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Epidermal Growth Factor (EGF) based cancer vaccine for NSCLC: immunological evaluation and impact on the EGFR signal transduction cascade

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Background: In Non Small Cell Lung Cancer (NSCLC), Epidermal Growth Factor Receptor (EGFR) expression correlates with reduced survival. Our approach consists in vaccination with one of the key EGF-R ligands, the Epidermal Growth Factor (EGF), coupled to a carrier protein in an attempt to induce a specific anti-EGF antibody response. This active immunotherapeutic procedure has been evaluated in small phase I trials intended to optimize vaccine dose and schedule.
Material and Methods: Two clinical trials using different vaccine schemes were conducted. Eighty advanced NSCLC patients were randomized to get vaccine or best supportive care (control arm) after chemotherapy. In a second study, 20 patients received 2 vaccine doses before first line chemotherapy and restarted vaccination after 4 chemotherapy cycles.
Results: Vaccination was safe. Most common adverse events consisted on grade 1 or 2 chills, headache and fever. The vaccine demonstrated to be immunogenic. More than 50% of vaccinated patients showed seroconversion in the chemotherapy-vaccine (ChV) trial and 78% of the patients vaccinated in the vaccine-chemotherapy-vaccine (VChV) study showed a very high antibody response. Moreover, vaccination resulted in an EGF immunodeprivation that was more pronounced in the VChV trial. The impact of the anti-EGF antibodies on the EGFR signal transduction cascade was also evaluated. Sera from vaccinated patients blocked the EGF/EGFR binding and inhibited the EGFR phosphorylation. On the contrary, sera from control subjects did not hamper receptor activation. The percentage of EGFR phosphorylation inhibition was significantly higher for patients vaccinated using the VChV schedule (75%) as compared to ChV scheme (19%). The percentage of EGF/EGFR binding inhibition was also significantly higher for subjects using VChV scheme (70%) as compared to ChV (23%). Finally, we characterized the immunodominance of the antibody response. In subjects that started vaccinated before chemotherapy there was a clear dominant response against the central peptide corresponding to loop B beta-sheet, which is the main region involved in the binding of EGF to the EGFR. Good antibody responses, EGF decrease, immunodominance by loop B as well as the EGF/EGFR binding blockade capacity were all significantly associated with better survival.
Conclusions: Vaccination is safe and generates anti-EGF antibodies that inhibit EGFR activation. Sera from patients vaccinated using the VChV scheme had a higher EGFR neutralizing capacity.

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Development of anti-angiogenic cancer vaccine using activated endothelium

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Background: Angiogenesis is essential for cancer development and progression. Recently, many efforts are done for the development of anti-tumor treatments based on anti-angiogenesis, that is believed to be potentially more effective than therapeutic approaches targeting directly tumor cells, since (1) endothelial cells are exposed to the internal lumen of vessels, and therefore can be easily achieved by therapeutics and immune effectors, (2) genetic mutations rarely occur in endothelial cells, and consequently, development of resistance to treatment is rare, (3) the endothelial cell population is significantly smaller than that of cancer cells. Thus, we have focused on the anti-angiogenic approach for cancer, and have developed a vaccine using activated endothelium, and tested it in pre-clinical animal tumor models, and also in patients with a progressive malignancy.
Materials and Methods: First, autologous activated endothelium was fixed or pulsed to dendritic cells, and its immunological as well as anti-tumor effects tested in mouse models of colorectal cancer. Second, safety and clinical effects of the vaccination were tested in a clinical study, by immunizing patients with progressive colorectal cancer or malignant brain tumors with fixed human umbilical vein endothelial cells (HUVECs).
Results: Endothelial cell vaccines effectively inhibited growth and metastasis of colorectal cancer in the mouse model through induction of specific immunity against tumor endothelium. Endothelial vaccines were confirmed to be clinically safe, since the immunized cancer patients showed neither adverse effects of vaccination nor aggravation of their clinical outcome, although specific humoral and cellular immune responses induced by vaccination could be detected. In addition, clinical tumor responses could be observed in part of brain tumor patients.
Conclusions: Endothelial cell vaccines were confirmed to have no harmful effects, and to induce specific immune responses against angiogenic endothelium. The use of adjuvants and/or antigen-presenting cells may help the development of more effective endothelial cell vaccines as a new strategy for immunotherapy of cancer, and studies are now ongoing.

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Conditioning of vaccine sentinel lymph node as adjuvant of autologous hemoderivative breast cancer vaccine

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Background: Tumor and Sentinel Lymph Node (SLN) configure the first interaction between malignant disease and patient's immune system. As consequence of this interaction, Tumor Associated Antigens (TAA) elicit a local immune response inside the SLN. Tumor's cytokines reach the SLN, conditioning its cellular microenvironment to produce local permissive immune responses. This local tolerogenic immunity is decisional because it starts a systemic and permissive immunity. The tumor progresses.
To counteract this mechanism, we have designed a medical procedure to create an Immunotherapeutic Site (ITS) that reproduces, distantly from the tumor, the local interaction tumor-immune system but producing tumor antiprogressive immunity. In this design, TAA source is a cancer vaccine and its draining lymph node is the Vaccine Sentinel Lymph Node (VSLN). Immuno-modulative adjuvants (IMAs) condition ITS cellular microenvironment, promoting local protective instead of permissive immune responses. Due to ITS decisional role, this local protective immunity starts a systemic anti-tumoral immune response.
Material and Methods: Forty breast cancer patients (T3N1M0), 4-group randomized, were submitted to Autologous Thermostable Hemoderivative-Cancer Vaccine (ATH-CV) according Lasalvia-Prisco et al (1995–2006). In Groups 2 to 4, ATH-CV was associated with 1 to 3 IMAs: Magnesium Silicate (Si), Granulocyte Macrophage-Colony Stimulant Factor (GM-CSF) and Etoposide (ETP). Thirty days after vaccination, the groups were compared exploring the VSLN immunophenotyping (IP) and the systemic immunogenic and immunotherapy vaccine effects (IG & IT). IP was evaluated as % of mean values reported in lymph nodes of cadaver samples. IG was assessed by percent of positive cases (>5 mm) in Delayed Type Hypersensitivity (DTH) tested with ATH-CV. IT was measured as percent of 30 days tumor growth (RECIST). Statistic assessment was performed by Student's t-test.
Results: See the table.

Group[a]	VSLN (IP)	IG & IT Effects
1: No adjuvant	Activated APC + 15; T-Reg Cells + 5	DTH+ (%) 30; Tumor Growth −26
2: Si adjuvant	Activated APC + 24; T-Reg Cells + 2	DTH+ (%) 33; Tumor Growth −34
3: SI + GM-CSF adjuvant	Activated APC + 44; T-Reg Cells + 3	DTH+ (%) 43; Tumor Growth −42
4: Si + GM-CSF + ETP adjuvant	Activated APC + 45; T-Reg Cells −20	DTH+ (%) 55; Tumor Growth −52

All groups: n = 10, treated with ATH-CV.

Conclusions: ITS, including ATH-CV, Si, GM-CSF and ETP, enhances the effects of ATH-CV only, immunogenicity and slowing tumor growth. These ITS effects are associated with a switch of VSLN cellular microenvironment, conditioning a local and systemic protective immunity.