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.

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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)

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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

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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.

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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,

comparison of the matching samples of cancer versus benign and cancer versus PIN showed that in 68% and 70% cases respectively, increased expression of PDEF was seen in cancer in comparison to the matching benign or PIN tissue.
Conclusions: This is the first report of the characteristics of PDEF protein expression in various stages of breast and prostate neoplasias. The data show frequent increase in the number and/or intensity of PDEF expressing epithelial cells in progression from benign breast or prostate tissue to carcinoma. These results together with: i) limited expression of PDEF in normal tissues; ii) its promotion of epithelial cell motility and invasiveness *in vitro* and tumorigenicity *in vivo*; and iii) immunogenicity of its mouse homologue support PDEF as a novel target in breast and prostate cancers.

120 POSTER

Transduction of tumor cell lines with adenoviral vectors expressing TLR ligands and scFv anti-IL-10 activates dendritic cell maturation and IL-12 secretion in the presence of IL-10

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In cancer patients and in mouse models, tumor progression has been associated with the accumulation of immature-type dendritic cells (DCs), known to be poor stimulators of Th1 cell responses and to participate in cancer-induced immunosuppression. The presence of "immunosuppressive" cytokines such as IL-10, secreted or induced by many types of tumors (melanomas, lung cancer, breast or cervical cancer, hepatocellular or renal carcinomas, lymphomas) contributes to maintain DCs in an immature state and profoundly affects their ability to produce IL-12 or to stimulate T cell-responses. In order to provide "danger" signals to DCs within tumors, we generated adenoviral (Ad) vectors driving the expression of TLR ligands in transduced cells. Thus, the outer membrane protein from Klebsiella pneumoniae (P40/OmpA) or Flagellin from Listeria monocytogenes, known to bind respectively TLR2 and TLR5, were expressed using Ad vectors in human tumor cell lines. Coculture with Ad-PAMP-transduced tumor cells was indeed able to induce the phenotypic maturation of human monocyte-derived DCs. With the goal of blocking the effect of IL-10 in the tumor milieu, we have constructed an Ad vector encoding a single-chain variable region (scFv) isolated from an anti-IL-10 blocking antibody. We show that in the presence of tumor cells transduced with the Ad-scFv vector, IL-10 is efficiently neutralized and LPS-stimulated human monocyte-derived DCs secrete increased levels of IL-12p70. Combined with Ad-PAMPs, Ad-scFv anti-IL-10 enhances the phenotypic maturation of DCs in the presence of IL-10. The combination of Ad-PAMP + Ad-scFv anti-IL-10 also restores IL-12p70 production by DCs in the presence of LPS and IL-10. The combination of an Ad-PAMP with Ad-scFv anti-IL-10 administered intratumorally may thus represent a promising strategy to reverse cancer-induced suppression of DC functions.

121 POSTER

Isolation and characterisation of anti-idiotypic scFv antibody fragments and llama VHH domains used as a surrogate tumour antigen to elicit an anti-HER-2 humoral response in mice

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HER-2 is a tumour antigen that is over-expressed in human breast tumours. Among the vaccine strategies developed to overcome immune tolerance to self-proteins, vaccination with anti-idiotypic (anti-Id) antibodies has been described as a promising approach for treatment of several malignant diseases. To develop an active immunotherapy for cancer patients positive for HER-2, we produced small molecular recognition units of Ab2beta type (scFv 40 and 69) and a single domain VHH antibody (VHH 1HE), specific for trastuzumab F(ab')$_2$ fragments (Ab1), a humanised anti-HER-2 monoclonal antibody. Using competitive ELISA and Biacore biosensor analysis, we showed that anti-Id scFv 40, scFv 69, and VHH 1HE could inhibit HER-2 binding to trastuzumab. Following vaccination of BALB/c mice with these molecules, Ab3 polyclonal antibodies, and among them Ab1' antibodies able to bind HER-2, were detected in the sera of the immunised mice. These results demonstrate that these anti-Id antibodies could act as a surrogate antigen for HER-2. The present study strongly suggests that the novel 30 kDa human mini-antibody and the 15kD llama VHH single domain could be used as an anti-idiotype-based vaccine formulation to induce an effective humoral response in patients bearing HER-2 positive tumours.

Chemoprevention

122 POSTER

An inhibitor of acetyl polyamine oxidase specifically blocks androgen induced oxidative stress and prevents occurrence of prostate cancer in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP)

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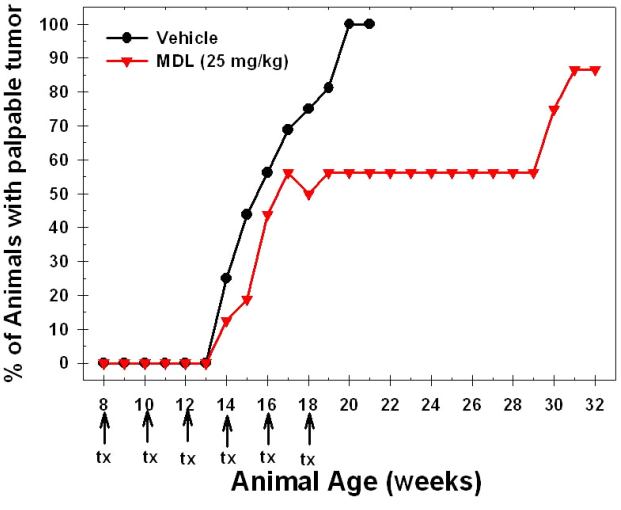
Background: Direct evidence linking oxidative stress with an increase in prostate tumor development both in human and in TRAMP animals has been reported. Reducing the oxidative stress in the prostate can be an effective strategy in preventing occurrence, recurrence and progression of prostate cancer. We have previously demonstrated that androgen treatment increases reactive oxygen species (ROS) levels in androgen dependent prostate cancer cells. The biochemical pathway involved in androgen induced ROS production in the prostate cells, however, remained unknown.
Method: DNA microarray, qRT-PCR, cell culture and cellular polyamine level determination by standard HPLC were carried out using androgen dependent LNCaP human prostate cancer cells grown with or without androgen analog R1881. Prostate tumor development in TRAMP animals was determined by tumor palpation and confirmed by micro-PET and micro-CT imaging.
Results: Our DNA microarray data validated by qRT-PCR results and confirmed by cellular polyamine and acetyl polyamine level determination demonstrate that R1881 treatment induces an overexpression of spermidine/spermine acetyl transferase (SSAT) mRNA by over fifty fold with a concomitant increase in SSAT activity in androgen dependent LNCaP human prostate cancer cells. Catabolism of polyamines spermidine and spermine produced in large excess by both normal and malignant prostate cells occurs through acetylation by SSAT followed by oxidation by acetyl polyamine oxidase (APAO) that generates H_2O_2 (ROS). Downregulation of SSAT gene expression by siRNA blocks R1881 induced peroxide production in LNCaP cells.



MDL significantly delays prostate tumor development in TRAMP mice.

We have identified a specific APAO inhibitor N1,N4-bis(2,3-butadienyl)-1,4-butanediamine (MDL) that blocks R1881 induced ROS production in LNCaP cells. MDL given 25 mg/kg i.p. once in two weeks is well tolerated by TRAMP animals without any overt sign of systemic toxicity. At this dose, MDL completely inhibits APAO activity in mice and reduced oxidative stress in the prostate *in vivo*. MDL treatment significantly increased overall survival and delayed time to prostate tumor development by over ten weeks.
Conclusion: Our results demonstrate that polyamine oxidation is one of the major causes of androgen induced oxidative stress in prostate cancer cells. To the best of our knowledge, this is the first report of an enzyme inhibitor MDL that blocks androgen induced oxidative stress specifically in the prostate and prevents spontaneous prostate tumor development.