Cancer vaccines

113 POSTER

Phase II trial to assess the activity of MVA5T4 (Trovax®) alone versus MVA5T4 plus granulocyte macrophage colony-stimulating factor (GM-CSF) in patients (pts) with progressive hormone refractory prostate cancer (HRPC)

R. Amato[1], M. Karediy[1], A. Cao[1], M. Khan[1], S. Naylor[2], M. McDonald[2], R. Harrop[2], W. Shingler[2], P. Chikoti[2], N. Drury[2]. [1]*The Methodist Hospital Research Institute, Genitourinary Oncology Program, Houston, USA;* [2]*Oxford Biomedia (UK) Ltd., Oxford, UK*

Background: MVA 5T4 consists of the highly attenuated modified vaccinia Ankara virus containing the gene encoding the human tumour associated antigen (TAA) 5T4 under regulatory control of a modified promoter, mH5 More than 85% of prostate cancers overexpress the 5T4 antigen. GM-CSF is involved in the enhancement of T-cell priming via effects on dendritic cells. An immunotherapy approach was evaluated using MVA 5T4 alone or in combination with GM-CSF to look at the comparative potency of these approaches to elicit an immune response and to further determine the impact of such a response on signs of clinical benefit as defined by PSA reduction and delay in time to progression (TTP). The humoral and/or cellular immune response to 5T4 will be correlated to clinical outcome.
Methods: Eligibility included HRPC pts with progressive disease based on at least 1 of the following; (a) 3 consecutive rising PSA levels, or (b) new or progressive measurable disease, or (c) new or progressive metastatic lesions on bone scan; serum testosterone level ($\leqslant$50 μg/mL), withdrawal from anti-androgen therapy, any prior therapy regimen, Karnofsky performance status (KPS) $\geqslant$60% and adequate physiologic parameters. The dosage regimen of MVA 5T4 consisted of intramuscular injections (5×10^8 pfu)on day 1 of week 1, 3, 6, 9, 17, 25, 33 and 41. GM-CSF is given at 250 μg/m^2 (maximum 500 μg) 14 days on, 14 days off by subcutaneous injection. Routine laboratory, PSA and imaging studies will occur every 8 weeks.
Results: 21 pts have been enrolled as of this review. Median age 70 (50−94) years. All pts received at least 2 prior therapies, including chemotherapy. Base-line PSA was 75.0 (4.0−2661.9). 1 pt was PSA only, 5 pts were PSA with bone involvement, 4 were PSA with measurable disease and 11 were PSA with bone involvment and measurable disease. All pts continue to receive therapy. Adverse events were assessed and included; grade 1 fever and bone pain. The clinical and immunologic analysis is in progress.
Conclusion: MVA 5T4 with or without GM-CSF is well tolerated in this group of patients. Reduction in PSA has been noted in 5/5 of the first patients treated with TroVax plus GM-CSF after only 2 weeks. The immune response along with clinical outcome will be presented. The study is still continuing to accrue.

114 POSTER

Effective inhibition of the EGF/EGFR binding by anti-EGF antibodies increased survival of advanced NSCLC patients treated with the EGF cancer vaccine

G. Beatriz[1], N. Elia[2], T. Ana[3], L. Idrissa[1], M. Rocío[1], V. Carmen[1], G. Gisela[1], M. Zaima[1], L. Agustín[1], C. Tania[1]. [1]*Center of Molecular Immunology, Clinical Trials, Havana, Cuba;* [2]*Ameijeiras Hospital, Chemotherapy, Havana, Cuba;* [3]*Celestino Hernández Hospital, Oncology, Villa Clara, Cuba*

Background: Previous studies have indicated that Epidermal Growth Factor (EGF) might be a suitable immunotherapeutic target in non-small cell lung cancer (NSCLC). Our approach consisted in active immunotherapy with the Epidermal Growth Factor. The aim of the present study was to characterize the humoral immune response and its relations with the clinical outcome of the treated patients.
Methods: Seventy-four NSCLC patients previously treated with first line chemotherapy were randomized to receive the EGF vaccine or best supportive care. Patients were vaccinated weekly for 4 weeks and then monthly. We choose 42 patients (26 vaccinated and 16 controls) to evaluate seric EGF concentration [EGF] and anti-EGF antibody titers. Then, we determined the capacity of the specific antibodies to inhibit the EGFR activation and the binding between EGF/EGFR.
Results: Eighty-four (84 %) of the vaccinated patients showed seroconvertion, while 56% of controls showed a 2 fold increase of the natural anti-EGF antibody titers. None of the controls developed a good antibody response (GAR) while 65% of vaccinated subjects did. In GAR patients, seric [EGF] was reduced below 168 pg/ml, which represents half of the mean [EGF] of healthy donors. In 58 % of vaccinated patients, the post-immune sera showed an EGF/EGFR binding inhibition capacity higher than 18% (range 18.9−60%). The mean EGF/EGFR inhibition percent of controls was significantly lower than the one from the vaccinated subjects at the same time points. In 46% of the vaccinated patients, post-immune sera inhibited more than 15% (range 16.31−62.5%) the EGFR phosphorylation induced by EGF as compared to day 0. Control patients showed a phosphorylation inhibition capacity lower than 10.4% at the same time points. A significant increase in survival was obtained for GAR patients in comparison to poor responders and control patients. A high correlation between anti-EGF antibody titers and EGFR phosphorylation inhibition was found. There was a significant increase of median survival for vaccinated patients in whom inhibition percent of EGF/EGFR binding was higher than 18% (13.17 months) as compared to those who did not achieve the referred inhibition capacity (5.63 months).
Conclusions: Immunization with EGF vaccine induced specific and neutralizing anti-EGF antibodies capable to inhibit EGFR phosphorylation. There is a significant correlation between the quality of anti-EGF antibodies and survival of advanced NSCLC.

115 POSTER

Enhanced growth inhibition of HER-2/neu overexpressing tumor cells by combining HER-2/neu specific polyclonal antibodies and other HER-2/neu targeting treatments

C. Gérard[1], N. Baudson[1], T. Ory[1], B. Keith[2], T. Gilmer[2], V.G. Brichard[1]. [1]*GlaxoSmithKline Biologicals, Rixensart, Belgium;* [2]*GlaxoSmithKline, Research Triangle Park, NC, USA*

Background: The HER-2/neu oncogene is overexpressed in 30% of breast cancer and other malignancies. As a transmembrane tyrosine kinase receptor this antigen can be targeted by different therapeutic approaches including HER-2/neu specific immunotherapy, passive transfer of HER-2/neu specific monoclonal antibody such as trastuzumab or of small molecules such as lapatinib, a dual EGFR(ErbB1)/HER-2/neu(ErbB2) tyrosine kinase inhibitor. We have developed a HER-2/neu antigen specific cancer immunotherapy (ASCI) that is based on the use of a recombinant HER-2/neu protein formulated in a strong GSK proprietary adjuvant. The HER-2/neu-ASCI has been shown previously to induce HER-2/neu specific T-cells and polyclonal antibodies with functional activity in animal models. The present studies illustrate that combination of the HER-2/neu specific polyclonal antibodies with the dual tyrosine kinase inhibitor lapatinib or with the humanized monoclonal antibody trastuzumab lead to enhanced growth inhibition of human tumor cells over-expressing HER-2/neu.
Methods: *In vitro* – HER-2/neu overexpressing cell lines were incubated with sub-optimal concentration of HER-2/neu specific polyclonal antibodies (pAb) and either lapatinib or trastuzumab. The % of growth inhibition was measured by 3H-thymidine incorporation. *In vivo* – BT474 tumor bearing SCID mice were treated with lapatinib or HER-2/neu specific polyclonal antibodies, or a combination of both. *In vivo* tumor growth was followed overtime.
Results: The *in vitro* combination of HER-2/neu specific polyclonal antibodies and lapatinib had at least an additive growth inhibitory effect on HER-2/neu overexpressing cell lines. Moreover, the combination of HER-2/neu specific polyclonal antibodies and trastuzumab also lead to improved growth inhibition, suggesting that inhibitory antibodies specific for other epitopes than the one recognized by trastuzumab have been generated by the immunization with the formulated HER-2/neu protein. In vivo, the administration of HER-2/neu specific polyclonal antibodies together with lapatinib was shown to enhance inhibition of BT474 tumor cell growth in mice.
Conclusions: Taken together these data provide evidence that combining different therapeutic approaches targeting the same HER-2/neu antigen could lead to a better control of the tumor growth which may translate into clinical benefit for the patient.

116 POSTER

A recombinant HER2 protein evaluated for cancer immunotherapy: induction of specific antibodies and T-cells

S.A. Limentani[1], M. Campone[2], T. Dorval[3], G. Curigliano[4], S. White[5], R. De Boer[6], J. Canon[7], T. Bachelot[8], F. Cormont[9], J. Louahed[9]. [1]*Blumenthal Cancer Center, Charlotte, NC, USA;* [2]*Centre René Ganducheau, Nantes, France;* [3]*Institut Curie, Paris, France;* [4]*Istituto Europeo di Oncologia, Milan, Italy;* [5]*Austin Hospital, Heidelberg, Australia;* [6]*Royal Melbourne Hospital, Parkville, Vic, Australia;* [7]*Centre Hospitalier Notre-Dame et Reine Fabiola, Charleroi, Belgium;* [8]*Centre Leon Bérard, Lyon, France;* [9]*GlaxoSmithKline Biologicals, Rixensart, Belgium*

Background: We designed an Antigen Specific Cancer Immunotherapeutic (ASCI) to induce a polyclonal antibody response and T-cells able to recognize HER2 epitopes.
Methods: The HER2 ASCI is a recombinant HER2 protein, including its extra and part of its intra-cellular domains (ECD/ICD), combined with

the potent immunological adjuvant AS15 (GlaxoSmithKline proprietary). Consecutive cohorts of patients with Stage II/III breast cancer received 20 (Cohort 1), 100 (Cohort 2) or 500 μg HER2 ASCI (Cohort 3) in the adjuvant setting. Treatment comprised of 6 injections over 14 weeks. Recall injections were given on weeks 34 and 38 in Cohort 3. The trial was extended to include an alternative immunization schedule (Cohort 4) 500 μg on days 0, 28 and 98. In an ongoing trial, patients with metastatic BC are receiving the 500 μg HER2 ASCI, and being assessed for clinical and immunological activity.
Results: The HER2 ASCI treatment was well tolerated, with no symptomatic cardiotoxicity. Increased doses showed no increase in the number or severity of adverse events. The induction of antibodies against ECD was dose-dependent, with 2/12, 9/15 and 14/16 immune responders in Cohorts 1, 2 and 3 after 6 immunizations, and 11/16 responders after 3 immunizations in Cohort 4. The anti-ECD antibody response of patients in Cohort 3 follows two main kinetic profiles. 7/14 patients show a maintained and predominant anti IgG antibody response after 4 immunizations. 6/14 patients have an antibody titer which drops to baseline level after the 4th immunization and have a poor switch to IgG. The alternative immunization schedule (Cohort 4) does not improve the immune response and switch to IgG. Preliminarly data in breast cancer, however, suggest that the anti-ECD is maintained during the immunization schedule. The anti-ECD antibodies in 11/14 patients (Cohort 3) bound HER2 overexpressing breast cancer cell lines. In sera from 2 patients tested so far, the gene-expression showed 70 and 20% similarity with that of Trastuzumab. An anti-ECD or ICD specific T-cell response was detected in about 50% of patients (Cohort 3).
Conclusions: The HER2 ASCI was well tolerated without major toxicity and induced a specific T-cell response and anti-ECD Ab against HER2. The alternative immunization schedule does not improve the immune response. The data of the metastatic study suggest that the Ab response is maintained over time of immunizations. Data on clinical activity are currently been evaluated.

117 POSTER

Activity of MVA 5T4 alone or in combination with either Interleukin-2 (IL-2), Interferon-alpha (IFN), or Sunitinib in patients (Pts) with Metastatic Renal Cell Cancer (MRCC)

R. Amato[1], A. Gary[1], A. Cao[1], J. Willis[1], M. McDonald[2], S. Naylor[2], R. Harrop[2], W. Shingler[2], P. Chikoti[2], N. Drury[2]. *[1]The Methodist Hospital Research Institute, Genitourinary Oncology, Houston, Texas, USA; [2]Oxford Biomedica(UK)Ltd., Oxford, UK*

Background: MVA 5T4 consists of the highly attenuated modified vaccinia Ankara virus containing the gene encoding the human tumour associated antigen (TAA) 5T4 under regulatory control of a modified promoter, mH5. 90% or more of RCCs overexpress the 5T4 antigen. A series of studies was conducted to evaluate the effectiveness of MVA 5T4 as a single agent or in combination with other agents in overcoming tolerance and potentiating an immune response to the 5T4 antigen.Humoral and cellular immune responses to 5T4 will be correlated to clinical outcome.
Methods: Eligibility included confirmed pathologic diagnosis of clear cell or papillary RCC, progressive measurable metastases, any prior therapy, adequate physiologic parameters, Karnofsky performance status (KPS) $\geqslant$ 80%, and no active CNS involvement. A regimen of MVA 5T4 alone or in combination with IFN or Sunitinib consists of an intramuscular injection of 5×10^8 pfu on day 1 of week 1, 3, 6, 9, 17, 25, 33 and 41. The standard dose of Sunitinib is used and the dose of IFN is 6×10^6 IU 3 times a week. MVA 5T4 in combination with IL-2 was given 14 days prior to the first cycle of IL-2 and repeated on days 0 and 28 of the first cycle. MVA 5T4 was repeated on day one of each 8 week IL-2 cycle. The schedule of subcutaneous IL-2 consists of an initial dose of 250,000 U/kg/dose for 5 days in week 1 followed by 125,000 U/kg/dose for 5 days in weeks 2–6, followed by a 2 week recovery.
Results: 16 patients all received MVA 5T4 with low dose IL-2 or IFN. 10 male/6 female, median age 54 (24−65) years. 6 pts had clear cell; 6 papillary; 3 mixed clear cell; and 1 mixed papillary. All pts had progressive MRCC. 10 pts had a KPS of 90%, and 6, 80%. Sites of disease included; lung, nodal, liver, bone, adrenal, and renal fossa. 4 pts had 1 metastatic site, 4 pts had 2 and the remaining 8 patients had 3 or more metastatic sites. 9 pts continue to receive therapy. 2 pts (both clear cell RCC) developed partial responses, 5 pts/stable for 3+ months and 4 pts are too early to be staged at this time. Median duration of therapy is 3.5+ months (1+−8+). No MVA 5T4 adverse related events have been reported. The immunologic analysis is in progress.
Conclusion: MVA 5T4 has promising anti-tumor activity demonstrated by objective responses and prolonged TTP. MVA 5T4 is well tolerated with each regimen. The immune responses will be presented along with the clinical outcome. The trials continue to accrue.

118 POSTER

Comprehensive preclinical model evaluating a protein-based MAGE-A3 specific cancer immunotherapy to fight against MAGE-A3 expressing tumors

C. Gérard, N. Baudson, T. Ory, R. Piccininno, V.G. Brichard. *GlaxoSmithKline Biologicals, Rixensart, Belgium*

Background: MAGE-A3 belongs to the family of tumor-specific antigens. This antigen represents an excellent target for immunotherapy. Its expression is shared by different tumor types. For some of these tumor types it has been shown that the MAGE-A3 expression is an unfavorable predictor for survival. The development of a MAGE-A3 antigen specific cancer immunotherapy (ASCI) able to induce strong T-cell responses would be a very targeted therapy and could provide significant benefits to a large number of cancer patients.
Methods: In these studies we used a murine tumor model genetically modified to express MAGE-A3. We characterized the immune response and anti-tumor effects induced by repeated injections of a MAGE-A3 recombinant protein formulated in a strong GSK proprietary adjuvant under different conditions (mice depleted of CD4 and/or CD8; IFNγ knock-out mice).
Results: The experiments conducted in mice demonstrated that the MAGE-A3 protein was weakly immunogenic by itself and that the addition of a strong adjuvant was required during the whole immunization schedule to induce a comprehensive immune response. This response included 1) the generation of MAGE-A3 specific antibodies with a TH1 isotypic profile, and 2) the induction of MAGE-A3 specific CD4 and CD8 T-cells that were able to proliferate *in vitro* in response to the antigen and to produce cytokines (IL2, IFNγ, IFNα). The immune response induced was systemic as it could be identified in all lymphoid organs and in the blood. Moreover, immunized mice were specifically protected against a tumor challenge with MAGE-A3 expressing tumor cells even when the challenge was applied long after the last immunization (2 months). Immunized mice remained tumor free for several months and they still resisted to a second challenge at 5 months after the first one, indicating that a long term immune memory has been generated. Experiments with mice depleted of CD4 and/or CD8 T-cells confirmed the importance of these cells in the protection process. In addition, experiments performed in IFNγ knock-out mice further emphasized the critical role of this cytokine in the effector mechanism.
Conclusions: Our preclinical experiments support the choice to use a strong GSK proprietary adjuvant in combination with the MAGE-A3 protein for future clinical development. Indeed, this immunotherapy consistently induced a comprehensive immune response and provided very good protection of mice against tumor challenge.

119 POSTER

Prostate derived Ets factor protein is frequently over expressed in breast and prostate tumors and is a novel target in these cancers.

A. Sood[1], R. Saxena[2], M. Desouki[3], J. Groth[2], K. Rodabaugh[4], C. Cheewakriang-Krai[1], L. Peng[1], J. Geradts[3]. *[1]Roswell Park Cancer Institute, Immunology, Buffalo, USA; [2]Roswell Park Cancer Institute, Pathology, Buffalo, USA; [3]Duke University Medical Center, Pathology, Durham, USA; [4]Roswell Park Cancer Institute, Gynecologic Oncology, Buffalo, USA*

Background: PDEF (prostate derived Ets factor) mRNA was previously reported to be over expressed in human breast tumors and shows highly restricted expression in normal human tissues. However, there is limited knowledge about the expression of PDEF protein in human tumors. The purpose of this study was to determine PDEF protein expression in various stages of breast and prostate neoplasias.
Materials and Methods: A new rabbit polyclonal antibody to PDEF was prepared and reacted with tissue microarrays (TMAs) consisting of 1 mm cores of 62 benign breast tissues (from cancer cases), 46 *in situ* carcinomas, 65 invasive ductal carcinomas and 39 invasive lobular carcinomas. The antibody was also similarly reacted with TMAs from 290 benign prostate tissues, 109 PIN (prostate intraepithelial neoplasia) samples and 230 prostate carcinomas from the same cohort of prostate cancer patients. The average nuclear staining intensity and the percentage of stained epithelial cells were evaluated, a combined score was calculated and a threshold for over expression was set.
Results: Relative over expression of PDEF was identified in 11 of 62 (18%) benign breast tissues, 23 of 46 (50%) DCIS lesions, 30 of 65 (46%) invasive ductal carcinomas and 20 of 39 (51%) invasive lobular carcinomas. Further, of the 9 matched samples of benign breast and tumor tissues from same patients, 8 showed an increase in the number and/or intensity of PDEF expressing epithelial cells in tumors. Relative over expression of PDEF was also identified in 79 of 290 (27%) benign prostate tissues, 36 of 109 (33%) PIN samples, 92 of 230 (40%) prostate carcinomas. Importantly,