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)

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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,