IL-12. In untreated mice, the tumor growth started 120 days after study initiation. In contrast, in mice immunized with the multi-epitope vaccine the tumor free interval was extended up to 140–190 days; the tumor free interval in mice immunized with the peptides and IL-12 was prolonged even up to 235 days. Once tumors developed, those mice immunized with peptides+IL-12 showed a significantly slower tumor progression than mice not or sham immunized. Characterization of the immune responses revealed that mice immunized with peptides+IL-12 displayed higher IgG2a levels in serum and Th1 biased immune responses (IFN γ) *in vitro*.
From our data, we conclude that immunization with a multi-epitope vaccine in conjunction with IL-12 is very effective in preventing progression of Her-2/neu overexpressing tumors. Such a vaccine could be used in humans together with chemotherapy and/or for prevention of metastases.

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Immunization with genetic vectors expressing rhesus CEA efficiently breaks immune tolerance in mice and rhesus monkeys

L. Aurisicchio, C. Mennuni, F. Calvaruso, M. Nuzzo, P. Giannetti, B. Cipriani, F. Palombo, P. Monaci, G. Ciliberto, N. La Monica. *IRBM "P. Angeletti", Molecular and Cellular Biology, Pomezia, Rome, Italy*

Background: CEA is a 180KDa glycoprotein over-expressed in a high percentage of adenocarcinomas, particularly those of the colon, pancreas, breast and lung. For this reason, it is currently under evaluation in clinical trials as target for immunotherapy in the treatment of colorectal cancer.
Materials and Methods: To demonstrate that genetic vaccination with vectors expressing this tumour antigen is capable of specifically breaking immunotolerance in non-human primates, it is necessary to use the equivalent of human CEA. Since the rhesus monkey (*macaca mulatta*) homologue of this human tumour associated antigen was not available, we have identified and cloned rhesus CEA (rhCEA) from colon tissue samples. rhCEA is an open reading frame of 2118 nucleotides encoding for a 705 aa polypeptide with 78.9% homology to human CEACAM-5 protein.
Results: Vaccination protocols using rhCEA expressing vectors were designed both for mice and rhesus monkeys.. To demonstrate the capability of xenogeneic vaccination to elicit an immune response against CEA as self-antigen in this model, we immunized CEA. Tg mice with vectors encoding either human (homogeneic) or rhesus CEA (xenogeneic).
After treatment of mice with DNA followed by EGT (Electro Gene Transfer) and adenovirus boosting, cross-reactive antibodies against human CEA protein were measured only in rhesus CEA immunized groups. Importantly, cellular immune-response against human CEA was observed upon immunization with rhesus CEA both in wild type and transgenic mice.
To further increase the level of antigen expression, we have constructed a synthetic codon usage optimized rhCEA cDNA (rhCEAopt). *In vitro* studies showed 10–50 fold greater protein levels than a similar vector carrying the native cDNA. Similarly, intramuscular injection of a DNA vector followed by EGT or Adenovirus expressing rhCEAopt in CEA. Tg mice resulted in greater protein levels than those detected upon injection of vectors encoding for rhCEA. Mice immunized with plasmid/ adenovirus vector mixed modality, both containing the cDNAopt showed strong cross reactive human CEA-specific antibody response, 300-fold higher than hCEA containing vectors. Cell mediated responses were two- and three-fold higher against rhesus or human protein, respectively, than using the vectors containing the native rhCEA.
To assess the efficiency of immunization of rhesus macaques with rhesus CEA, we injected vectors encoding for rhCEA or rhCEAopt in twelve monkeys. Both Ad vectors alone or in combination with DNA were efficient in breaking immune tolerance to CEA in immunized rhesus monkeys and maintain over time elicited immune response.
Conclusions: Our data show that use of rhesus CEA and development of modified expression cassettes that result in increased potency of Adenovirus, plasmid DNA and other gene delivery vaccine approaches may have significant impact on vaccine development against neoplastic malignancies expressing CEA.
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Synergistic antitumor activity of interleukin 23 and interleukin 2

C. Lo[1], P. Wu[1], S. Tang[2], M. Tao[1]. [1]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan;* [2]*Graduate Institute of Medical Technology, National Taiwan University, Taipei, Taiwan*

The recently discovered IL-23 shared with IL-12 similar heterodimeric structures and overlapping but distinct functions in the regulation of both innate and adaptive immunity. IL-12 has been shown to confer potent antitumor activity in a variety of murine tumor models. In our previous study, we showed that IL-23 also possessed potent antitumor activity. CT26 colon adenocarcinoma cells transduced with retroviruses carrying a single-chain IL-23 gene (CT26/IL-23) grew progressively until day 26 to an average size of 521 ± 333 mm^3 and then tumors started to regress in most animals, resulting in a final 70% rate of complete tumor rejection. In the present study, we seek a possible cooperative antitumor effect of IL-23 and IL-2. CT26 cells engineered to secrete both IL-23 and IL-2 (CT26/IL-23/IL-2) produced only a transient tumor growth, followed by complete rejection in all animals. Most significantly, transduction of both IL-23 and IL-2 resulted in significant reduction of lung tumor metastasis and led to 60% of mice survived the challenge, while all animals challenged i.v. with IL-23- or IL-2-transduced CT26 cells eventually died of lung metastasis. *In vivo* depletion experiment showed that rejection of CT26/IL-23/IL-2 tumor cells required both $CD4^+$ and $CD8^+$ T lymphocytes. Immunohistochemical analysis revealed tumor moderate infiltration of $CD4^+$ and $CD8^+$ T cells, and abundant infiltration of granulocytes ($Gr\text{-}1^+$) and macrophages ($Mac\text{-}1^+$) when tumors were in regression. We are currently investigating whether granulocytes and macrophages play a role in the IL-23/IL-2-mediating antitumor activity.

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Strong Melan-A/MART-1 specific CD8+ T cell responses to peptide vaccination in young melanoma patients

D. Speiser[1], D. Lienard[3], V. Rubio-Godoy[1], E. Devevre[1], A.M. Krieg[2], J.-C. Cerottini[1], P. Romero[1]. [1]*Ludwig Institute for Cancer Research, Div. of Clinical Onco-Immunology, Lausanne, Switzerland;* [2]*Coley Pharmaceutical Group, Wellesley, MA, USA;* [3]*Multidisciplinary Oncology Center, Univ. Hospital CHUV, Lausanne, Switzerland*

Tumor vaccines aim to induce strong specific T cell activation in vivo, which may well result in enhanced immune protection. It remains poorly understood why T cell responses are detectable in some but not all patients. We have previously shown that tumor-driven CD8+ T cell pre-activation is one of the factors associated with increased T cell responsiveness to peptide vaccination. In search for further parameters, we analyzed whether patient age may play a role. Eight stage III/IV melanoma patients (34–75 years old) were treated with four monthly low dose vaccinations with CpG oligodeoxynucleotide 7909, mixed with Melan-A analog peptide and Incomplete Freund's Adjuvant. We used fluorescent HLA-A2/Melan-A multimers (tetramers) to measure T cell frequency ex vivo in circulating blood by flow cytometry. High percentages (between 0.07 and 3.42%) of Melan-A peptide specific CD8+ T cells were found after vaccination, revealing strong T cell responses in all eight patients. Interestingly, we found a statistically significant (P <0.01) inverse correlation between T cell responses and patient age. Thus, besides tumor-driven T cell pre-activation, young patient age is an additional parameter predicting T cell responsiveness to vaccination with tumor peptides.

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First clinical evidences of antigen spreading in metastatic melanoma patients treated with a NGcGM3/VSSP/Montanide ISA 51 vaccine: A Phase I/IIb study

G. Saurez[1], M. Osorio[2], E. Rodríguez[2], A. Carr[3], L. Anazagasti[4], M.C. Arango[5], A. Torrella[5], J. González[6], R. Pérez[3], L. Fernández[7]. [1]*Center of Molecular Immunology, Clinical Immunology, Havana City, Cuba;* [2]*National Institute of Oncology and Radiobiology, Experimental Chemotherapy, Havana City, Cuba;* [3]*Center of Molecular Immunology, Research and Development Division, Havana City, Cuba;* [4]*National Institute of Oncology and Radiobiology, Research Division, Havana City, Cuba;* [5]*National Institute of Oncology and Radiobiology, Immunology Department, Havana City, Cuba;* [6]*National Institute of Oncology and Radiobiology, Peripheral Tumors Service, Havana City, Cuba;* [7]*Center of Molecular Immunology, Vaccine Department, Havana City, Cuba*

Background: N-glycolyl GM3 ganglioside immunodominant epitope is expressed on human breast and melanoma tumours but absent from normal melanocytes. This unique feature renders this antigen very attractive for immunotherapy. NGcGM3 was non-covalently incorporated in the natural outer membrane vesicles of *Neisseria meningitidis* to form very small sized proteoliposomes (VSSP) and emulsified with Montanide ISA 51. In previous studies the immunogenicity and safety of this vaccine have been documented but in breast cancer patients. With this phase I/IIb study we intended to evaluate, in metastatic melanoma patients, the immunogenicity and safety of the preparation at two different dose levels. Patient's monitoring for clinical responses was also planned.
Methods: Twelve and nine metastatic melanoma patients received 0.2 or 0.4 mg of the vaccine in each of the 9 immunisations, respectively. The first 5 IM doses (induction phase) at two weeks intervals, while the remaining