

## 4.11 Progress report of subproject 11

**Project title:** Development of strategies for immunological and genetherapeutic vaccines for malignant lymphomas

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### 4.11.1 Summary

The major goal of the project during the progress report period was the identification of T-cell epitopes of lymphoma-associated cancer testis antigens (CTA). We succeeded in defining 3 HLA-A2 restricted CD8<sup>+</sup> T-cell epitopes of HOM-TES-14/SCP-1, the CTA with the most frequent expression in malignant lymphomas. Moreover, we also identified an MHC-II restricted epitope, the exact restriction of which is currently being determined. For all these peptide epitopes the occurrence of their natural processing has yet to be demonstrated. However, since the epitopes were selected following the SYPEITHI algorithm, which includes predictions with respect to their processing by proteasomes, we assume that the occurrence of the natural processing of the epitopes can be proven.

The second major goal of the project during the last year was the establishment of a eukaryotic expression system for the SEREX (serological analysis of recombinantly expressed clones) approach. We succeeded in establishing an expression system in yeast. This novel expression system is well suited for the expression of defined antigens on the surface of yeasts and in this respect is superior to the conventional SEREX system using bacteria because it can be modified for flow cytometry and thus allows for a significantly higher „through-put“.

### 4.11.2 Results and ongoing activities

The expression of defined CTA on the surface of yeast was used to do a serological analysis of antibodies in the serum of patients directed against the CTA NY-ESO-1. This analysis demonstrated a great concordance of the yeast expression system compared to the phage assay and Western blot. However, the yeast system proved to be not only faster due to the use of flow cytometry. Our results indicate that it is also more sensitive than the two other detection systems. Problems were encountered when we tried to sue the yeast system for expression cloning of cDNA libraries.

Using the yeast surface expression system an unexpectedly high proportion of out-of-frame clones were identified. This indicates that the respective antigen/antibody reactions are artifacts caused by yeast-specific posttranslational modifications such as hyperglycosylations.

The analysis of the „out-of-frame“ problem represents currently our major effort of the project. Its solution is the prerequisite for the intended analysis of eucaryontically expressed lymphoma antigens, one of the major goals of the subproject in the current funding period.

#### **4.11.3 Cooperation within the network**

By defining the scientific basis for vaccine development of a broad spectrum of different lymphomas, this project will make a significant contribution to the central goal of the network, which is the development of more efficacious and more specific therapeutic strategies for malignant lymphomas. The prosecution of this project within the network frame provides added value in a two-way fashion: This project can successfully be executed only within the network frame, because it is only within the network frame that the results of our immunological investigations can be correlated with the histologic diagnosis (SP3) and chromosomal status (SP13), the pretherapeutic characteristics and the clinical course of patients treated within prospective trials of four different cooperative study groups (GHSG, DSHNHL, GLSG, GCLLSG) via SP1 and SP2. On the other hand, only our group possesses the prerequisites to pursue the goals of the project. This includes, besides the scientific know-how, the availability of and access to the large spectrum of transfectants, the extensive serum and tissue bank from patients treated within prospective trials, which was collected during the last ten years, and - no less important with respect to future vaccine development - the legal properties for the clinical use of the lymphoma-associated antigens. Hence this project offers the unique chance to evaluate the clinical relevance of specific immune responses of patients with malignancies against their tumours using human lymphomas as a model. Moreover, the results of this project will enable various clinical study groups to implement novel therapeutic approaches in their phase I and phase II-trials in the foreseeable future. The development of such strategies within the network frame assures rapid translation of our results into the clinics. The application and clinical evaluation of these strategies in different types of lymphomas will result in multiple synergies between basic and clinical research in malignant lymphomas.

Similarly, since our project aims at the definition and development of broadly applicable vaccines, the application of our results will enable several study groups to perform phase I and phase II immunotherapy studies in patients with a wide spectrum of different lymphomas, thus creating added value and synergisms that go beyond the immediate interactions within this network.

#### 4.11.4 Other cooperation

The project participates in a worldwide effort to define the human cancer immunome, i. e. the entire spectrum of antigens expressed by human malignant tumours and recognized by the patients immune system. The cancer immunome project is an initiative of the Ludwig Institute of Cancer Research and open to all researchers involved in the definition of human tumour antigens.

The comparative analysis of methods available for the detection of serum antibodies against human CTA was done in cooperation with Prof. Knuth's group at the Nordwest-Krankenhaus in Frankfurt and with Elisabeth Stockert / Lloyd Old at the LICR (Ludwig Institute for Cancer Research) in New York. For the analysis of antigens by Western blot and immunohistology, the availability of monoclonal antibodies is a prerequisite. Such antibodies will be produced in cooperation with the Shanghai Branch of the LICR by immunizing mice with recombinantly expressed antigens.

For the demonstration of reactive T-cells in the peripheral blood of lymphoma patients with the HLA-A2 haplotype, T-cells will be stimulated according to protocols established in our lab. The relevant MHC-I and MHC-II-binding peptides have been predicted using the SYFPEITHI program of H.G. Rammensee's group, synthesised and confirmed in an MHC binding assay. Moreover, in cooperation with H.G. Rammensee's group, the peptides are checked for processing by the proteasome.

#### 4.11.5 Publications

- Xie X, Schmits R, Renner C, Preuss D, Kubuschok B, Pfreundschuh M: Systematic search and molecular characterization of the antigenic targets of myeloma immunoglobulins: A monoclonal IgA from a female patient targeting sperm-specific cyclicin II. *Cancer Immunity* (2002) 1: 11-19
- Ayyoub M, Stevanovic S, Sahin U, Guillaume P, Servis C, Rimold D, Valmori D, Romero P, Cerrotini JC, Rammensee HG, Pfreundschuh M, Speiser D, Levy F: Proteasome-assisted identification of a SSX-2-derived epitope recognized by tumor-reactive CTL infiltrating metastatic melanoma. *J Immunol* (2002) 168: 1717-22
- Xie X, Wacker H-H, Huang S, Regitz E, Preuss K-D, Romeike B, Parwaresch R, Tiemann M, Pfreundschuh M: Differential expression of cancer testis genes in histologic subtypes of non-Hodgkin's lymphomas. *Clin Cancer Res* (2003) 9: 167-173
- Huang S, Preuss KD, Xie X, Regitz E, Pfreundschuh M: Analysis of the antibody repertoire of lymphoma patients. *Cancer Immunol Immunother* (2002) 51: 655-662
- Mischo A, Wadle A, Wätzig K, Jäger D, Stockert E, Santiago D, Ritter G, Regitz E, Jäger E, Knuth A, Old LJ, Pfreundschuh M, Renner C: Recombinant antigen expression on yeast surface (WAYS) for the detection of serological immune responses in cancer patients. *Cancer Immunity* (2003, in press)
- Neumann F, Wagner C, Kubuschok B, Stevanovic S, Rammensee H-G, Pfreundschuh M: Identification of an antigenic peptide derived from the cancer testis antigen NY-ESO-1 binding to a broad range of HLA-DR subtypes (2003, submitted for publication)

#### **4.11.6 Further objectives for the current funding period**

The objectives for the current funding period remain the same as specified in our application from 4/2002. The “out-of-frame problem” of the expression cloning using the yeast expression system was not expected and we hope to solve it by summer 2003. Alternatively, a mammalian expression system will be pursued, assuring that the identification of eucaryontically expressed lymphoma antigens can be accomplished by 12/2004.

Similarly, we continue with the definition of T-cell defined peptide epitopes of lymphoma-associated CTA, in particular HOM-TES-14/SCP-1 and HOM-MEL-40/SSX-2. As described above, we have already identified several candidate peptides that have elicited the CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses, respectively, and with this part of the project are ahead of the original time schedule. The demonstration of their natural processing and the more detailed analysis of their MHC restriction should be accomplished by the end of 2003 and leave us enough time for defining additional epitopes from other lymphoma-associated antigens during the rest of the current funding period.