

### Section 3

## Midterm Report of Running Projects and Application for Continued Funding

### Title Page

Network title: Competence Network Malignant Lymphoma

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## **Part A – General Statements about the Project**

### **A.1 Subject**

Development of strategies for immunological and genetherapeutic vaccines for malignant lymphomas

### **A.2 Co-investigators**

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## Part B – Results from the First Funding Period

### B.1 Summary

Even though the clonal idiotypes of the B-cell lymphoma associated immunoglobulins have been successfully employed in clinical lymphoma vaccine trials, the need for tailor-made individual vaccines has compromised their widespread use. In contrast, cancer-testis (CT) genes [Chen et al., *Proc Natl Acad Sci USA* 1997; 94: 1914-1918] are expressed in a variety of human cancers, but not in normal tissues, except for testis, and represent promising targets for immuno- and gene therapeutic approaches. Aiming at the development of a broadly applicable vaccine, the main focus during the first period of this project was the analysis of the expression of CT antigens in Non-Hodgkin's lymphomas. We investigated the composite expression of 9 CT genes (MAGE-3, MAGE-4, NY-ESO-1, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TES-14/SCP-1, and HOM-TES-85) in 78 B- and 15 T-NHL specimens. Only 1/7 CLL cases expressed a CT gene (HOM-TES-14/SCP-1), and 10 follicular lymphomas were negative for all CT genes tested. In B-cell lymphomas, the most frequent expression of CT genes was observed in diffuse large cell lymphomas: 7/28 cases expressed HOM-TES-14/SCP-1 and 5/28 SSX-1, respectively. CT-7 was expressed in 2/28 cases, HOM-MEL-40/SSX-2, HOM-TES-85 in 1/28 cases each. Only 1/8 Burkitt's and 1/7 lymphoblastic lymphomas expressed a CT gene. A majority (9/15) of T-NHL (9 peripheral T-cell lymphomas, 2 lymphoblastic T-cell lymphomas and 4 cases of AILD) expressed HOM-TES-14/SCP-1. Thus, HOM-TES-14/SCP-1 and to a lower degree SSX-1 and CT-7 are good candidates for lymphoma vaccine development. However, the identification of additional tumour-specific antigens with a frequent expression in lymphomas is warranted to allow for the development of widely applicable polyvalent lymphoma vaccines.

With respect to T-cell responses, two HLA-A2 restricted CD8 T-cell epitopes of SSX-2 have been identified, work on SCP-1 epitopes is ongoing. In order to discover new lymphoma antigens, we modified the classical SEREX approach and screened a testis-derived cDNA library with the sera of 25 lymphoma patients and identified 23 different antigens, of which 4 were encoded by unknown genes and 1 represents a new CT antigen.

The major goals for the 2<sup>nd</sup> period of the project are the identification of new lymphoma antigens by the development and use of a eukaryotic SEREX expression system and the further delineation of the T-cell epitopes of the respective antigens as the prerequisite for specific lymphoma vaccine development.

### B.2 Original aims of the project

The aims at the starting point of the project had been defined as:

1. Analysis of the expression of cancer testis antigens in lymphomas;
2. Correlation of expression with defined lymphoma sub-types;
3. Analysis of antibody and T-cell responses in lymphoma patients;
4. Identification of antigens useful for vaccine development;
5. Identification of novel lymphoma associated antigens.

### B.3 I. Scientific results (until 02.2002):

**1. Analysis of the cancer testis antigen expression in Non-Hodgkin's lymphomas:** In cooperation with our network partner at the Institute for Hemato-pathology of Kiel University we investigated the expression of 9 cancer testis genes (MAGE-3, MAGE-4, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TES-14/SCP-1, and HOM-TES-85) in 78 lymphomas by RT-PCR using specific primers.

*Expression of individual CT genes in Non-Hodgkin's lymphomas:* NY-ESO-1 was negative in all lymphoma samples tested. HOM-MEL-40/SSX-2, HOM-TES-85, MAGE-3 and MAGE-4 were expressed in only one, and SSX-4 in two cases, respectively. SSX-4 was expressed in 6/78 B-cell lymphomas, but was absent in T-cell lymphomas. Similarly, CT-7 was found in only 4/78 B-cell lymphomas, but not in T-cell lymphomas. The most frequently expressed CT gene was HOM-TES-14/SCP-1. It was expressed in 15/78 (19%) of the B-cell and 9/15 (60%) of the T-cell lymphomas.

*Expression of CG genes according to histologic sub-type:* MALT and follicular lymphomas were completely negative for all CT genes tested, and only 1/7 CLL cases expressed SCP-1. The most frequent expression of CT genes in B-cell lymphomas was observed in the diffuse large cell lymphomas, where 7/28 (25%) expressed SCP-1, 5/28 SSX-1 and 3/28 expressed CT-7. In contrast, expression of CT genes was rare in Burkitt's and B-lymphoblastic lymphomas, where SSX-1, SCP-1 and HOM-TES-85 were expressed in only 1/15 cases. In T-cell lymphomas, SCP-1 was expressed in the majority of the cases (9/15 or 60%). Apart from SCP-1, there was a rare expression of SSX-4, MAGE-3 and MAGE-4 (one case each). All other CT genes were not expressed in any of the 15 T-cell lymphoma cases tested.

*Coexpression of multiple CT genes in lymphomas:* Expression of more than one CT gene was observed in only 4/28 (14%) cases with diffuse large B-cell lymphomas and in 2/15 (13%) of the T-cell lymphomas. Coexpression of three CT genes occurred once among the 15 T-cell lymphomas, and coexpression of 4 CT genes was observed in only one case with diffuse large B-cell lymphoma [Xie et al., submitted].

**2. Correlation of CT antigen expression with sub-type:** These results demonstrate that the expression of certain CT antigens is associated with defined NHL sub-types. T-NHL express SCP-1 in >50% of cases, while of the B-NHL only diffuse large B-cell NHL express CT antigens at a frequency that would allow for vaccination of a significant proportion of patients with this sub-type of NHL.

**3. Analysis of T- and B-cell responses against CTA in lymphoma patients:** The analysis of the B-cell responses against CTA in lymphoma patients will be completed within the next months. With respect to T-cell responses, we identified two HLA-A2 restricted CD8 T-cell epitopes of SSX-2. In 2/2 HLA-positive patients with a SSX-2 positive tumour we were able to demonstrate CTL with specific reactivity to HLA-2 binding peptides of the SSX-2 antigen. The analysis of other SSX-2 epitopes and other antigens, especially the frequently expressed SCP-1 are ongoing and are one of the major goals of the remaining time of this and the next financial period of the project.

**4. Identification of antigens useful for lymphoma vaccine approaches:** Of the known cancer testis antigens, only SCP-1, and to some extent SSX-1, SSX-2, SSX-4 and CT-1 are promising candidates for lymphoma vaccine development, because only these antigens are expressed in malignant lymphomas at reasonable frequencies.

**5. Identification of novel lymphoma associated antigens:** The paucity of tumour-specific antigens that are expressed in lymphomas with an acceptable frequency to justify a vaccine development made it imperative to search for additional lymphoma-associated antigens. Because this can not be achieved using the classical SEREX approach developed by us [Sahin et al., *Proc Natl Acad Sci USA* 1985; 92: 11810-1183], we modified SEREX using a cDNA library derived from normal testis, which should express the entire spectrum of cancer testis antigens [Türeci et al., *Proc Natl Acad Sci USA* 1998, 95: 5211-5216]. We immunoscreened this library with the 1:1000 diluted sera of 25 (allogeneic) patients with

NHL. Of  $1.6 \times 10^6$  clones tested with these 25 sera, 42 clones were positive. These 42 clones represented 23 different antigens. 19 antigens were encoded by known genes, and 4 represented novel transcripts. Most of the antigens were broadly expressed in both normal and malignant tissues. However, HOM-NHL-6, HOM-NHL-8 and HOM-NHL-21 were shown to be cancer testis antigens representing SCP-1, NY-ESO and a new CTA, respectively. Antibodies against 11/23 antigens were detected in the sera from both patients and healthy controls, while antibodies against 3/23 antigens were found only in the individual serum from the NHL patient used for the immunoscreening. However, 9/23 antigens reacted only with the sera from patients with lymphomas suggesting their potential usefulness for diagnosis and follow-up of lymphomas.

**6. Identification of target antigens of myeloma proteins:** In order to establish the SEREX modifications, we used the 1: 1 000 000 diluted sera from myeloma patients. Screening  $1 \times 10^5$  clones from the testis-derived cDNA, we were able to identify the sperm-specific cylicin II as the target of the IgA myeloma protein of a woman with plasmacytoma. Even though not being an original aim of this project, this finding is interesting with respect to the pathogenesis of this B-cell disease in the respective woman and demonstrates that the modified SEREX approach is also a straightforward strategy for the identification of target antigens of B-cell neoplasms other than plasmacytomas [Xie et al., *Cancer Immunity* 2001; 1:11].

## **II. Work plan for the rest of the financial period: (03.2002 to 09.2002):**

The work plan for the rest of the financial period (03.2002 to 09.2002) consists of two major goals: 1<sup>st</sup>, further analysis of the T-cell responses against lymphoma-associated antigens; and 2<sup>nd</sup>, first steps towards the establishment of a eukaryotic SEREX system. With respect to the 1<sup>st</sup> goal, the analysis of other SSX-2 epitopes and other antigens, especially the frequently expressed SCP-1 is ongoing and will be pursued by one of the Ph. D. students and the technician using the described methods. In parallel, the second Ph.D. student addresses the establishment of the eukaryotic SEREX system following the methodological strategy described below.

**Standing of the project with respect to the initial proposal and international standard:** With respect to the analysis of the expression of cancer testis antigens in lymphomas, the study performed within this project is the first in the field, except for a small study in cutaneous T-cell lymphomas [Häfner et al., *Int J Cancer* 2002; 97: 668-670]. Similarly, with respect to the definition of the B-cell repertoire of lymphoma patients against antigens expressed by their tumours, to date only a small study in cutaneous T-cell lymphomas has been published [Eichmüller et al., *Proc Natl Acad Sci USA*. 2001; 98: 629-634]. With respect to B-cell lymphomas, no results of other groups have been published and we are not aware that such work is in progress in any other group. This is understandable in the light of the technical problems with the classical SEREX approach associated with the expression of immunoglobulins by B-cell lymphomas. Thus, the results obtained in this project represent the international state-of-the-art with respect to the analysis of human lymphoma antigens recognised by the immune system of lymphoma patients.

All the milestones envisaged in the initial proposal were accomplished in due time. The project is even ahead of time regarding the identification of novel lymphoma antigens using a modified SEREX approach, which had been envisaged only for the next financial period of the project.

## B.4 Publications and patents

### Articles:

Xie X, Schmits R, Renner C, Preuss D, Kubuschok B, Pfreundschuh M: Systematic search and molecular characterisation 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 recognised by tumour-reactive CTL infiltrating metastatic melanoma. *J Immunol* 2002; 168: 1717-22

<sup>1</sup>Xie X, Wacker HH, Huang S, Preuss D, Parwaresch R, Tiemann M, Pfreundschuh M: Expression of cancer testis genes in malignant lymphoma (submitted).

<sup>1</sup>Huang S, Xie X, Tiemann M, Preuss D, Regitz E, Pfreundschuh M: Analysis of the B-cell repertoire of patients with malignant lymphomas (submitted).

### Abstracts:

<sup>1</sup>Xie X, Wacker HH, Huang S, Preuss D, Parwaresch R, Tiemann M, Pfreundschuh M: Identification of targets for immunotherapy of lymphomas. *Eur J Cancer* 37 (suppl. 6), 11

<sup>1</sup>Xie X, Wacker H-H, Huang S, Regitz E, Preuss K-D, Parwaresch R, Tiemann M, Pfreundschuh M: Expression of shared tumour / cancer testis antigens by lymphomas. *Proc Amer Assoc Cancer Res* (in press)

Bormann C, Neumann C, Frederich N, Schmidt W, Stefan Stvanovic, Ayyub M, Speiser D, Kubuschok B, Pfreundschuh M: Identification of HLA-A\*0201 binding antigenic peptides that stimulate CTL from patients with SSX.2 positive tumours. *Proc Amer Assoc Cancer Res* (in press)

### Reviews:

Türeci Ö, Sahin U, Zwick C, Neumann F, Pfreundschuh M: Exploitation of the antibody repertoire of cancer patients for the identification of human tumour antigens. *Hybridoma* 18: 23-28 (1999)

Türeci Ö, Sahin U, Zwick C, Neumann F, Pfreundschuh M: Identification of human tumour antigens using the antibody repertoire of cancer patients. *Gann Monograph on Cancer Res* 48: 93-101 (1999)

Renner C, Trümper L, Pfreundschuh M: Tumour Vaccines: a new immunotherapeutic approach in oncology. *Ann Hematol* 79: 651-659 (2000)

Renner C, Kubuschok B, Truemper L, Pfreundschuh M: Clinical approaches to vaccination in oncology. *Ann Hematol* 80: 255-266 (2001)

Pfreundschuh M: Exploitation of the B cell repertoire for the identification of human tumour antigens. *Cancer Chemother Pharmacol* 46 (suppl): S3-S7 (2000)

<sup>1</sup> represents the joint activities of two or more network groups.

## **B.5 Networking**

As can be seen from the publication list, this project was only possible within the network frame. In particular, it was only within a close cooperation with the network partner from the Institute of Hemato-pathology, Kiel University, that enough lymph node material was available for the analysis of the CTA expression in lymphomas. Moreover, the expertise of the Kiel hemato-pathologists was a prerequisite for the correlation of antigen expression with defined histological sub-types (SP3). Since the majority of both serum and biopsy materials stems from patients treated within prospective trials of the GLSG, DSHNHL and the German CLL Study Group, a correlation of antigen expression and/or immune responses against defined antigens in lymphoma patients will be possible with the network partners from the biometric centres of the respective study groups (SP2). Correlations between chromosomal changes of the lymphomas (SP13\_new) and the expression of defined antigens (SP13\_new) will also be investigated.

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.

## Part C – Follow-Up Proposal

### C.1 Aims

The research objectives as set down in the initial project proposal are still relevant and achievable. With SCP-1 we have already identified an antigen that is a suitable target for immunotherapeutic approaches in malignant lymphomas, in particular T-cell lymphomas. By the end of the second financial period we expect to have antigens identified that can be used as targets for widely applicable vaccines also in B-NHL. To achieve this goal, the following aims will be pursued during the 2<sup>nd</sup> period of this project:

**1. Establishment of a eukaryotic SEREX expression system and analysis of eukaryotically expressed lymphoma antigens:** Our analysis of the B-cell repertoire against lymphomas using the conventional SEREX approach with a bacterial expression system shows that the human *lymphoma immunome*, i.e. the sum of all the proteins expressed by lymphomas and recognised by the patients' immune system, is limited. While our analysis is still ongoing, we must expect that antigens that have escaped detection by the conventional SEREX approach to date are very likely to have a very limited expression spectrum, thus lacking our criterion of having the potential for a widely applicable vaccine.

Moreover, it is eye-catching that very few of the lymphoma antigens detected to date are located on the cell surface. A modified SEREX approach using a eukaryotic expression system should open a whole new dimension of novel antigens, since the spectrum of antigens to be discovered will not be limited to genomically determined proteins, but will include all posttranslational modifications of these molecules. Posttranslational modifications play an important role not only for the function of many proteins, but also for their immunogenicity. Hence, the characterisation of such modifications is also very important for the development of recombinant protein-based vaccines [Chen et al., *J. Exp Med.* 1999; 189: 1757-1764]. The spectrum of posttranslational modifications covers a wide range spanning from glycosylation, lipidation, phosphorylation, methylation, acylation, citrullination, and deimination to posttranslational truncation [Doyle & Mamula, *Trends Immunol* 2001; 22: 443-9;] and modification of peptides [Stoltze et al., *Nature Immunol* 2000; 1: 413-418; 2000; Skipper et al., *J Exp Med* 1996; 183: 537-534; Valmori et al., *Cancer Res* 1999; 59: 4050-4055]. Aberrant glycosylation of glycosphingolipids and glycoproteins in malignant cells is an important mechanism in the pathogenesis of many neoplasias, since it influences the function of many adhesion and signal transduction molecules in these cells [for review see Hakamori, *Cancer* 1996; 56: 5309-118]. Recent evidence indicates that up to 0.1% of the antigenic peptides presented by MHC-I molecules are glycosylated [Haurom et al., *J Exp Med* 1999; 190: 145-150].

**2. Continuing identification and further characterisation of the T-cell responses against lymphoma-associated antigens as the basis for lymphoma vaccine development:** Antigens with a specific expression spectrum suggestive of their potential use as widely applicable vaccines will be analysed for their capacity to induce T-cell responses in patients with lymphomas expressing the respective antigens. First choice will be peptides with MHC-I and MHC-II binding motifs that are processed by the proteasome. Since our analysis of the B-cell repertoire is restricted to high-titered B-cell responses that require cognate T-cell help, we expect that T-cell responses in patients with high antibody titers to the respective antigen should be readily demonstrable.

## C.2 Methodological approach

**Establishment of a eukaryotic expression system for the SEREX analysis of lymphoma antigens:** The eukaryotic expression system will be established in COS cells. To this end, the cDNA will be cloned into a eukaryotic expression vector that expresses the encoded protein under the control of a CMV promoter. Even though we have never detected antibody responses against allogeneic HLA molecules using the conventional SEREX system, we have to expect such responses in a eukaryotic expression system. Therefore, the establishment of the eukaryotic expression system will be pursued in an autologous system using the serum of a lymphoma patient with a known high-titered IgG response against bacterially expressed SCP-1.

Lymphoma-derived or testis-derived cDNA will be cloned into a eukaryotic expression vector, which will express the encoded sequence under the control of a CMV promoter and will present the antigen on the cell surface. The transformed COS cells will be analysed by flow cytometry and positive clones will be sorted into single vials. Alternatively, positive cells will be picked by a micromanipulator under a fluorescence microscope. Thereafter, the plasmid DNA will be isolated and expanded in bacteria. After monoclonalisation the DNA will be sequenced using an automatic sequencer and the positive reactivity will be confirmed using the identical indicator serum from the same patient both in the eukaryotic and the bacterial expression system in order to check, whether the reactivity of the antibody is directed against the protein backbone of the antigen or, alternatively, against a posttranslationally modified structure. Identified sequences will be compared with sequences from BLAST in data banks (Genbank, EMBL, SEREX database) and checked for homologies.

Each new lymphoma antigen will undergo a basic characterisation which includes 1) sequence analysis, 2) analysis of the expression spectrum in a range of normal and malignant tissues and 3) determination of antibody reactivities against the respective antigen in the sera of lymphoma patients, patients with other malignant or autoimmune diseases and healthy controls. Lymphoma antigens, which according to the results of this initial characterisation appear to be promising vaccine candidates, will undergo further characterisation. This includes the analysis of posttranslational modifications, chromosomal allocation, analysis of the genomic organisation of the encoding gene, as well as the cloning of full-length transcripts and analysis of the protein product by Western blot and immunohistology. Putatively posttranslationally converted peptides will be analyzed in cooperation with Dr. Stevanovic and Prof. Rammensee (Tübingen), sugar structures with F.G. Hanisch (Institut für Biochemie der Universität zu Köln), and lipids with G. Ritter (Ludwig Institute for Cancer Research, New York).

The analysis of the expression spectrum of new antigens will be performed by Northern blot, RT-PCR and Real-Time-PCR. For these investigations a tissue bank with ca. 800 tumours and normal human tissues is available.

Antibody reactivity in the sera of patients and healthy controls will be investigated using the SEREX phage screening assay for protein antigens and the respective eukaryotic screening assay for posttranslationally modified antigens.


The chromosomal allocation of new genes will be accomplished in cooperation with K. Zang and E. Meese from this institution by FISH using fluorescence-labelled cDNA probes. For analysis of the genomic structure, DNA will be isolated from normal tissue and lymphomas, digested with restriction enzymes, blotted and hybridised with cDNA inserts as probes.

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 Dr. Elisabeth Stockert / Dr. Lloyd Old, LICR New York by immunizing mice with recombinantly expressed antigens.

**Analysis of T-cell responses against lymphoma 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 will be predicted using the SYFPEITHI program of Prof. Rammensee's group, synthesised and confirmed in an MHC binding assay. Moreover, in cooperation with Dr. Rammensee's group, the peptides will be checked for processing by the proteasome. The peptides will then be used for stimulation of the T-cells, using an IFN- $\gamma$ -ELISPOT assay as the basic read-out system.

**Methodological strategy and availability of techniques:** The methodological approach has not been changed from that in the initial proposal. With the exception of the eukaryotic SEREX expression system, which still has to be firmly established, all methods and techniques needed to achieve the goals of this project are well established in our own lab or in the labs of our network partner in Kiel and the other collaborators.

### C.3 Work plan

	2002	2003				I-IX.2004		
	4.Q	1.Q	2.Q	3.Q	4.Q	1.Q	2.Q	3.Q
<b>Schedule</b>	<i>Establishment of eukaryotic SEREX</i>							
	<i>Anti-SCP-1 T-cell responses</i>			<i>Antigen identification by eukaryotic SEREX</i>				
				<i>Analysis of other T-cell responses</i>				
								2 <sup>nd</sup> Report 

The work plan spans 2 years. The first milestone will be the establishment of the eukaryotic SEREX expression system. This will be pursued by one of the Ph.D. students (Doktoranden) employed for the project, and should be accomplished within the first year of the next financial period. The next milestone to be achieved by this Ph.D. student will then be the detection and characterisation of new lymphoma-associated antigens by screening of eukaryotically expressed cDNA libraries, which will be her/his major task for the second year of the financial period.

The second Ph.D. student employed for the project will continue with the analysis of T-cell responses against lymphoma-associated antigens. This includes both CD8 and CD4 responses. We expect the experiments concerning SCP-1 to be completed within the 1<sup>st</sup> year of the second financial period and hope to accomplish the definition of T-cell responses in lymphoma patients against 2 or 3 additional antigens during the 2<sup>nd</sup> year of the financial period.

Throughout the financial period the technician employed for the project will be responsible for cell culture work necessary to establish and maintain the eukaryotic SEREX expression system. In addition, she/he is responsible for the asservation and documentation of primary patient materials, e.g. serum, blood cells and lymphoma tissue, the typing of the HLA haplotypes, sequence analyses as well as histologic and immunohistologic investigations.

## C.4 Networking

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, that 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 (DHSG, DSHNHL, DSNHL, CLL Cooperative Group) 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 – not 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.