

IMMUNOTHERAPY AND IMMUNOMONITORING

The main objective of this line of research is the development of novel T cell immunity-based strategies that can be translated into clinical application. The focus is on treatment of patients with solid tumors, especially melanoma and renal cell carcinoma. A second line of research concerns immunomonitoring. This work is performed under supervision of Dr F Vyth-Dreese. Its primary aim is to evaluate specific- and cytokine-based immunotherapies, using advanced technologies for characterization of immune responses in peripheral blood and at the tumor site. These studies are conducted together with the Schumacher lab, NKI-AVL, national and international collaborators.

DNA vaccination for the treatment of cancer

Phase I study in melanoma patients

Induction of immunity following DNA vaccination is generally considered a slow process. We have shown that DNA delivery to the skin results in a highly transient pulse of antigen expression. Based on this information, we developed a novel, rapid and potent intradermal DNA vaccination method. By short-interval intradermal DNA delivery, robust T cell responses, of a magnitude sufficient to reject established subcutaneous tumors, are generated within 14 days. These results were confirmed in a non-human primate model (in collaboration with BPRC, Rijswijk). We could show that DNA tattooing results in 10-100-fold increase in vaccine-specific T cells compared to intramuscular vaccination.

Together with Dr. B Nuijen (Pharmacy) and Profs. W Hennink and G Storm (University of Utrecht) we have developed an *ex vivo* human skin model to optimize DNA tattoo vaccination for human skin and to create a platform for testing novel DNA formulations for transfection and targeting of human skin cells. Results from these studies have been crucial for clinical application of this strategy. A clinical grade DNA vaccine has been produced in the GMP DNA plasmid production unit (Amsterdam-Biotherapeutics Unit) at the NKI-AVL/Slotervaart Hospital pharmacy department. In 2009 and 2010, six stage IV melanoma patients have been enrolled in a first phase I clinical trial with DNA tattoo vaccination. The study was amended in 2010 and recently opened in the Leiden University Medical Center as well.

DNA vaccination for the treatment of high risk human papilloma virus (HPV) associated cancers

Human papilloma virus infection (serotypes 16 and 18) is strongly associated with the development of squamous cell cancer of the cervix, but also of penis, vulva, anus en oropharynx. Because the persistence of oncogenic high-risk HPV proteins E6 and E7 is required for carcinogenesis, these viral antigens are exquisite targets for immunotherapeutic interventions. Indeed, therapeutic vaccinations targeting these viral antigens have shown some promise in woman suffering from cervical cancer. In the next years (in collaboration with Prof. G. Kenter, Dr. M. van Beurden en Prof. S. Horenblas (all Division of Surgical Oncology), we will perform a phase I/II study in patients with HPV 16-positive squamous cell cancer of the penis and cervix, using our novel and potent intradermal DNA vaccination strategy. In preclinical studies, we have developed highly immunogenic and safe HPV 16 E6- and E7-containing DNA vaccines for which we have started GMP production in 2010 for a first clinical trial.

Adoptive immunotherapy program

TIL therapy

Adoptive therapy with Tumor-infiltrating Lymphocytes (TIL) is based on results from the Surgery Branch, NIH (Bethesda, MD, USA) and results from the Sheba Medical Center (Tel Aviv, Israel) showing a 50% objective response rate in heavily pretreated stage IV melanoma patients. This treatment combines the *ex vivo* culture of melanoma-reactive T cells from resected metastases with non-myeloablative chemotherapy and high dose bolus IL-2. Our goals are to show that this treatment can be given safely at the NKI-AVL, to demonstrate in an randomized controlled phase II trial that this treatment improves progression-free survival compared to standard chemotherapy and to perform a comprehensive analysis of the T cells



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Publications

Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, Kaiser AD, Pouw N, Debets R, Kieback E, Uckert W, Song JY, Haanen JB, Schumacher TN. *Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy*. *Nat Med*. 2010;16:565-70

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- Vyth-Dreese FA, Sein J, Van De Kastele W, DelleMijn TA, Van Den Bogaard C, Nooijen WJ, De Gast GC, Haanen JB, Bex A. *Lack of anti-tumour reactivity despite enhanced numbers of circulating natural killer T cells in two patients with metastatic renal cell carcinoma*. *Clin Exp Immunol*. 2010;162:447-459

specificities of the melanoma-reactive TIL prior and after adoptive transfer. We expect to start treatment of the first patients early 2011.

T-cell receptor gene therapy

In close collaboration with the Schumacher lab, we have selected a highly avid T cell receptor (TCR) specific for melanocyte differentiation antigen MART-1₂₆₋₃₅. This TCR, called 1D3, has been cloned into a retroviral vector (MP-71) and has been produced by a German GMP manufacturer. This TCR has been equipped with extra safe guards to prevent mispairing with endogenous TCR chains after transduction of peripheral T cells to prevent potential side effects. The process of clinical grade culturing and transduction of peripheral T cells with the 1D3-MP-71 retrovirus is now being validated step-by-step in our GMP facility in order to start a phase I clinical study in melanoma patients in 2011.

Immunomonitoring of DNA tattoo vaccination trial Recently, we have analysed samples from the first two cohorts of 6 melanoma patients treated in the DNA tattoo vaccination trial. Biopsies, taken from the vaccination site, were partly used to culture and recover T lymphocytes and partly for immunohistological analysis. Culture of skin pieces in the presence of high dose IL-2 resulted in outgrowth of MART-1-specific CD8 T lymphocytes in 10-500 fold higher numbers compared to healthy donor skin samples. In 3 out of 5 cases, yields of 30-50% of MART-1 specific CD8 T cells were observed compared to less than 0.1% in control skin. Immunohistological analysis showed the presence of CD8 T lymphocytes and activated DCs near the basal membrane. This indicated that MART-1 DNA tattoo vaccination induced migration of MART-1 specific CD8 T cells to the vaccination site. Virtually no therapy-induced MART-1 specific CD8 T cells were detected in the peripheral blood. ELISpot analysis showed therapy-induced IFN γ responses to MART-1 in 1 out of 6 and to TTFc peptides in 2 out of 6 patients. These results show that DNA tattoo vaccination can induce local CD8 T cell responses. In this trial 2 more cohorts, including 9 patients, will be treated.

Immune infiltrates in renal cell carcinoma (RCC) treated with anti-angiogenic agents Patients with metastatic RCC are currently treated with targeted therapy consisting of tyrosine kinase and mTOR inhibitors, and anti-VEGF mAb. These therapies are based on inhibition of angiogenesis, as well as direct tumor-targeting and may potentiate anti-tumor immune responses. Tumor specimens obtained from RCC patients treated with Sunitinib, Avastin or IFN α show increased immune cell infiltration and apoptosis of tumor and vasculature compared with untreated patients. These analyses will be extended to additional patients.

Detection of mHag-specific T lymphocytes in human tissues

We have successfully applied *in situ* tetramer staining for the detection of minor Histocompatibility antigen (mHag)-specific T cells in a human *ex vivo in situ* skin explant model of Graft versus Host reactivity (in collaboration with groups at the Leiden University Medical Center and Utrecht University). Recently, we have developed and validated a method, using so-called MHC-dextramers, to directly enumerate specific T lymphocytes in cryopreserved tissues. Currently, we are implementing this technique in Graft versus Host Disease to detect mHag-directed T lymphocytes in patient skin lesions.

Human ex vivo in situ skin model for vitiligo and melanoma In collaboration with the Amsterdam Academical Medical Center and Leiden University Medical Center, a human *in situ* skin model has been developed to study immune factors involved in the development of vitiligo and potential therapy of melanoma. Using melanocyte specific T cell clones or bulk T cells obtained from vitiligo lesions co-cultured with normal skin tissues, the induction of vitiligo could be mimicked *ex vivo*. Currently, presence of tumor specific T cells in peripheral blood of melanoma patients is compared to tumor infiltration profiles.