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THERAPEUTIC VACCINATION AND TUMOR EXPRESSION PROFILING GROUP

Cancer cells express tumor-specific antigens that can be targeted by cytolytic T lymphocytes (CTL). These antigens are small peptides derived from endogenous proteins presented at the surface of tumor cells by HLA molecules. In vitro, cytolytic T lymphocytes (CTL) lyse selectively tumor cell lines that express their cognate antigen. Our group has developed small scale clinical immunotherapy trials in which patients with advanced cancer, often metastatic melanoma, have been treated repeatedly with a vaccine containing one or several defined tumor antigens that are expressed by their tumor (Fig. 1). Different immunization modalities, such as vaccination with peptides like MAGE-3.A1 and NA17.A2, or with the MAGE-3 recombinant protein, both with or without adjuvant, or with an ALVAC recombinant viral vector, have already been tested. They are all devoid of severe toxicity. A minority of vaccinated melanoma patients (about 10 to 20%) showed regression of metastatic lesions (Fig. 2). This frequency is far beyond the reported incidence of spontaneous regressions of melanoma metastases, estimated at 0.2-0.3%, indicating that these regressions are linked to the vaccinations. However, only 5% of the patients experience a true clinical benefit. Some of the remissions have lasted for several years. There is no evidence that one of the vaccines tested is more effective against the tumors than the others. The most likely explanation for the poor effectiveness of cancer vaccines is the fact that tumors have acquired the ability to resist destruction by anti-tumoral T cells, following repetitive in vivo challenge with spontaneously occurring immune responses. The molecular mechanisms of tumor resistance remain largely unknown, despite the many candidates that have been proposed. Importantly, we have observed that tumor-infiltrating lymphocytes (TIL) purified from melanoma metastases can rapidly recognize and kill autologous tumor cells in vitro, indicating that tumor resistance is a local effect in the tumor environment. We are following two different approaches to try to improve these results: find more immunogenic vaccines, and combine vaccines with treatments that modify the tumor environment in favor of effective tumor rejection.

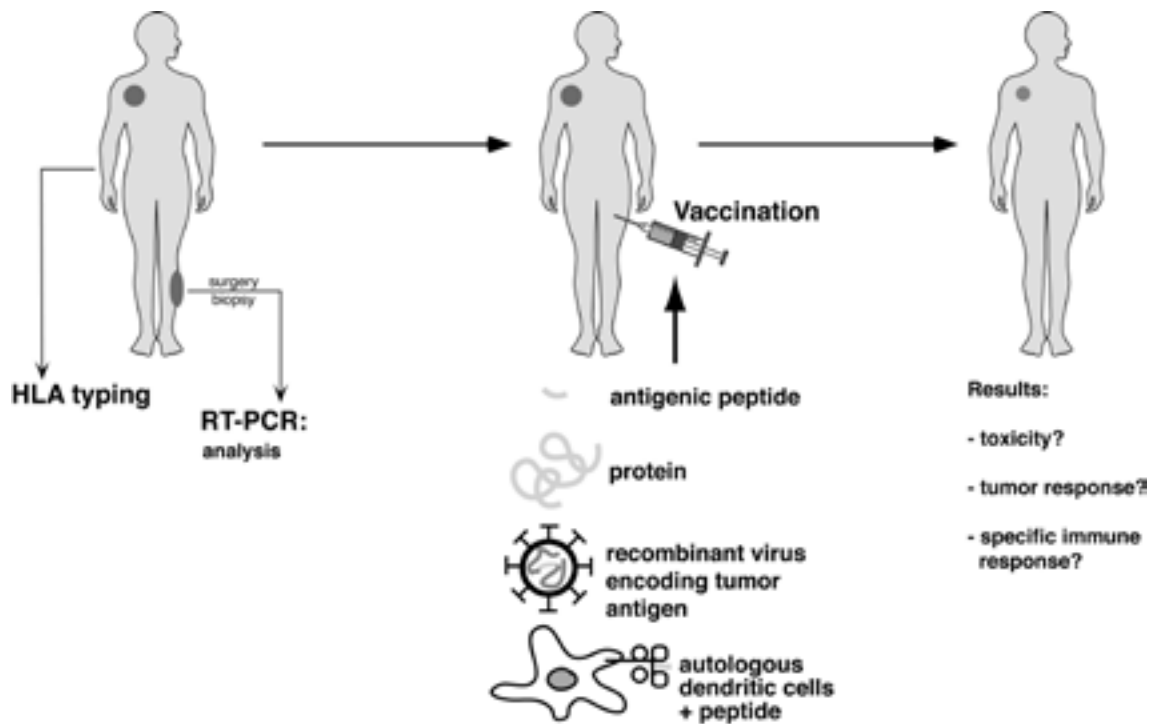


Fig. 1. Principle of anti-tumor vaccination with a defined antigen : The first step is to determine if the patient's tumor cells express the tumor antigen. This can be determined by HLA typing, and by RT-PCR analysis of a tumor sample. Selected patients receive repeated injections of a vaccine with the antigen. Usually this vaccine is a synthetic peptide, a recombinant protein, a recombinant virus coding for the antigen, or dendritic cells derived from the patient's blood and forced to express this antigen. The effect of vaccinations on tumor progression is then assessed. Their immunogenicity is analyzed by comparing the frequency of anti-vaccine CTL in the pre and post-immune blood..

VACCINATION OF MELANOMA PATIENTS WITH THERAVAC, A NEW VACCINE CONCEPT

In collaboration with the groups of J.F. Baurain (Centre du Cancer, Cliniques Universitaires St Luc), P. Coulié, B. Van den Eynde, and Cl. Leclerc (Institut Pasteur, Paris France).

In a recently started phase I clinical trial, we are testing the safety, immunogenicity and anti-tumoral effect of a new promising vaccine called Theravac, developed at Institut Pasteur. Theravac is a recombinant chimeric protein vaccine aimed at targeting dendritic cells (DC) in vivo, and force them to express a Tyrosinase.A2 antigen, a peptide derived from the melanocyte and melanoma-specific tyrosinase pro-

tein. Theravac is derived from CyaA, a bacterial toxin that binds specifically to CD11b, an adhesion molecule expressed by dendritic cells and macrophages. Upon binding, a portion of the toxin is internalized and neutralizes its target cell in order to turn off innate immunity at the infectious site. In the recombinant vaccine protein, the toxin activity has been inactivated by insertional mutagenesis, and coupled to the Tyrosinase.A2 peptide. Thus, the unique advantage of this vaccine is its ability to target dendritic cells in vivo, with a putative higher immunogenicity as a consequence. Preclinical experiments have shown that Theravac has a very potent capacity to activate Tyrosinase.A2-specific CTL. In our clinical trial, patients with tyrosinase-expressing metastatic melanoma are immunized with repeated injections of Theravac, at increasing doses, in a classical phase I

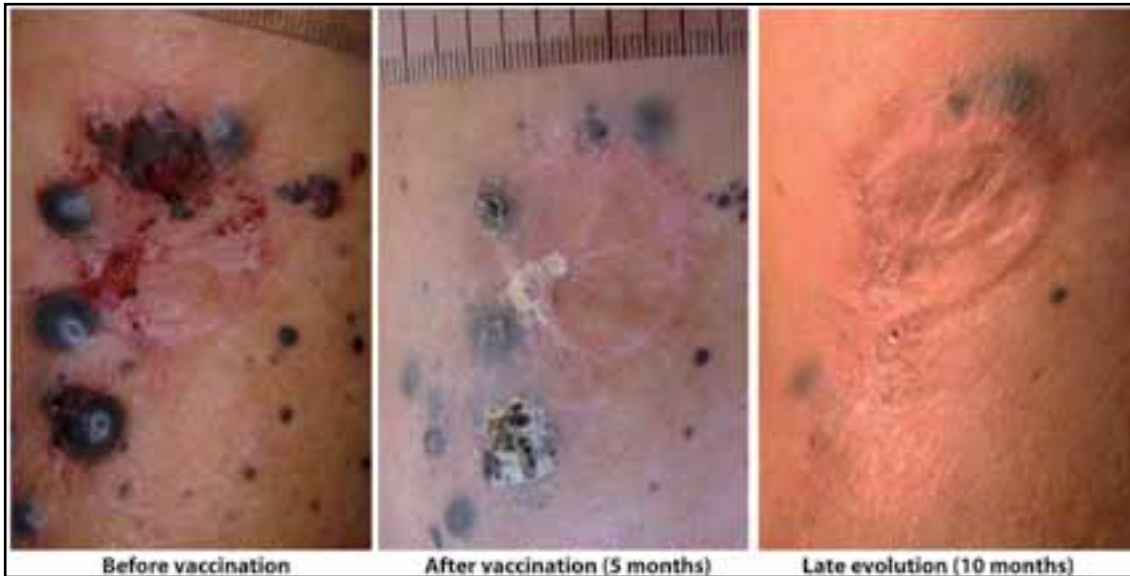


Fig. 2. Example of a complete regression of cutaneous metastases in a melanoma patient after 4 priming vaccinations with an ALVAC recombinant virus expressing the MAGE-3.A1 and MAGE-1.A1 epitopes following by 3 booster vaccinations with the corresponding peptides.

clinical trial design. During the treatment, clinical signs of side effects of the vaccine, including depigmentation occurring as a consequence of anti-melanocyte immune activity, will be recorded, and the size of the metastases will be followed to detect anti-tumoral effect of the vaccine. Blood lymphocytes will be collected before and after the vaccinations to measure the anti-Tyrosinase.A2 immune response. If successful, this new vaccine modality could have a much broader application than in melanoma vaccines.

VACCINATION OF MELANOMA PATIENTS WITH PEPTIDES ASSOCIATED WITH IMMUNOMODULATION OF THE TUMOR ENVIRONMENT

In collaboration with the groups of J.F. Baurain (Centre du Cancer, Cliniques Universitaires St Luc), P. Coulié and T. Boon

In another ongoing clinical trial, melanoma patients with superficial metastases are being vaccinated with a peptide vaccine, either

MAGE-3.A1 or NA17.A2, matching the antigenic profile of their tumor. Each of these peptides was previously tested in clinical vaccine trials, and was shown to be well tolerated and associated with tumor regression in some patients. In addition to the vaccine, the patients receive repeated peritumoral injections of a cocktail of pro-inflammatory cytokines and a TLR ligand, in one or two superficial metastases. This local treatment is aimed at inducing a “spark” effect in the tumor environment that could modify it in favor of effective tumor rejection. The same cocktail has been tested in the H-Y mouse model of skin graft rejection, in which it is able to induce effective tissue rejection (see the contribution of T. Boon in this report). As with the other clinical trials run by the group, great attention is given to the collection of biological material (tumor and blood samples), which will allow to study the effect of the treatment on the anti-tumoral immune responses.

VACCINATION OF MELANOMA PATIENTS WITH PEPTIDES ASSOCIATED WITH A GALECTIN-3 INHIBITOR

In collaboration with the groups of J.F. Baurain (Centre du Cancer, Cliniques Universitaires St Luc), P. Coulie and P. van der Bruggen

Recent work in the laboratory has shown that the state of anergy that characterizes tumor-associated T cells can be reversed pharmacologically (see the contribution of P. van der Bruggen in this report). Inhibitors of galectin-3, a protein produced by cancer cells that is able to interfere with effective T cell activation, have been able to reactivate anergic T cells in vitro. In a new clinical trial, in preparation, melanoma patients will receive the same peptide vaccine as in the previous study, in association with repeated infusions of an experimental drug called Davanat®, a plant-extracted oligosaccharide that binds to and inhibits galectins. Galectin-3 is a protein produced by cancer cells that is able to inhibit T cell activation. The group of Pierre van der Bruggen has shown that the anergy that characterizes tumor-associated T cells can be reversed with galectin-3 inhibitors including Davanat®. We hope that this combined treatment will result in the induction of anti-tumoral CTL responses by the vaccine, in synergy with the inhibition of tumor resistance by the galectin-3 inhibitor.

STUDY OF THE INFLAMMATORY ENVIRONMENT IN MELANOMA METASTASES

In collaboration with the groups of P. Coulie (Cellular Genetics Unit, de Duve Institute)

Using the microarray technology, we have established the gene expression profile of a series of tumor samples, mainly cutaneous metastases, obtained from melanoma patients.

This approach is combined with systematic immunohistological or immunofluorescence analysis of adjacent cryosections, using antibodies directed against tumor cells, T and B cells, macrophages, blood vessels, and various molecules involved in inflammatory reactions (Fig. 3). In addition, adjacent cryosections are analyzed by performing laser capture microdissection of selected areas, e.g. T cell rich areas, followed by RT-qPCR analysis of T

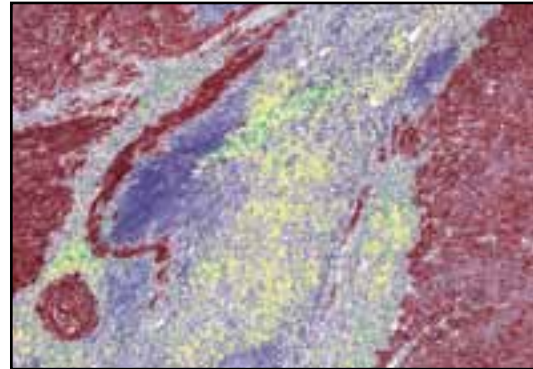


Fig. 3. Example of immune cell infiltration in a melanoma metastasis. The T lymphocytes surround the tumor mass without infiltrating it. This image was reconstructed by superposition of digital microscope images obtained after staining with an antibody directed against melanoma cells (in red) and counterstaining the nuclei with hematoxylin (in blue), followed by elution of antibody and dyes, and by immunofluorescence staining with antibodies directed at CD8+ T lymphocytes (green) and CD4+ T lymphocytes (yellow).

cell, macrophage, melanoma cell and inflammation associated genes. These complementary approaches help us to characterize the inflammatory events that take place inside the metastases, and to understand the interaction between the tumor cells and the inflammatory cells at the tumor site. We are currently characterizing an inflammatory signature that is detected in most tumor samples, and that is associated with T cell activation. We also analyze lymphoid structures present in tumors in which B cell responses seem to occur. The informations gathered from these analyses help us to understand the immune pathways that are active or silent in the tumor environment.

ANALYSIS OF MELANOCYTE-DE-RIVED TUMORS BY NON-LINEAR OPTICS TECHNIQUES.

Our group collaborates with several other European groups in a project aimed at developing innovative imaging microscopy and endoscopy approaches that might improve cancer diagnosis. These approaches are based on spectroscopical analysis of tissue sections or samples illuminated with one or several laser beams of selected frequencies, using so-called Raman and Coherent Anti-Stokes Raman Spectroscopy (CARS) microscopes. The Raman and CARS effects involve light reflection that depends on the molecular bonds present in the illuminated sample. The objective is to identify spectral signatures associated with tumor cells, which would allow to detect and quantify these cells in conventional microscope preparations without staining. Eventually, this technique coupled to an endoscope might allow to detect the presence of cancer cells in vivo. The current project is focused on melanoma and benign naevus samples, and is at an early, proof-of-feasibility stage of development.

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