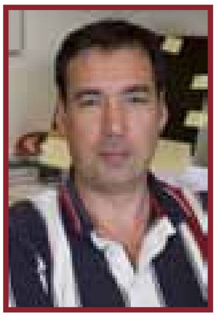

Nicolas van BAREN, Senior Investigator

Francis BRASSEUR, Associate Investigator
Jérôme DEGUELDRE, Clinical Research Associate
Christian VERFAILLE, Project Manager
Madeleine SWINARSKA, Technician
Marjorie MERCIER, Technician

THERAPEUTIC VACCINATION AND TUMOR EXPRESSION PROFILING GROUP

Cancer cells express tumor-specific antigens that can be targeted by cytolytic T lymphocytes (CTL). 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. Whilst these vaccines have shown no toxicity and have been associated with evidence of tumor regression in some patients, their overall anti-tumoral effect is poor. It is thought that this limited effectiveness is a consequence of the acquisition and selection by the tumor of immunosuppressive features, that allow it to resist immune mediated rejection. We are now focusing on new therapeutic approaches that combine a vaccine and an immunomodulatory treatment that is aimed at reverting the immunosuppressive tumor environment.

THERAPEUTIC VACCINATION WITH MAGE TUMOR ANTIGENS

In collaboration with J.F. Baurain (Centre du Cancer, Cliniques Universitaires St Luc) and the group of P. Coulie (Cellular Genetics Unit, de Duve Institute). The clinical trial program was set up and a large part of it was carried out by M. Marchand.

Many if not all human cancers express tumor antigens that can be recognized by T cells. 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 the cognate antigen. MAGE antigens are examples of tumor-specific antigens. They are encoded by MAGE genes, which are expressed in many different tumor types, such as melanoma, non-small cell lung cancer, bladder cancer, head-and-neck cancer and multiple myeloma. These genes are not expressed in normal somatic tissues. They are expressed in germline cells such as spermatogonias, which are devoid of surface HLA class I molecules, and thus can not present MAGE antigens on their surface. Thus, MAGE antigens are good candidates for cancer vaccines, because they are strictly tumor-specific, and are shared by various cancers.

Based on these findings, we have launched phase I/II clinical trials in which patients with advanced cancer, mainly melanoma, were repeatedly immunized with one or more tumor-specific antigens (Figure 1). These trials have two main objectives. First, the effectiveness of various vaccination modalities can be assessed by following the clinical evolution of the tumor, by analyzing whether a specific CTL res-

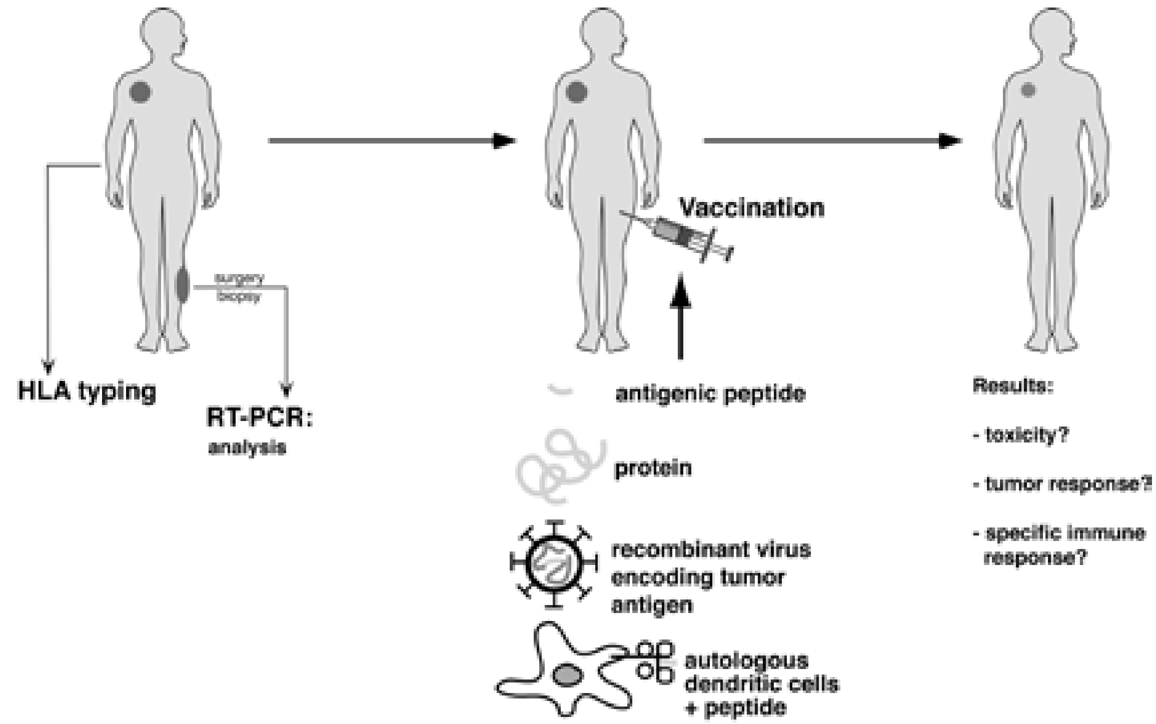

Figure 1. Principle of anti-tumor vaccination with a defined antigen : The first step is to determine if the patient's tumor cells express the tumor antigen. This can be determined by HLA typing, and by RT-PCR analysis of a tumor sample. Selected patients will receive repeated injections of a vaccine 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.

ponse to the vaccine antigen occurred, and by determining whether immunological and clinical responses are correlated. Secondly, these trials allow crucial biological material to be collected from vaccinated patients. Blood samples provide T cells, which allow to analyze the spontaneous and vaccine-induced immune response against tumor antigens. Tumor samples allow to analyze the interaction between cancer cells and immune cells in the tumor environment.

Different immunization modalities, such as immunization with peptides, or with the MAGE-3 recombinant protein, both with or without adjuvant, or with the 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. 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 (Figure 2). 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. CTL responses were detected in a minority of patients vaccinated either with peptides or with the ALVAC virus. These responses were often weak, and, in the case of the MAGE-3.A1 antigen, were observed mostly in patients who had tumor regressions.

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

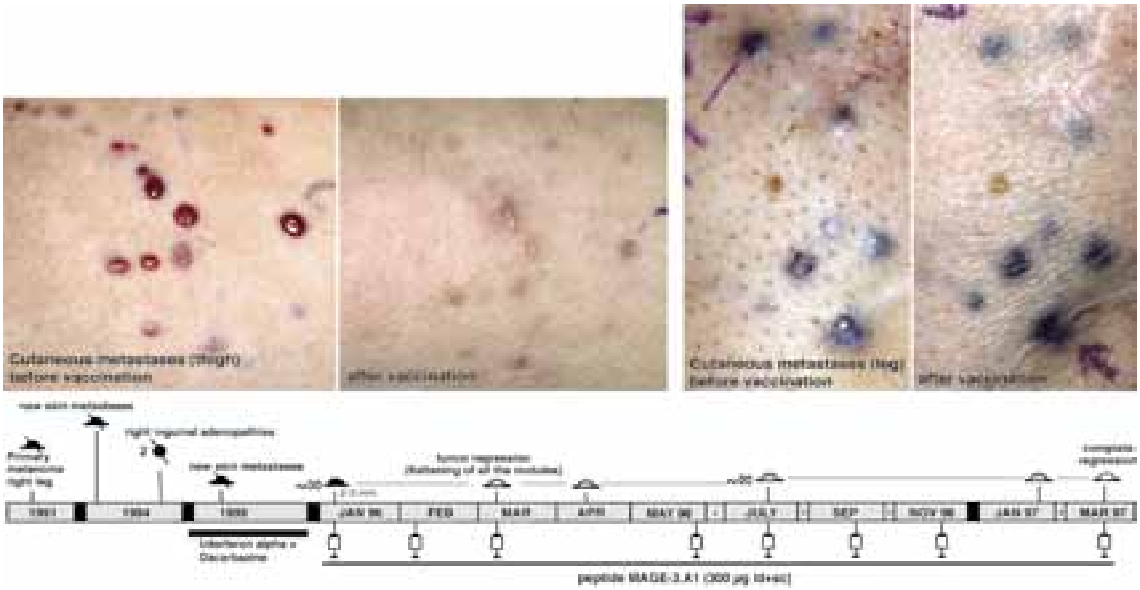

Figure 2. Example of a complete regression of cutaneous metastases in a melanoma patient after repeated vaccinations with the MAGE-3.A1 peptide given without immunological adjuvant.

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 consequence of local immunosuppression in the tumor environment.

We are now setting up a new clinical trial in which patients with advanced melanoma, including superficial metastases, will receive repeated peptide vaccinations, as previously, but now combined with a local immunomodulatory treatment. The latter will associate immunostimulatory cytokines and/or TLR ligands, administered in or close to 1 or 2 superficial metastases. The precise treatment will be chosen on the basis of skin graft rejection experiments performed in a murine model that mimics the situation observed in tumors. The vaccine is aimed at inducing new anti-tumoral T lymphocyte responses, and the local treatment is aimed at modifying the tumor environment in favor of effective tumor rejection.

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 Pierre 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. We are currently developing a new clinical trial, in which patients with advanced melanoma will receive a treatment combining a peptide vaccine and an experimental drug that inhibits galectin-3. 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.

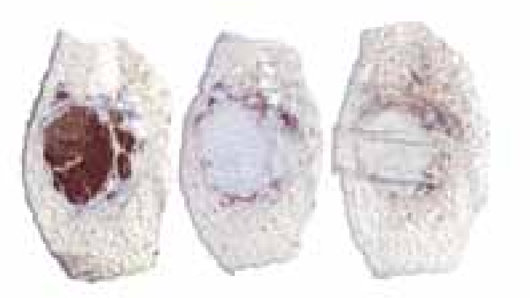

Figure 3. Cryosections obtained from a cutaneous melanoma metastasis were stained with antibodies directed against either melanoma cells (left), T lymphocytes (middle) and macrophages (right), and counterstained with hematoxylin. The corresponding cells appear in red. These images show clearly that the inflammatory cells do not infiltrate, but rather surround the tumor mass.

EXPRESSION PROFILING OF TUMOR SAMPLES FROM VACCINATED PATIENTS

In collaboration with the group 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 analysis of adjacent cryosections, using antibodies directed against tumor cells, T and B cells, macrophages, blood vessels, and various molecules involved in inflammatory reactions (Figure 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 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.

ANALYSIS OF MELANOCYTE-DERIVED 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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Nicolas van Baren
Ludwig Institute for Cancer Research
Av. Hippocrate 74-75
B - 1200 Brussels

[T] +32 02 764 75 08
[F] +32 02 764 65 65
[E] Nicolas.Vanbaren@bru.licr.org
[W] http://www.deduveinstitute.be/therapeutic_vaccination.php