatocyte growth factor (HGF), an immune-modulating cytokine implicated in MM pathogenesis.
Patients and Methods: Thirty-four patients with plasma cell dyscrasia (27 newly diagnosed or relapsed MM, 4 smoldering MM and 3 MGUS) were enrolled in this study. Tryptophan and KYN were measured both in patients' serum and in bone marrow fluid with RP-HPLC. FoxP3-expressing Treg cells and NY-ESO-1+CD8+ T cells were quantitated with multiparameter flow cytometry. Conventional ELISA allowed the measurement of HGF levels both systemically and in the bone marrow microenvironment.
Results: The KYN-to-tryptophan ratio was significantly higher in patients compared with healthy controls, and correlated with serum β2-microglobulin, frequency of FoxP3+ Treg cells and HGF release. Interestingly, the frequency of NY-ESO-1-specific CD8+ T cells was significantly lower in IDO+ MM patients compared with the IDO- ones, and inversely correlated with the frequency of Treg cells. Myeloma cells, but not in vitro-expanded bone marrow stromal cells (BMSC), constitutively expressed IDO1, promoted the conversion of naïve allogeneic CD4+ T cells into Treg cells and inhibited the development of Th1, Th2 and Th17 cells. These effects were significantly albeit incompletely reverted by 1-methyl-tryptophan, suggesting that they were mediated by IDO1. In vitro mechanistic assays with IDO- MM cell lines showed the up-regulation of IDO1 expression by exogenous HGF. At variance with IDO- MM cells, IDO+ MM cells released high quantities of KYN in the culture supernatant and constitutively expressed phosphorylated Akt, an intermediate of HGF intracellular signaling.
Conclusions: We propose that IDO1 expression induced by HGF contributes to immune suppression in patients with MM and possibly other HGF-producing cancers. The HGF-IDO1 interaction represents a therapeutically exploitable molecular circuit to restore anti-tumor immunity.

Key Words: Multiple myeloma, Regulatory T cell, Indoleamine 2, 3-dioxygenase 1.

Cancer Testis Antigens as Prognostic Biomarkers for Breast Cancer Patients

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We have previously reported that the presence of a distinct immune function gene signature network at the tumor lesions of patients with early stage breast cancer could predict relapse-free survival following conventional therapies. We hypothesized that expression of cancer testis antigens (CTA) may be responsible for converting weakly immunogenic breast tumors into highly immunogenic tumors, and result in relapse-free survival. To test this hypothesis, we performed qRT-PCR analysis of RNA extracted from tumor lesions of patients with breast cancer from which we compared CTA expression levels of those who relapsed within 1 to 3 years with those who remained relapse-free during 5 to 7 years follow-up. We detected an increased expression of a number of CTA in tumor lesions of patients who remained relapse-free but not in those with tumor relapse. We also showed that treatment of human breast tumor cell lines with a demethylating agent, Decitabine, induced expression of CTA in the tumors. Altogether, these data suggest that lack of CTA expression in tumor lesions of breast cancer patients at the time of diagnosis may predict high risk of tumor relapse, and that using Decitabine in a neoadjuvant setting may convert patients with high risk into those with low risk of tumor relapse.

Key Words: Breast cancer, Cancer immunotherapy, relapse.

Phase I Study of Intravenous Recombinant Human Interleukin-15 (rh IL-15) in Adults With Metastatic Malignant Melanoma and Renal Cell Carcinoma

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Interleukin-15 (IL-15) is a cytokine with unique biological features and may have increased potential as an immunotherapeutic compared to IL-2 due to its capacity to maintain the activation of central and effector memory CD8 T-cells without augmentation of T regulatory cell (TReg) function. Our group has demonstrated the activity of IL-15 in syngeneic murine tumor models (CT26 and MC38) and conducted a pharmacology toxicology assessment in rhesus macaques to support this first in human trial. A phase I, single institution, dose escalation trial with a standard 3+3 design to determine the safety, toxicity and maximum tolerated dose (MTD) in subjects with metastatic melanoma or renal cell carcinoma was initiated. Eight subjects have been treated to date and enrollment continues. Subjects were to receive a 30 minutes intravenous (IV) infusion of rh IL-15 at doses of 3, 7, 10, 15, 20 or 25 mcg/kg daily for 12 doses. After dose limiting toxicities (DLTs) occurred in 2 of the first 5 subjects, the protocol was amended to

add a 1 and 0.3 mcg/kg dose level. Most subjects treated at the 3 mcg/kg exhibited a common spectrum of treatment related side effects of fevers, rigors, decreased blood pressure (BP) with the nadir characteristically 4 1/2 to 5 hours after treatment. Nausea/vomiting and brief asymptomatic periods of decreased oxygenation were seen in 3 subjects. The 3 subjects treated to date at the 1 mcg/kg dose level have not shown any significant changes in their BP or oxygenation during treatment. No responses by RECIST criteria have observed, but disease stabilization and regression of some marker lesions has occurred most notably in the first subject treated at the 1 mcg/kg dose level who had near complete disappearance of one of his marker pulmonary lesions. Analysis of the inflammatory cytokines IL-6, interferon gamma (IFN γ), IL-1β, tumor necrosis factor alpha (TNFα) showed maximal levels for all these cytokines at the 4 hour post treatment time point. The pharmacokinetic (PK) analysis of serum IL-15 concentration showed maximum levels (Cmax of 20,000 to 90,000 picograms/mL) at the 10 minute time point with a rapid decline in IL-15 and short half life (t $\frac{1}{2}$ alpha) of approximately 30 minutes and a terminal t $\frac{1}{2}$ (β phase) of 2 to 3 hours. No subject developed anti-IL-15 antibodies. Substantial increases in the absolute lymphocyte count (1.5 to 4×), CD8 (1.5 to 3×) and NK cells (4 to 10×) numbers were seen in all multidose subjects.

Key Words: Interleukin-15.

Phase I Clinical Trials in Cancer Vaccine Development Do Not Determine Dose Neither Based on Safety Nor on Biological Activity

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Phase I clinical trials are generally conducted to identify the MTD and the optimal efficacious dose in a traditional dose escalation design. However, this design may not apply to certain therapies such as cancer vaccines, given their unique mechanism of action and the profile of their clinical outcome. Based on these factors FDA is in the process of establishing the guidelines for therapeutic cancer vaccines development. Nevertheless, the question of whether the conventional design could still be used is a challenge. To address this question we analyzed the toxicity profile in 241 therapeutic cancer vaccine phase 1, phase 1/2, and pilot clinical trials conducted between 1990 and 2011. In trials that used dose escalation design we addressed the relationship between vaccine dose and toxicity and the ability of dose escalation to determine biologically active dose (BAD). Amongst 241 trials 62 grade 3/4 vaccine related systemic toxicities were reported in 4952 treated patients (1.25% toxicity rate). The number of grade 3/4 toxicities was also analyzed in relation to the number of the administered vaccines in 206 trials out of the 241 trials. Based on this analysis, 4024 patients received 21,835 vaccines and experienced 43 grade 3/4 systemic vaccine related toxicities (0.2% toxicity rate). In order to study the dose-toxicity relationship, we analyzed all trials that used dose escalation design (127/241 trials). Twenty-two of 127 dose escalating trials reported 40 grade 3/4 systemic vaccine related toxicities with only 10 toxicities occurred at the highest dose level. Interestingly, only 3 trials out of 127 dose escalating trials reported DLT. One out of 17 allogeneic vaccine trials reported a DLT related to the adjuvant, and two out of 37 bacterial vectors vaccine trials reported DLTs related to the vaccines. Furthermore, we analyzed the dose-immune response relationship in 106 trials that included immune response as a secondary endpoint out of the 127 dose escalating trials. We also included 10 additional trials designed to determine BAD by immune response as a primary endpoint. Out of 116 trials, only 2 trials showed a statistically significant dose immune response correlation (a peptide vaccine and an anti-idiotypic vaccine). Our analysis suggests that potential serious toxicity in vaccines therapy is extremely low and the toxicity or biologic activity do not correlate with dose levels based on the traditional dose escalation design. Accordingly, conventional dose escalation phase I design is not suitable for cancer vaccine studies with few exceptions. Alternative designs to determine vaccine dose should be developed. We will explore alternative designs to address BAD based on immunologic activity.

Key Words: Phase I, Cancer Vaccine, Toxicity.

UNCOUPLING NEGATIVE REGULATION IN THE TUMOR MICROENVIRONMENT

Hypoxia Determines CD137 Functional Expression on Tumor Infiltrating T Lymphocytes

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The response to hypoxia modulates the expression of multiple genes. The tumor microenvironment of transplanted and spontaneous mouse tumors is profoundly deprived of oxygen as confirmed by PET imaging. CD8 and CD4 tumor infiltrating T lympocytes of transplanted colon carcinomas, melanoma and spontaneous breast adenocarcinomas are CD137 positive, as opposed to their counterparts in tumor draining lymph nodes and spleen. Expression of CD137 on activated T lymphocytes is markedly enhanced by hypoxia and the prolyl hidroxilase inhibitor DMOG. Importantly, hypoxia does not up-regulate CD137 in inducible HIF-1alpha$^{-/-}$ T cells, and such HIF-1alpha deficient T cells remain CD137 negative even when becoming tumor infiltrating lymphocytes, in clear contrast with co-infiltrating HIF-1alpha- sufficient T cells. The fact that CD137 is selectively expressed on TILs was exploited to confine the effects of immunotherapy with agonist anti-CD137 mAb to the tumor tissue, thereby avoiding liver inflammation, while still permiting synergistic therapeutic effects with PD-L1/B7-H1 blockade.

Key Words: CD137 (4-1BB), micrenvironment, Hypoxia.

Inflammation-induced Immunological Soil and Prevention of Breast Cancer Brain Metastasis

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As therapies for systemic cancer improve and patients survive longer, the risk of brain metastases increases, yet we lack predictors of and effective therapies for brain metastases. Brain metastases of cancers, therefore, are major obstacles that must be overcome before cancers can be cured by any means. To study whether brain metastasis can be mediated by primary tumor-induced immunological pre-conditioning in the brain, we conducted the following studies using Balb/c mice and syngeneic 4T1 mammary adenocarcinoma cells. Mice bearing 4T1 tumors in the mammary pad present with accumulation of CD11b+Gr1+ myeloid cells, which are likely to be myeloid-derived suppressor cells (MDSCs), in the brains prior to any detectable tumor cell metastasis. We have also demonstrated that S100A8/A9, serum amyloid A (SAA)3 and CCL2, but not other common inflammatory cytokines, are up-regulated in the brain prior to metastatic spread of 4T1 cells. On the other hand, neither accumulations of MDSCs, nor up-regulation of S100A8/A9 and SAA3 is detected in the brains of mice bearing JC breast cancer cells which are not metastatic. Systemic treatment of 4T1-bearing mice with cyclooxygenase-2 (COX-2) inhibitor, celecoxib, reduces both CD11b+Gr1+ myeloid cell accumulation as well as expression levels of S100A8/A9, SAA3 and CCL2 in the pre-metastatic brains

of 4T1 bearing mice. Furthermore, celecoxib treatment starting on Day 2 following the 4T1 cell inoculation in the mammary pad significantly inhibits brain metastasis of 4T1 cells detected on Day 30. Systemic treatment with anti-CCL2 (C1142) or anti-Gr1 (RB6-8C5) monoclonal antibodies (mAb) also reduces CD11b+Gr1+ myeloid cell accumulation as well as expression levels of S100A8/A9 and SAA3 in the pre-metastatic brains. Our results strongly suggest, for the first time, that tumor-derived inflammatory responses, including the induction of CCL2, may be responsible for priming the "pre-metastatic soil" in the brain, thereby promote metastasis. Celecoxib, anti-CCL2 or anti-Gr1 mAb treatment may be used to prevent the formation of pre-metastatic immunological soil. In particular, celecoxib may be useful for the prevention of brain metastasis in patients with breast cancer. Further understanding of the mechanisms underlying the immunological soil will allow us to develop more effective strategies to prevent brain metastasis of breast cancer.

Key Words: brain metastasis, breast cancer, myeloid-derived suppressor cells (MDSCs).

Tissue Imaging Visualizes Lytic Deficits in Tumor-infiltrating CD8 T Lymphocytes in Situ and Combined With in Vitro Models Uncovers a Pivotal Role of the Tumor Microenvironment in Causing Cell Deviations Related to Tumor Immune Escape

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Human renal cell carcinoma is densely infiltrated with CD8 lymphocytes. Yet, tumors are not rejected suggesting that the tumor environment limits effector cell efficacy to control tumor growth. To unravel deficits multiparameter fluorescence staining and confocal microscopy was performed determining the status of lymphocytes in direct physical contact with malignant cells and under the control of the local microenvironment. All CD8 lymphocytes in tumor tissue (CD8-TILs) were found equipped with lytic granules, yet more than 60% lacked perforin and granzyme B. A special image analysis, modeled on the process of lytic granule exocytosis, was applied to identify CD8-TILs with active tumor recognition. Synaptic lytic granule topology, a pattern which was associated with lytic function in in vitro models, was seen among perforin-positive but not perforin-negative CD8-TILs. Although some TILs appeared actively engaged in tumor recognition there was no evidence that any CD8 cell was stimulated to produce IFNγ. Compared to control tissues with histopathologically verified lytic tissue destruction, RCC had significantly more perforin-negative and fewer functionally active CD8 T cells revealing a shift towards T cells with poor functional quality in RCC. Assessing the perforin status of CD8-TILs in relation to their spatial distribution within the tumor revealed a pivotal role of the tumor microenvironment: Most CD8-TILs that had extravasated into the tumor parenchyma were perforin-negative while those still residing in the tumor vasculature were largely perforin-positive. Thus, CD8 cells appear to arrive at the tumor site functionally proficient and become compromised within the tumor environment. Ex vivo analyses identified TCR signaling alterations in CD8-TILs compared to CD8 T cells of non-tumor kidney which were associated with failure to degranulate. These deviations were reversible concomitantly with gain in perforin and function. Application of in vitro models, which mimic conditions of solid tumors, identified tumor lactic acidosis as one potent factor abrogating TCR-stimulated IFNγ production by inhibition of p38 and JNK/c-Jun activation. Unidentified tumor cell-secreted factor(s) appear to cause loss of perforin and lytic function. The results reveal perforin paucity and inhibition of CD8-TIL function imparted by the tumor environment as important mechanisms of immune escape in RCC. Identified alterations indicate options to modulate the tumor environment allowing maintenance of CD8-TIL function which could enhance the efficacy of immunotherapy.

Key Words: Tumor milieu, cytotoxic lymphocytes, functional deficits.

Intracellular Expression of the Co-inhibitory Molecule, B7-H4, in NSCLC Cell Lines: Is it Real? What Does it do?

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The family of B7 ligands interact with the CD28 family of receptors on T cells to deliver either co-stimulatory or co-inhibitory signals. Shifting the balance of these signals during cancer immunotherapy likely impacts whether the developing anti-cancer immune response is tumor destructive or tolerized. B7-H4 is one of the B7 family members that has co-inhibitory activity and has been associated with poor immune responses and/or worse clinical outcome in melanoma, renal, ovarian, esophageal and gastric cancers, leading to the suggestion that it has a role in tumor immune evasion. Given our interest in NSCLC we evaluated a panel of 13 NSCLC cell lines for expression of B7-H4 and found only one cell line over expressed the gene. The other cell lines exhibited a similar expression level as RNA from normal lung. We next analyzed surface expression by flow cytometry and found 0/13 cell lines with detectable levels of the protein. Since a number of recent reports have identified intracellular expression of B7-H4, we stained for intracellular levels of B7-H4 and found that 100% (10/10) of the cell lines were strongly positive for B7-H4 expression. Current efforts are focused on confirming expression findings and evaluating whether these cell lines secrete B7-H4.

Key Words: NSCLC, B7-H4 Co-inhibitory molecule, immune escape.

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