CCRI

Children´s Cancer Research Institute

Scientific Report 2004/2005

St. Anna Kinderkrebsforschung

Jahresbericht 2004/2005

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Univ. Doz. Dr. Heinrich Kovar
Mag. Marion Zavadil
Ing. Ulrike Monschein

Vorwort

Dem Forschungsinstitut für krebskranke Kinder ist es im Zeitraum 2004/05 gelungen, eine Reihe von Strukturveränderungen, die schon seit längerem angedacht oder angekündigt waren, umzusetzen. So wurde einerseits durch die Etablierung einer kaufmännischen Leitung eine aussagekräftige Transparenz der auftretenden Kosten und Leistungen in den verschiedenen durch Projekte vernetzten Arbeitsgruppen ermöglicht, und andererseits auch die Voraussetzungen für die Qualitätssicherung im – nach 2-jähriger Vorlaufzeit – eröffneten Tochterunternehmen „Labordiagnostik für krebskranke Kinder", kurz „Lab-Dia" bezeichnet, geschaffen. Der diagnostische Zweig zweier Arbeitsgruppen, der Zytogenetik und der Molekulargenetik der hämatologischen Neoplasien wurden in diese 100%ige Tochtergesellschaft ausgelagert und die Gruppenleiter sind zu ärztlichen Direktoren avanciert. Wie bereits in einem früheren Jahresbericht dargelegt, ist das Ziel dieser gemeinnützigen GesmbH, brauchbare methodische Entwicklungen zu vermarkten, die aus dem unmittelbaren Forschungsbereich ausgelagert werden und damit neue Ressourcen und Arbeitsplätze für die Forschung sichern.

Die Arbeitsgruppe der Tumorimmunologie die sich schwerpunktmäßig auf die Herstellung von Impfstoffen für Tumorvakzination konzentriert hat, wurde in eine eigene Organisationsform mit der Bezeichnung I-Med (GesmbH) umgewandelt.

Ende 2005 ist es gelungen, den schon lange angekündigten Spatenstich zum Bau eines neuen Institutsgebäudes am Zimmermannplatz (auf der Rückseite des St. Anna Kinderspitals), nach einvernehmlicher Klärung aller von den Anrainern geäußerten Sorgen und Befürchtungen, festzulegen. Nach einer etwa einjähriger Bauzeit einer Tiefgarage (Beginn Mitte Februar 2006) durch einen eigenen Unternehmer (Garagenerrichter) soll anschließend aufbauend auf diese Garage der Hochtrakt des Forschungsinstitutes errichtet werden (Finanzierung durch Leasingvertrag). Die Bezugsnahme des zweistöckigen Hochbaues mit ca. 2.000 m² Forschungsfläche ist für die zweite Hälfte des Jahres 2008 vorgesehen. Durch die Schaffung der neuen Räume wird der seit Jahren zunehmenden Raumnot im Forschungsinstitut Rechnung getragen und die Voraussetzungen für eine weiterhin erfolgreiche Arbeit geschaffen. An dieser Stelle sei der Bezirksvorsteherin Frau Martina Malyar für die wertvolle Unterstützung bei der Realisierung dieses Projekts, der Gemeinde Wien für die Überlassung des Grundstückes, sowie für den großzügigen finanziellen Beitrag zur Errichtung des Neubaues, sowie allen, die zur Planung und Umsetzung beigetragen haben, gedankt.

Erwähnenswert ist auch, dass der in den vergangenen Jahren vollzogene Schritt hin zu einer stärkeren Professionalisierung der PR-Aktiviäten sehr erfreuliche Erfolge gebracht hat. Dadurch scheint das Fortbestehen dieser für die krebskranken Kinder im Osten Österreichs, sowie für spezielle Diagnostik und Therapieformen für ganz Österreich wichtigen Einrichtung gesichert zu sein, und wir können positiv in die Zukunft blicken.

Abschließend möchte ich die Gelegenheit dieses Leistungsberichtes nutzen, allen zu danken, die uns bisher geholfen haben, unsere Ziele erfolgreich zu verfolgen. Besonders gilt dies für alle Mitarbeiterinnen und Mitarbeiter für ihren tatkräftigen Einsatz, die kooperierenden Institutionen und externen Mitarbeiter die zu der Umsetzung unserer Forschungsvorhaben Wesentliches beigetragen haben, die verschiedenen Wissenschaftsförderungsfonds für die finanzielle Unterstützung und auch die Vorstandsmitglieder und Mitglieder des Fördervereines „ St. Anna Kinderkrebsforschung", die seit Jahren uneigennützig ihre Zeit und Expertise dem Forschungsinstitut zur Verfügung stellen. Ein besonderer Dank gilt natürlich den unzähligen Spenderinnen und Spendern, die uns seit vielen Jahren die Treue halten und damit die Kinderkrebsforschung am Leben erhalten.

Univ.-Prof. Dr. Helmut Gadner
Institutsleiter

Preface

In the last two years, the Children´s Cancer Research Institute has translated into action structural changes which had been announced already some time ago. On the one hand, a new financial management has been established which makes investment and costing transparent and clear. On the other hand, a new quality management has been introduced and implemented for our spin-off company Labdia Labordiagnostik GmbH which, after an establishing phase of two years, has finally been founded. The diagnostic branches of two of our CCRI laboratories (Cytogenetics and Molecular Genetics of Haematological Neoplasia) have been subsumed under this new name and are now a subsidiary company of the CCRI of which the respective group leaders have been promoted to medical directors. As already mentioned in an earlier scientific report, it is the aim of this limited corporation to capitalize on useful methodological developments which can thus be outsourced from the scientific branch of the CCRI thereby creating new jobs and opportunities.
In addition, part of the tumor immunology group that focuses on the production of tumor vaccines has also been turned into a company of its own and called I-Med limited.
The construction work for our new building at the Zimmermannplatz (at the rear side of the St. Anna Children´s Hospital) has been initiated at the end of 2005. There will be a subterranean garage on which our new institute building will then be erected (financed by lease contract). In the second half-year of 2008 we will hopefully be able to move into our new home, two floors, approximately 2000m^2 of space dedicated to science which will finally end the crowded situation which the CCRI has been struggling with for some time now. At this point I should like to thank all who have contributed to the planning and realization of this project and especially Mrs. Martina Malyar of the Vienna municipal administration for providing the premises and granting generous financial support for the erection of the building. I should also like to inform you that due to introducing new concepts and strategies with regard to public relation and fundraising, our future looks very bright which is especially important for children with cancer here in the eastern provinces of Austria and generally for all those in Austria who depend on special diagnostics and therapies.
Finally, I would like to thank everybody who has helped and supported us and my special thanks go to the staff of the CCRI but also to all external co-workers, cooperating institutions and to the various funds for their financial support. And of course many thanks to the managing committee and members of the "Verein St. Anna Kinderkrebsforschung" who for years have been giving us their time and expertise. Last but not least, I should like to thank all the private donors for their loyalty without which the Children´s Cancer Research Institute would not be able to exist at all.

Univ. Prof. Dr. Helmut Gander
Director

Einleitung

Innerhalb nur weniger Jahre hat zuletzt der Wissenschaftsstandort Wien vor allem im Bereich der „Life Sciences", und hier vor allem auf dem Gebiet der biomedizinischen Forschung, an Attraktivität und Vielfalt gewonnen. Es kam zu Neugründungen von Forschungsinstituten und vielerlei Biotechfirmen. Auch die Erlangung der Unabhängigkeit der Medizinischen Fakultät von der Universität Wien und die Neuordnung der staatlichen Fördermittelvergabe haben wesentlich zu einer Umstrukturierung der biomedizinischen Forschungslandschaft in Österreich beigetragen. Diese Entwicklung ist auch für die St. Anna Kinderkrebsforschung von großer Bedeutung. Einerseits wächst der Wettbewerb um Fördergelder, deren Erhöhungen nicht mit der gewachsenen Nachfrage Schritt hält, andererseits ergeben sich viele neue und interessante Kooperationsmöglichkeiten. Um in diesem Umfeld erfolgreich zu sein und die neuen Ressourcen auch für eine international kompetitive Kinderkrebsforschung effizient zu nutzen, haben wir im Berichtszeitraum verstärkt Maßnahmen gesetzt, die zur nationalen und internationalen Netzwerkbildung beitragen. So beteiligte sich die St. Anna Kinderkrebsforschung erfolgreich am österreichischen Genomforschungsprogramm GEN-AU und im Rahmen des Framework Program 6 der Europäischen Union an mehreren internationalen Projekten, zum Teil in führender Rolle. Zuletzt ging das Forschungsinstitut mit dem Institut für Molekulare Pathologie (IMP), der Medizinischen Universität Wien und zwei kleinen Biotechfirmen eine Partnerschaft mit einem neu gegründeten Ludwig Boltzmann Institut für Krebsforschung (LBI-CR) ein, welche uns Zugang zur Entwicklung und Nutzung neuer Mausmodelle für präklinische Forschung an Kinderkrebserkrankungen ermöglichen soll. Dem von uns vorgegebenen Ziel, den Großteil unserer Forschungsprojekte einer unabhängigen Begutachtung zu unterziehen, konnte weitgehend entsprochen werden, wie aus der steigenden Erfolgsrate der Einwerbung von Drittmitteln, vornehmlich vom Österreichischen Forschungsförderungsfonds FWF und anderen Förderorganisationen, ersichtlich ist. In der Folge wurde dem Forschungsinstitut nach eingehender Evaluation am Ende des Jahres 2004 vom international besetzten Wissenschaftlichen Beirat eine hohe Qualität der wissenschaftlichen Leistungen bescheinigt. Qualitätskontrolle und Qualitätsmanagement waren auch wichtige Themen der organisatorischen Arbeit der St. Anna Kinderkrebsforschung gemeinsam mit dem Kinderspital und mündeten in der Zertifizierung eines neu geschaffenen GMP (Good Manufacturing Practice) Labors und der Einrichtungen zur Zelltransplantation. Verbesserungen im administrativen Bereich, wie die Anstellung einer Qualitätsmanagerin und eines „Grantmanagers" sollen in Zukunft ein qualitativ hohes Niveau diagnostischer und therapeutischer Leistungen, welche durch die hundertprozentigen Töchter des Forschungsinstitutes, der LabDia und der I-Med, erbracht werden, und eine effiziente Nutzung von öffentlichen Forschungsgeldern garantieren und die Forscher selbst im administrativen Bereich entlasten. Wir sind davon überzeugt, dass alle genannten Entwicklungen zu einer weiteren Steigerung des wissenschaftlichen Outputs der St. Anna Kinderkrebsforschung beitragen werden.

Assoc. Prof. Dr. Heinrich Kovar
Scientific Director

Introduction

Within a few years only, Vienna has significantly gained in diversity and attraction in Life Sciences. Particularly in the field of biomedical research several new research institutes and small biotech companies have been founded. In addition, the medical faculty of the Vienna University has become independent and the system of public grant money distribution has been reorganized. These developments have had a significant impact on biomedical research in Austria in general and have also become very important for the Children´s Cancer Research Institute (CCRI). On the one hand, there is increased competition for national grant money, which has not been sufficiently raised to accomodate the increasing demand, on the other hand opportunities for interesting collaborations have broadened. In order to be successful in this changing environment and exploit the novel ressources to remain at the forefront of international pediatric cancer research, we have taken a number of actions during the reporting period aiming at increased national and international networking. Examples are the sustained involvement of the CCRI into the Austrian genome research program GEN-AU and the contribution, partly in a leading role, to a number of international projects funded within the 6th framework program of the European Union. Most recently, we, together with the Institute for Molecular Pathology (IMP), the Medical University of Vienna, and two small biotech companies, joined into a partnership with the newly founded Ludwig Boltzmann Institute for Cancer Research (LBI-CR) which will enable access for the CCRI to the development and use for preclinical research of innovative pediatric cancer mouse models. For the reporting period, our self imposed requirements to subject all our research projects to independent evaluation before their start were largely met, as can be seen from the increasing number of successful grant applications mainly supported by the Austrian Research Fund (FWF) and other funding institutions. As a consequence, the international Scientific Advisory Board attested a high scientific quality of research to the CCRI during its site visit in fall 2004. Quality control and quality management have also been major issues for the organisational work at the CCRI together with the St. Anna Children´s Hospital, and resulted in the certification of a new laboratory for good manufacturing practice (GMP) and all other facilities involved in cellular transplantation. Further improvements in the administrative structure of the CCRI like the recent recruitment of an expert in quality management and of a grant manager will help to maintain the high quality level of diagnostic and therapeutic output of the two CCRI spin-offs, the LabDia and the I-Med, and to guarantee an efficient utilisation of public grant money. These developments shall also relieve laboratory researchers from increasing adminstrative burdens. We strongly believe that these measures will contribute to a further increase in the scientific output of the CCRI.

Assoc. Prof. Dr. Heinrich Kovar
Scientific Director

Part I

Group Descriptions CCRI

Zytogenetik hämatologischer Neoplasien und
Molekulargenetik von Tumorprädispositionssyndromen

Cytogenetics of Hematologic Neoplasia and
Molecular Genetics of Tumor Predispositions Syndromes

Gruppenleiter (Group Leader):	Univ.-Prof. Dr. Oskar A. HAAS (M.D., Professor)
Wiss. Mitarbeiter (Staff Scientists):	DI Anne MORITZ (M.S.) (seit/since 07/04)
	Dr. Sabine STREHL (Ph.D.)
	DI Dr. Andreas WEINHÄUSEL (Ph.D.) (bis/until (05/04)
	Dr. Petra ZEITLHOFER (Ph.D.) (seit/since 10/04)
	[Doktorandin / Ph.D. Student bis/until 09/04]
Doktoranden (Ph.D. Student):	Mag. Karin NEBRAL (seit/since 11/04)
	[Diplomandin / diploma student bis/until 04/04]
Klin. Mitarbeiter (Clin.Collaborator):	Dr. Andishe ATTARBASCHI (M.D.) (St. Anna Kinderspital)
Technische Mitarbeiter (Technicians):	Eva BERGENDI (bis/until 06/04)
	Ulrike ENGEL
	Roland HAHN (bis/until 09/04)
	Eva Maria HÖRANDER (bis/until 10/04)
	Brigitte GRIMM
	Margit KÖNIG
	Mag. Susanna KOSKELA (M.S.) (seit/since 07/04)
	Elisabeth LANG (bis/until 02/04)
	Gudrun NEUMANN
	Bettina NISTLER
	Bettina NOCKER
	Gertrude PASS
	Dr. Hendrati PIRC-DANOEWINATA (Ph.D.) (bis/until 05/04)
	Sigrid RIEDL-HOFFMANN (von/from 07/04 bis/until 11/05)
	Barbara SPITALER (seit/since 01/05)
	Eva WINKLER

Abstracts:

1.1. FISH screening for leukemia-associated genetic abnormalities
S. Strehl, K. Nebral, M. König, B. Spitaler, A. Attarbaschi, O.A. Haas

5.1. Diagnostic evaluation of the Beckwith Wiedemann Syndrome with a semi-quantitative methylation-sensitive PCR assay
P. Zeitlhofer, A. Weinhäusel, O.A. Haas

5.2. X nondisjunction and inactivation patterns in hyperdiploid acute lymphoblastic leukemia (ALL)
P. Zeitlhofer, A. Weinhäusel, M. König, S. Strehl, O.A. Haas

Diese Arbeitsgruppe beschäftigt sich mit der Diagnostik und Erforschung von genetischen Veränderungen bei hämatologischen Neoplasien und bestimmten familiären Tumor-Prädispositionssyndromen. Für unsere Untersuchungen setzen wir zytogenetische, die Fluoreszenz in situ Hybridisierung (FISH) und molekulargenetische Methoden ein. Die zytogenetische Untersuchung von Knochenmark- und Blutproben dient nicht nur der Diagnose und Differentialdiagnose hämatologischer Neoplasien, sondern bildet darüber hinaus die wesentliche Grundlage für unsere Forschungstätigkeit, deren Schwerpunkt in der molekularzytogenetischen und molekulargenetischen Analyse von spezifischen Leukämie-assoziierten genetischen Veränderungen liegt. Diese Untersuchungen erfolgen im Rahmen von diversen Forschungsprojekten in enger Zusammenarbeit mit vielen anderen Arbeitsgruppen sowohl des CCRI, als auch solchen aus anderen nationalen und internationalen Institutionen. Die umfassende Analyse spezifischer genetisches Merkmal macht das biologische Verhalten und den klinischen Verlauf einer neoplastischen Erkrankung vorhersehbar und spielt somit eine wichtige Rolle für die Prognose und die Behandlungsstrategie der Patienten. Deshalb ist ein weiteres Ziel unserer Forschungsarbeit die kontinuierliche und dem neuesten Stand der Technik entsprechende Weiterentwicklung unseres methodischen und diagnostischen Spektrums. In diesem Zusammenhang bilden viele der von uns im Rahmen unserer Forschung etablierten Untersuchungen bereits einen integralen Bestandteil der klinisch relevanten Spezialdiagnostik.

Wir haben in den letzten Jahren unsere Forschungsarbeit auch in Richtung Grundlagenforschung intensiviert und uns z.B. mit der X-Inaktivierung in hyperdiploiden akuten lymphoblastischen Leukämien mit Trisomie oder Tetrasomie X beschäftigt. Durch diese Forschungsarbeit ist es uns im humanen System zum ersten Mal gelungen ein Zweifarben FISH System, das den gleichzeitigen Nachweis von X-chromosomaler DNA und XIST RNA erlaubt zu etablieren. Diese Methode ermöglicht die eindeutige Evaluierung der Anzahl aktiver und inaktiver X-Chromosomen auf dem Einzelzellniveau.

This group is involved in the diagnosis and research of hematological neoplasms and particular forms of tumor-predisposition syndromes. For these analyses we apply various cytogenetic, fluorescence in situ hybridization (FISH) and molecular genetic methods. The cytogenetic analyses are not only important for diagnostic and differential diagnostic purposes, but also provide the basis for our research activities that are focused on the molecular cytogenetics and molecular analyses of specific leukemia-associated genetic aberrations. These investigations are performed in the context of various research projects and in close cooperation with several other groups in the CCRI as well as with other national and international laboratories. The comprehensive analyses of specific genetic markers are predictive of the biological and clinical behavior of the neoplastic disease, and thus play a crucial role for prognosis and therapeutic strategies. Therefore, one of our main research interests focuses on the further development of state of the art diagnostic procedures and the expansion of our methodological and diagnostic spectrum. In this context, during the recent years we have established a variety of new techniques that already have become integrated into and supplement our routine cytogenetic studies.

Over the last years we have also intensified our basic research program and have e.g. studied X-inactivation in hyperdiploid acute lymphoblastic leukemia with trisomy or tetrasomy X. We have, for the first time in the human system, developed a simultaneous dual-color DNA and XIST-RNA FISH assay that enables the unequivocal enumeration of active and inactive X chromosomes on a single cell level.

Biologie der Leukämien

Biology of Leukemias

Gruppenleiterin (Group Leader):	Univ. Prof. Dr. E. Renate PANZER-GRÜMAYER (M.D., Professor)
Postdoktoranden (Postdoct. Res. Fellow):	Dr. Christopher DIAKOS (M.D.) (bis/until 06/04 and again seit/since 07/05)
	Dr. Martina PEHAM (M.D.)
DoktorandInnen (Ph.D. Students):	Dipl.Ing. Alexander DOHNAL (M.S.) (bis/until 10/05)
	Mag. Andrea INTHAL (seit/since 07/04)
	Mag. Gerd KRAPF (seit/since 01/05)
	Heide NIEDERLEITHNER (bis/until 06/04)
Diplomanden (M.Sci. Students):	Martin KIENBERG (09/03-06/04)
Fachhochschule (College Student):	Dominik BECK (seit/since 07/05)
Techn. Mitarbeiterinnen (Technicians):	Dr. Eva CSINADY (seit/since 09/04)
	Susanna FISCHER
	Marianne KONRAD (bis/until 04/04)
	Ing. Ulrike MONSCHEIN

Abstracts:

1.6. Immune receptor rearrangements and leukemia development
S. Fischer, U. Monschein, E. Csinadi, S. Hübner, M. Peham , ER. Panzer-Grümayer

2.6. Modeling critical events in childhood acute lymphoblastic leukemia
C. Diakos, A. Inthal, G. Krapf, D. Beck

3.5. Anti leukemia-specific immune response in children with T-lineage acute lymphoblastic leukemia
A. Dohnal, S. Hübner, ER Panzer-Grümayer

4.1. Analysis of minimal residual diseases in children with acute lymphoblastic leukemia
S. Fischer, U. Monschein, E. Csinadi, M. Konrad, S. Hübner, M. Peham, ER. Panzer-Grümayer
In collaboration with the Austrian ALL and SCT Study Groups (G. Mann, C. Peters, H. Gadner), the German and International BFM-ALL and SCT Study Groups and the European Study Group for the detection of MRD

Unsere Arbeitsgruppe beschäftigt sich sowohl mit der Entwicklung als auch mit dem Entstehungsmechanismus der akuten lymphoblastischen Leukämie (ALL) im Kindesalter. ALL ist die häufigste Krebserkrankung im Kindesalter und kann bis zu 80% mit modernen Behandlungsschemata geheilt werden. Die erste Mutation entsteht in einer Stamm- oder Vorläuferzelle bereits während der Entwicklung im Mutterleib. Weitere Mutationen sind jedoch notwendig, damit eine Leukämieerkrankung entsteht. Während die Information über die Gene, die an chromosomalen Translokationen beteiligt sind, rasch zunimmt, ist die Funktion der daraus resultierenden Fusionsgene praktisch unbekannt. Weiters ist nicht bekannt, welche Ereignisse als sekundär in Frage kommen.

Unser Hauptforschungsgebiet betrifft drei Aspekte der t(12;21) positiven ALL. Wir möchten die funktionelle Bedeutung des *ETV6-RUNX1* Fusionsgens für die Leukämie erfassen, den Beitrag potentieller sekundärer Veränderungen für die Leukämieentstehung untersuchen und schließlich die Immunrezeptorumlagerungen als klon-spezifische Marker für die Untersuchung der klonalen Evolution in der Leukämie nutzen. Diese Studien sollen nicht nur zu einem besseren Verständnis der Leukämieentstehung und der Entstehung von Rückfällen führen, sie werden auch Einblicke in die Mechanismen, mit denen die unterschiedlichen Mutationen ihre spezifische Funktion ausüben, geben. Weiters erwarten wir, dass wir die Fusionsgene, bzw. die von ihnen regulierten Gene, spezifisch ausschalten können. Ein solches Vorgehen birgt nicht nur die Möglichkeit die große Masse der leukämischen Zellen, sondern auch die seltenen Leukämiestammzellen, und darüber hinaus auch die noch nicht vollständig bösartigen Zellen, die als Quelle für die Rückfallerkrankung angenommen werden, zu vernichten.

In einem weiteren Projekt untersuchen wir, ob die T-Zell-Leukämie, eine kleine Untergruppe der ALL mit einem besonderen Risiko für frühe, therapie-resistente Rückfälle im Kindesalter eine spezifische Immunantwort auslösen kann. Unsere bisherigen Daten unterstützen bereits die Aktualität neuer immuntherapeutischer Ansätze.

Als klinisch relevanten Beitrag unseres Labors führen wir den Nachweis der minimalen Resterkrankung (MRD) bei allen Kindern mit ALL aus Österreich durch. Diese Diagnostik wird im Rahmen einer Europäischen Zusammenarbeit mittels der Genumlagerungen von Immunglobulin- und T-Zellrezeptoren als klonspezifischen Marker durchgeführt. Die Menge an MRD stellt einen integralen Bestandteil der Stratifizierung innerhalb der laufenden Therapiestudien dar (BFM ALL 2000, REZ-2002). Dementsprechend wird die Therapieintensität den individuellen Bedürfnissen jedes erkrankten Kindes angepasst. Als Referenzlabor für Österreich nehmen wir regelmässig an Europa-weiten Qualitätskontrollen teil und verfeinern und erweitern darüberhinaus die angewandte Methode.

Our laboratory is interested in the etiology of childhood acute lymphoblastic leukemia (ALL) as well as the pathomechanism of this disease. ALL is the most frequent malignancy in children and can - with contemporary treatment protocols – be cured in about 80%. The first mutation occurs in a stem or progenitor cell frequently already during in utero development, but further molecular events need to occur to lead to the clinical manifestation of leukemia. While increasing knowledge is gained on individual genes that are involved in chromosomal translocations, one of the initiating events in leukemia development, little is known on the function of the corresponding fusion genes as well as on the nature of the pivotal secondary events.

Our major emphasis is on three aspects of ALL carrying the t(12;21). We will evaluate the function of the *ETV6-RUNX1* fusion gene in the leukemia, the role of potential secundary events in leukemia development as well as the immune receptor rearrangements for studying the clonal evolution of leukemia. These studies will not only contribute to a better understanding of leukemia development and the emergence of relapses but also to the elucidation of the mechanisms by which the mutations exert their specific function. It is further expected that we discover whether the particular fusion gene or its downstream genes can be specifically targeted. Such an approach would provide the opportunity to eradicate not only the bulk of leukemic cells but also the rare leukemic stem cells as well as the preleukemic cells, the proposed sources for relapse.

In another project we address the immunogenicity of childhood T-ALL, a small subgroup of leukemias with a particular risk for early, resistant relapse. The identification of novel leukemia-associated proteins (LAA) as well as the induction of a specific T-cell response by one of these LAA support new approaches for immunotherapy.

As a clinically relevant part of our laboratory work we provide minimal residual disease (MRD) diagnostics for all children treated for ALL in Austria. This analysis is performed within a network of European collaboration that uses immunoglobulin and T cell receptor gene rearrangements as leukemia clone-specific markers. The results from MRD analyses during the first months of treatment are an integral part of the stratification system in the current ALL protocols (BFM ALL 2000, REZ-2002). Accordingly, the intensification of chemotherapy is adapted to the individual needs of the child. As the reference laboratory for Austria we are participating in regular European-wide quality control rounds and further refine and expand the applied method.

Solide Tumoren
Solid Tumors

Molekulare Zytogenetik und Biologie Solider Tumoren
Molecular Cytogenetics and Biology of Solid Tumors

Gruppenleiter (Group Leader):	Univ. Doz. Dr. Peter F. AMBROS (Ph.D. Assoc. Professor)
Wiss. Mitarbeiterinnen (Staff Scientists):	Dr. Ingeborg M. AMBROS (M.D.) Dr. Cornelia STOCK (Ph.D.)
Postdoktoranden (Postdoctoral Res. Fellows):	Dr. Eva BOZSAKYOVÁ (seit/since 10/05)
Technische Mitarbeiterinnen (Technicians):	Bettina BRUNNER (seit/since 03/04) Andrea LUEGMAYR Elisabeth VITASEK (bis/until 02/04)
Doktoranden (Ph.D. Students):	Mag. Eva BOZSAKYOVÁ (bis/until 10/05) Mag. Agata KOWALSKA Mag. Rita NARATH (bis/until 01/05)
Klin. Mitarbeiterinnen (Clinical Collaborators):	Univ. Doz. Dr. Ruth LADENSTEIN (M.D.), St. Anna Kinderspital, Wien Dr. Ditha MODRITZ (M.D.), St. Anna, Wien

Abstracts:

1.2. Automatic telomere length measurements in interphase nuclei by IQ-FISH
R. Narath, T. Lörch, K.M. Greulich-Bode, P. Boukamp, P.F. Ambros

1.3. Linkage of sequence data with chromosomal data
P.F. Ambros, A. Kowalska, T. Ramsauer, T. Lörch, Z. Trajanoski

1.4. Visualization of Episomal and Integrated Epstein - Barr Virus DNA by Fiber FISH
J. Reisinger, S. Rumpler, T. Lion and P.F. Ambros

2.1. Induction of tumor cell senescence in cell lines with gene amplification
R. Narath, I.M. Ambros, D. Printz, P. Boukamp, P.F. Ambros

4.3. Detection of tumor cells in the bone marrow and aphaeretic product
D. Modritz, R. Ladenstein, A. Luegmayr, I.M. Ambros, U. Pötschger, H. Gadner, P.F. Ambros

Neben dem langjährigen Ziel der Arbeitsgruppe biologische/genetische Marker zu identifizieren und anzuwenden, die sowohl eine exakte Diagnose als auch eine Einschätzung des klinischen Verhaltens einer Tumorerkrankung erlauben, widmen wir uns der Aufklärung der Mechanismen der Tumorzellalterung und deren möglichen Anwendung als neues therapeutisches Verfahren.

Beim Neuroblastom, dem häufigsten Tumor des frühen Kindesalters, dient vor allem das Vorhandensein einer Amplifikation des *MYCN* Onkogens als Entscheidungsgrundlage für das therapeutische Vorgehen in nationalen wie auch internationalen Studien. Zusätzlich zur routinemäßigen Analyse aller Neuroblastome von Kindern aus Österreich und einer Reihe anderer europäischer Länder, werden auch andere pädiatrische Tumoren verschiedenen histogenetischen Ursprungs auf diagnostisch/prognostisch relevante genetische Veränderungen untersucht. Die Validierung bzw. Qualitätskontrolle dieses wie auch anderer genetischer Marker wird im Rahmen eines EU-Projektes unter der Leitung des CCRI nach europäischem Standard.
durchgeführt. Um die genetischen Parameter eindeutig bestimmen zu können, haben wir die Erkennung und Quantifizierung von genetischen Veränderungen automatisiert. Es ist somit erstmals gelungen auch kritische Regionen, wie die Telomerregionen von individuellen humanen Tumorzellen, zu quantifizieren, was eine Reihe von wertvollen Ergebnissen geliefert hat.

Weiters erlaubt die Visualisierung intakter viraler Genome (EBV) mittels Mehrfarben-FISH, die erstmals erfolgreich durchgeführt werden konnte, neue Einsichten in den Aufbau des viralen Genoms.

Ein weiteres Ziel unseres Labors liegt im eindeutigen Nachweis von Tumorzellen im Knochenmark, Blut und Apphereseprodukten. Wir führen diese Untersuchungen mit einem vollautomatischen Fluoreszenzmikroskop – einem Detektionssystem, das sich durch eine unvergleichlich hohe Sensitivität bei höchst möglicher Spezifität auszeichnet – an allen Blut und Knochenmarkproben österreichischer Kinder mit Neuroblastomen und einer Vielzahl von Proben von Kindern aus anderen europäischen Ländern durch. Weiters fungiert unser Labor als Referenzzentrum in der Hochrisiko-Neuroblastom-Studie an der 19 europäische Länder teilnehmen (Swerts et al, 2005).

Maligne Tumoren mit amplifizierten Onkogenen, wie das Neuroblastom mit *MYCN* Amplifikation, sind äußerst aggressive Tumoren mit hohem Metastasierungspotential. Obwohl eine große Anzahl dieser Tumoren initial auf zytotoxische Behandlung reagiert, sind Rezidive häufig und daher die Entwicklung neuer, zusätzlicher Behandlungsmöglichkeiten notwendig. Die Erkenntnis, dass sogar diese hochmalignen Tumorzellen wieder in den Alterungsprozess (zelluläre Seneszenz) eintreten können, ist fast so neu wie der Forschungsbereich der zellulären Seneszenz in der Onkologie selbst. In vorangegangenen Arbeiten konnten wir spontane Seneszenzprozesse in Onkogen-amplifizierten Neuroblastomzelllinien beobachten und den Anteil seneszenter Tumorzellen durch Behandlung mit Hydroxyharnstoff auf bis zu 100% verstärken. Die seneszenten Zellen weisen eine ausgeprägte Reduktion oder sogar ein völliges Fehlen von überzähligen Onkogenkopien auf, zeigen Änderungen im Expressionsmuster und Phänotyp, sind nicht mehr teilungsaktiv und exprimieren den Seneszenz-Marker β-Galactosidase. Ziel weiterführender Arbeiten ist es, unsere Ergebnisse mit einer Serie von zusätzlichen Neuroblastomzelllinien zu bestätigen und unsere *in-vitro*-Experimente auf eine große Anzahl von Tumorzelllinien auszudehnen.

Besides our group's long-standing aim to identify and apply biological/genetic markers which facilitate an exact diagnosis as well as say something about a tumor's clinical behavior, we are trying to reveal the mechanism of tumor cell-senescence and its possible application as new therapeutic strategy.

In neuroblastoma, the most frequent tumor in early childhood, therapeutic decisions as recommended in national as well as international studies are based on the presence of an amplification of the *MYCN* gene. In addition to routine analyses of all neuroblastomas from children from Austria and from a number of other European countries, other pediatric tumors of various histogenetic origin are also being analyzed with respect to diagnostically/prognostically relevant genetic alterations. The validation and quality control of this and other genetic markers are being performed according to European standards in an EC-project headed by the CCRI. In order to be able to unambiguously define the genetic parameters, we have automated the identification and quantification of genetic aberrations. Thus, it has been made possible for the first time to quantify critical regions, like the telomere-regions of individual human tumor cells, which has yielded a number of highly valuable results.

Furthermore, the visualization of intact viral genomes (EBV) via multi-color-FISH, which could be performed successfully for the first time, brought us new insights into the make-up of the viral genome.

An additional aim of ours is to provide unequivocal evidence of tumor cells in the bone marrow, blood and apheretic products. We perform this analysis via a fully automated fluorescence microscope – a detection system which is characterized by its extraordinarily high sensitivity combined with the highest possible specificity – on all blood- and bone marrow samples from Austrian children with neuroblastoma and on quite a number of samples from children from other European countries. Moreover, our laboratory acts as the reference center for the High Risk-Neuroblastoma-Study in which 19 European countries take part (Swerts et al, 2005).

Malignant tumors with oncogene amplification belong to the most aggressive types of neoplasms. They are characterized by rapid proliferation, high metastatic potential and, although they initially respond to cytotoxic agents, they often evade treatment.
Today, the field of cellular senescence is a novel research area in tumor biology/oncology which could offer new additional treatment possibilities especially to pediatric and adult patients with highly aggressive neoplasms which are frequently associated with gene amplifications. In previous work, we have identified spontaneous senescence processes in oncogene amplified neuroblastoma cell lines and the possibility of inducing and enhancing this pathway to up to 100% of senescent cells after six weeks of treatment with hydroxyurea. The senescent cells show a marked reduction or even lack of supernumerary oncogene copies, changes in expression pattern and phenotype, cease to divide and test positive for the senescence marker β-galactosidase. The aim of this project is to confirm our results on a series of additional neuroblastoma cell lines and to extend our in vitro experiments to a large number of cell lines from tumors of other histogenetic origin.

Molekularbiologie Solider Tumoren

Molecular Biology of Solid Tumors

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Abstracts:

2.2. Functional genomics of the Ewing´s sarcoma translocation t(11;22) -
Identification of target genes for oncogenic EWS-FLI1 in its authentic cellular milieu
J. Ban, C. Siligan, G. Jug, C. Hutter, H. Kovar

2.3. Structural aspects of germline and chimeric EWS and development of inhibitory tools
D. Aryee, R. Bachmaier, K. Mühlbacher, H. Kovar

2.4. Approaches to CD99 function in Ewing´s sarcoma family tumors
M. Kreppel, R. Joas, H. Kovar

Unterschiedliche Krebserkrankungen sind auf unterschiedliche Entstehungsmechanismen zurückzuführen. Sie sind das Ergebnis eines Wechselspiels zwischen genetischem Hintergrund und Art, gewebsspezifischem Zusammenhang und Zeitpunkt des Auftretens spezifischer Mutationen. Obwohl individuelle Tumor-assoziierte Veränderungen allgemeine Mechanismen der Zellentwicklung und –integrität betreffen können, sind wir überzeugt, dass individuelle Aberrationen nur im Bezug zueinander und im Zusammenhang des spezifischen Zellhintergrundes verstanden werden können. Deshalb sind unsere Forschungsaktivitäten vorzugsweise auf die umfassende Analyse einer Tumorart, und nicht auf die spezielle Untersuchung einzelner Tumorwachstumsmechanismen in verschiedenen heterogenen Krebserkrankungen ausgerichtet. Aus diesem Grund steht die Familie der Ewing Tumoren (Ewing´s sarcoma family of tumors – ESFT) seit Bestehen des Molekularbiologie Labors im Mittelpunkt unserer Untersuchungen. Wir interessieren uns für die onkogenen Mechanismen der ESFT spezifischen Chromosomentranslokationen und für ihre Interaktionen mit anderen molekularen Eigenheiten des Tumors. Unsere Ziele sind die Identifikation von Ansatzpunkten für neue therapeutische Ansätze und von prognostischen Merkmalen. Um diese Ziele zu erreichen, wenden wir das gesamte Arsenal moderner molekularbiologischer Werkzeuge an. Die Anwendung sehr teurer Technologien wird durch Zusammenarbeit mit anderen akademischen Forschungseinrichtungen ermöglicht. Deshalb nimmt unsere Forschungsgruppe – zum Teil in führender Rolle - aktiv an nationalen und internationalen Netzwerkprogrammen teil (dem österreichischen Genomforschungsprogramm GEN-AU, dem 6. EU Forschungsrahmenprogramm, dem Vienna Array Consortium). Unser wichtigstes Modell für funktionelle Studien sind 27 genetisch gut charakterisierte ESFT Zelllinien. Zugang zu primärem Tumormaterial soll über die Leitung der molekularen Begleitstudien der internationalen multizentrischen EuroE.W.I.N.G. klinischen Studie und die Teilnahme an dem von der Europäischen Union geförderten Forschungsprojekt PROTHETS erreicht werden, Zugang zu Mausmodellen ermöglicht die Partnerschaft mit dem neu gegründeten Ludwig Boltzmann Institut für Krebsforschung. Unsere Forschungsaktivitäten innerhalb des Berichtszeitraumes konzentrierten sich auf das für die Entwicklung von ESFT verantwortliche Fusionsgenprodukt EWS-FLI1, seine Zielgene, Struktur und Modifikationen, und Interaktionen mit Komponenten genereller Mechanismen der Tumorentstehung wie z.B. p53. Außerdem studierten wir die Funktion eines Zelloberflächenproteins, CD99, welches charakteristischer Weise in ESFT in besonders hohen Mengen vorhanden ist. Die durchgeführten Forschungsarbeiten wurden im Berichtszeitraum von Förderungen des Österreichischen Forschungsförderungsfonds (FWF), der österreichischen Regierung (GEN-AU Programm), der österreichischen Nationalbank, und der Europäischen Union unterstützt.

The etiology of cancer varies with the individual cancer types. It results from the interplay of genetic background, type and developmental timing of tumor associated mutations, and tissue specific context. While individual tumor specific mutations may impact on general mechanisms of cellular integrity (proliferation control, cell differentiation and death, cellular communication and immunogenicity) it is our belief that we have to understand individual aberrations in relation to each other within their specific histogenetic context. We therefore aim at the comprehensive analysis of a single tumor entity rather than to study selected mechanisms of aberrant growth control in various heterogeneous malignant diseases. For this reason, the Ewing´s sarcoma family of tumors (ESFT) has been the focus of our research ever since the establishment of this lab. We aim at understanding the oncogenic activities of the ESFT specific chromosomal translocations and how they interact with other molecular features of ESFT. Our goal is to identify sites of potential therapeutic intervention and prognostic markers. To this end we exploit the whole arsenal of modern molecular biological tools. The application of highly expensive technologies (microarrays, proteomics) is made possible by academic collaborations. To this end, the group actively participates in network programs (The Austrian Genome program GEN-AU, Vienna Array Consortium, EU 6^{th} framework program). Access to mouse models is achieved through partnership in the newly founded Ludwig Boltzmann Institute for Cancer Research. Our major model system to perform functional studies is a panel of 27 genetically well characterized ESFT cell lines. Access to primary patient material will be made possible through the active participation in a leading role in the multi-centric EuroE.W.I.N.G clinical study and through an EU supported international collaborative specific targeted research project (PROTHETS). The research activities in the report period centre around the investigation of the ESFT characteristic fusion gene product EWS-FLI1, its target genes, post-translational modifications, and interactions with components of general mechanisms of tumor development, i.e. p53. In addition, we have been studying the function of the cell surface antigen CD99 which is characteristically highly expressed in ESFT. In the reporting period the group´s research has been supported by the Austrian science fund (FWF, 3 grants), the Austrian government (GEN-AU program), the Austrian Nationalbank, and the European Union.

Tumorimmunologie

Tumor Immunology

Gruppenleiter (Group Leader):	Dr. Thomas FELZMANN (Dr. med)
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	Petra PAUL
Techn. Mitarbeiter (Technicians)	Christina EICHSTILL (seit/since 12/04)
	Dagmar WAGNER
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Abstracts:

3.1. Biology of tumor antigen presentation by dendritic cells
Generation of potent anti-tumor immunity in mice by interleukin-12-secreting dendritic cells
S. Chang-Rodriguez, M. Lindbauer, R. Luger, K. Hüttner, S. Breuer, T. Felzmann

3.2. Biology of tumor antigen presentation by dendritic cells
Semi-mature IL-12 secreting dendritic cells present exogenous antigen to trigger cytolytic immune responses
A. Dohnal, P. Paul, P. Kinross, T. Felzmann

3.3. Cancer vaccination using IL-12 secreting dendritic cells
T. Felzmann, A. Dohnal, H. Hügel, D. Wimmer, C. Eichstill, D. Wagner, G. Ressmann

Die Arbeitsgruppe Tumorimmunologie beschäftigt sich mit der Entwicklung neuer immunologischer Strategien zur Behandlung von Krebserkrankungen. In humanen in vitro Modellsystemen und in Maustumormodellen untersuchen wir die Interaktion von Krebszellen mit Zellen des Immunsystems. Auf Basis dieser Grundlagenforschungsprojekte entwickeln wir neue Konzepte für die Krebsimmuntherapie.

Im Zentrum unserer Grundlagenforschungsprojekte steht das Studium der Biologie der Dendritischen Zellen. Diese sind die zentralen Regulationsstellen im Immunsystem. Eine Dendritische Zelle entscheidet, ob gegen ein bestimmtes Antigen eine Immunreaktion ausgelöst wird, oder ob das Immunsystem sich gegenüber diesem Antigen tolerant verhält. Tumorantigene sind körpereigen und die Dendritische Zelle unterdrückt daher normalerweise die Immunreaktion gegen Tumorzellen. Der entscheidende Schritt in der Auslösung einer Antitumorimmunreaktion ist, diese Toleranz zu durchbrechen. Eine weitere Aufgabe der Dendritischen Zellen ist die Polarisierung des Immunsystems. Dabei steht entweder die Bildung von Antikörpern oder die Aktivierung von Killerzellen im Vordergrund. In der Antitumorimmunität sind die Killerzellen von besonderer Bedeutung, da nur sie in der Lage sind, Tumorzellen direkt zu zerstören. Ein weiterer Forschungsschwerpunkt ist daher die Untersuchung der Steuerung der Immunpolarisierung in Richtung einer Aktivierung von Killerzellen.

Neben der biomedizinischen Grundlagenforschung ist die Umsetzung unserer Forschungsergebnisse in die klinische Anwendung von zentraler Bedeutung. Seit mehreren Jahren führen wir daher klinische Studien durch, welche die Wirksamkeit der Behandlung mit einem Tumorimpfstoff nachweisen sollen. Dabei standen zunächst Fragen der prinzipiellen Durchführbarkeit und der Ausschluss möglicher Nebenwirkungen im Vordergrund. Diese Phase wurde erfolgreich absolviert und als nächster Schritt soll nun die Wirksamkeit der Tumorimpfung mit Dendritischen Zellen nachgewiesen werden.

The tumor immunology laboratory works on the development of new immunologic strategies for the treatment of cancer. In human in vitro model systems and in mouse tumor models we study the interaction of cancer cells with cells of the immune system. On the basis of these research projects we aim to extend the understanding of mechanisms to trigger anti-tumor immunity.

The main target of our research is the biology of the dendritic cell. These cells are the central regulators of immunity. The dendritic cells decide whether an immune response against a specific antigen will be induced, or whether the immune system will be tolerant against this antigen. Tumor antigens are autologous and normally the dendritic cell will not raise an immune response against them. To overcome this tolerance is a key step in triggering anti-tumor immunity. Another function of dendritic cells is the polarization of the immune system towards antibody production or killer cell activation, respectively. In anti-tumor immunity, killer cells are of particular importance because they have the capacity to directly destroy tumor cells. Thus, one important goal of our work is to drive immune polarization in such a way that killer cells will be activated.

In addition to conducting basic research we apply new concepts to the induction of anti-tumor immunity in clinical trials. Since several years we are conducting clinical trials to study the potential of cancer vaccines based on dendritic cells. First our focus was the establishment of the feasibility and the lack of toxicity of a cancer vaccine. After successful completion of this phase we are now focusing on the efficacy of the treatment with a dendritic cell based cancer vaccine.

Transplantationsimmunologie

Transplantation Immunology

Gruppenleiter (Group Leader):	Univ. Doz. Dr. Andreas HEITGER, (M.D., Assoc. Professor)
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	Dr. Markus SEIDEL (M.D.) (seit/since 01/04)
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	Mag. Ursula HAINZ (M.S.) (bis/until 10/05)
	MMag. Birgit JÜRGENS (M.S.) (seit/since 02/05)
Diplomstudentin (Diploma student):	Mag. Birgit JÜRGENS (M.S.) (bis/until 01/05)

Abstracts:

3.7. The tolerogeneic potential of indoleamine 2,3-dioxygenase (IDO) in antigen presenting cells
U. Hainz, B. Jürgens, A. Heitger

3.8. Studies on a possible cooperation of interleukin-10 and rapamycin on the induction of tolerance in a human mixed lymphocyte reaction
B. Jürgens, M. Battaglia, M.G. Roncarolo, A. Heitger

3.9. $CD4^+CD25^+$ T cells after pediatric hematopoietic stem cell transplantation express the putative regulatory T cell marker FOXP3
M. Seidel, U. Ernst, A. Heitger

Diese Arbeitsgruppe beschäftigt sich mit der Erarbeitung von Strategien zur Generation von T-Zell Populationen, die gegen Allo-Antigene des Spenders tolerant jedoch gegen Pathogene der Umwelt (z.B. Viren, humanpathogene Pilze, etc.) funktionell aktiv sind. Diese Zellen eigenen sich hypothetisch optimal für einen adoptiven Zelltransfer nach hämatopoietischer Stammzelltransplantation (HSZT), indem sie ohne das Risiko einer Spender-gegen-Empfänger Krankheit (Graft-versus-host-disease, GvHD) zu erhöhen, die immunologische Kompetenz des Knochenmarkempfängers verbessern, bis die Integrität des Immunsystems durch körpereigene Regeneration wieder hergestellt ist.

In den letzten beiden Jahren haben wir unsere Aktivitäten zur Toleranzinduktion über die Beeinflussung des Tryptophanstoffwechsels intensiviert. Den Aspekt des Einsatzes von IL-10 zur Gewinnung von Typ-1 regulatorischen Zellen haben wir aufgrund ähnlicher mit maximalem Einsatz betriebener Aktivitäten des Labors von M.G. Roncarolo (Mailand), mit dem wir bisher auf diesem Gebiet kollaboriert haben, zunächst hintangestellt. Die Möglichkeit einer klinischen Anwendung dieses Verfahrens bei pädiatrischen Patienten scheint noch nicht, wie erhofft, in greifbarer Nähe.

Der derzeitige Fokus unserer Forschungsaktivität ist die Toleranzinduktion über eine gesteigerte Metabolisierung der für aktivierte T-Zellen essentiellen Aminosäure Tryptophan. Der Metabolismus von Tryptophan wird wesentlich durch die enzymatische Aktivität der Indoleamine-2,3-dioxygenase (IDO) bestimmt. Einem neu entwickelten Konzept zufolge führt die Immunstimulation über Antigen-präsentierende Zellen (APZ), die einen gesteigerten Tryptophanabbau, d.h. eine erhöhte IDO Aktivität aufweisen, interzellulär zu einem Tryptophanmangel und Anhäufung von Tryptophanabbauprodukten (z.B. Kynurenin) und dadurch zu einer Tolerisierung von T-Lymphozyten. Diesbezüglich haben wir unsere Untersuchungen über die immunsuppressive Aktivität von Monozyten nach KMT, dem zumindest partiell ein gesteigerter Tryptophanmetabolismus zugrunde liegt, was schlussendlich zu einer Einschränkung der Funktion von T-Lymphozyten führt, abgeschlossen. Derzeit sind wir bestrebt, den Tryptophanmetabolismus in Monozyten und dendritischen Zellen gezielt zu aktivieren, um das tolerogene Potential von APZ auszuschöpfen. Des weiteren beschäftigen wir uns mit den zellbiologischen Auswirkungen eines erhöhten Tryptophanmetabolismus auf T-Zellen. Das zugrunde liegende klinisch relevante Ziel dieses Ansatzes ist es, die Reaktivität einer T-Zellpopulation so zu modulieren, dass sie keine immunologische Aktivität gegen die Spenderzellen jedoch volle Aktivität gegen Pathogene aufweisen mit dem ultimativen Ziel das Immunsystem nach HSZT sicher und effektiv unterstützen zu können.

This group aims at improving the immune competence after hematopoietic stem cell transplantation (HSCT). Specifically we attempt to generate cell populations of donor T-lymphocytes which are tolerant against the recipient but retain full immunological activity against environmental pathogens. The hypothesis is that such modified T-cell populations are suitable for adoptive cell transfer procedures to improve the post-transplantation immunity without bearing the risk of inducing graft-versus-host disease (GVHD).

In the past two years the approach of inducing T-cell tolerance via modulation of tryptophan catabolism has become the center of our research efforts. Our previous second effort of addressing the tolerogeneic potential of interleukin-10 (IL-10) was halted, as similar experimental approaches that would have to follow from our as yet performed experiments (diploma thesis of B. Jürgens) will be done with maximum effort by the group of M.G. Roncarolo (Milano), which we collaborated with. Overall, the approach of inducing tolerance by exposure of donor T cells to IL-10 in order to tolerize them against recipient cells is not as close to be clinically applied as we anticipated.

The current focus of our research efforts employs the tolerogenic potential of antigen presenting cells (APC) via interference with APC tryptophan metabolism. The rate of tryptophan metabolism is controlled by the enzymatic activity of indoleamine-2,3-dioxygenase (IDO), which is expressed by activated monocytes and dendritic cells (DC). A recently developed concept proposes that a stimulation of T cells by APC that display an enhanced tryptophan metabolism, i.e. high IDO activity, will, by tryptophan depletion and accumulation of tryptophan breakdown products, finally induce tolerance. To this point we have completed our investigation of monocytes that after HSCT display an accelerated tryptophan breakdown and thereby act to suppress T cells. Based on the conclusion of this study, which is that an enhanced tryptophan metabolism may contribute to tolerance induction after HSCT, we are now investigating whether DCs, the most potent APCs, can be induced to express high amounts of IDO and thus promote T-cell tolerance. Our current experiments aim at (i) defining conditions of optimal IDO induction in DCs, (ii) to understand IDO-mediated effects on T cells and (iii) to demonstrate antigen-specific tolerance induction. The ultimate goal is to obtain evidence that T cells can be educated to permanently mount only negligible immune reactions against allo-antigens but preserve full immune competence against viral or fungal antigens. We envision that thus tolerized T cells can be transferred to HSCT recipients safely to support the host immunity with a negligible risk of GVHD until natural immune regeneration has taken place.

Normale und Maligne Hämatopoese
Normal and Malignant Hematopoiesis

Stammzellforschung und
Durchflusszytometrische Phänotypisierung und
Immunologische Leukämiediagnostik

Stem Cell Research and
Flow Cytometric Phenotyping and
Immunological Leukemia-Diagnostics

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Wiss. Mitarbeiter (Staff Scientist):	Dr. Zvenyslava HUSAK (Ph.D.) Dr. Waltraud OGRIS (Ph.D., seit/since 12/05) Dr. Josefa PICHLER (Ph.D., bis/until 10/05)
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Abstracts:

2.5. Functions of CD99 in neoplastic and normal B-lymphopoiesis
Z. Husak, M. Dworzak

3.6. Cytomegalovirus (CMV)-specific immunotherapy by pp65-loaded semi mature DC1 (sm-DC-pp65) after allogeneic stem cell transplantation (SCT) from CMV seronegative donors
J. Pichler, T. Felzmann, J. Stemberger, G. Fritsch

4.2. Immunological detection of minimal residual disease in childhood acute leukemia
A. Schumich, M. Dworzak

6.5. Molecular Diagnosis of Invasive Fungal Infections: Species Identification and Quantitative Analysis by Broad-spectrum PCR Assays
C. Schermann, G. Fritsch

Die in den letzten Jahren durch unsere Arbeitsgruppe untersuchten Schwerpunktthemen aus pädiatrischer Hämato-Onkologie wurden im Zeitraum 2004/2005 intensiv weiterverfolgt. In diesem Zusammenhang widmeten wir uns weiterhin der Anwendung der von uns entwickelten Methode zur Erfassung minimaler residueller Erkrankung bei akuter Leukämie im Kindesalter in der Durchführung von international konzertierten Patientenstudien.

Zusätzlich wurde ein Grundlagenforschungsprojekt zur Bedeutung des CD99 Moleküls in der frühen B Zell Entwicklung, unterstützt durch den FWF, weitergeführt.

Bei der klinischen Routinediagnostik lag der Arbeitsschwerpunkt weiterhin in der Analyse der verschiedenen Leukozyten Subpopulationen, vornehmlich der allogen knochenmarks-transplantierten Patienten, sowie beim Sortieren der Zellen für deren anschließende Genotypbestimmung mittels PCR oder FISH. Im Rahmen der Qualitätskontrolle beteiligte sich unser Labor regelmäßig an Rundversuchen der österreichischen und deutschen Qualitätskontrollstudien welche von INSTAND (Quantifizierung CD34 positiver Zellen) und von ÖQUASTA (Immunstatus und Quantifizierung von CD34+ Zellen und Restleukozyten) organisiert werden. Eine Diplomarbeit verglich verschiedene Methoden der Detektion CMV-spezifischer T Lymphozyten und etablierte eine durchflusszytometrische Multiparameter Analyse auf Basis hochspezifischer Tetramere.

Wichtige Änderungen gab es im Bereich der klinischen Zellmanipulation: Im März 2004 bezogen wir das neu geschaffene GMP Labor nachdem wir die „Betriebsbewilligung gemäß §63 Arzneimittelgesetz" vom *Bundesministerium für Gesundheit und Frauen* erhalten hatten. Zudem sind wir seit März 2005, gemeinsam mit der *Station für Allogene Stammzelltransplantation* und der *Apherese Gruppe* des St. Anna Kinderspitals, als erstes Kinderspital Europas nach den JACIE (Joint Accreditation Committee ISCT & EBMT) Richtlinien zertifiziert. Die unter diesen Vorgaben durchgeführten klinischen Zellmanipulationen wurden im Berichtszeitraum um eine weitere Methode erweitert: Die CD3/CD19 Depletion kann nun anstelle der CD34 Positivselektion bei HLA Unverträglichkeit zwischen Spender und Empfänger eingesetzt werden.

Im Bereich der adoptiven Immuntherapie wurde eine Erfolg versprechende Methode zur zellulären Behandlung von CMV Infektionen in transplantierten Patienten etabliert.

Durch den Erwerb eines neuen Cell-Sorters wurde es ab 2004 möglich, die Sort-Routine auf die 8-Farben-Technik auszuweiten. Die Zahl der durchflußzytometrischen Anwendungen stieg weiter an und lag 2005 bei 2800 Zell-Sorts und 44000 Zell-Analysen. Aufgrund der verwendeten und für das gesamte Institut bereitgestellten Methoden hat das Labor seit 2005 den Status einer *Core Facility*.

Within the last two years, our established methods for immunological detection of minimal residual disease in pediatric acute leukemias have been used for the continued prospective evaluation of a large patient series in internationally concerted studies.

Our research project investigating the functions of the CD99 molecule in early B-lymphopoiesis has been continued and is supported by the Austrian Research Fund (FWF).

In terms of the routine work of the clinical laboratory, the main focus has again been on flow cytometric analysis of leukocyte subtypes as well as on cell sorting for subsequent PCR- or FISH-based determination of donor/recipient genotype. Quality Control (QC) was addressed by participating in the Austrian and German QC trials organized by ÖQUASTA and INSTAND, for the flow cytometric analysis of the immune status and for the enumeration of CD34+ cells and of residual leukocytes in blood and blood components. In a diploma thesis, different methods were compared to detect CMV-specific T lymphocytes, and a sensitive, tetramer-based flow cytometric multi parameter analysis was established.

Important changes occurred in the field of clinical cell manipulation: In March 2004, we moved into our new GMP facility after having received the operating approval from the *Bundesministerium für Gesundheit und Frauen*. Together with the stem cell transplantation unit of the St. Anna Childrens´s Hospital and the apheresis group, we obtained, as the first European Children´s Hospital, JACIE (Joint Accreditation Committee ISCT & EBMT) accreditation for allogeneic transplantation, cell collection and cell processing in March 2005. Under these specifications, we validated an additional cell manipulation procedure: In case of HLA-mismatched donor-recipient pairs, we can now employ T- and B-cell depletion instead of CD34 positive selection. Regarding adoptive immunotherapy, we established a promising cell therapeutic procedure to treat CMV infections in transplanted patients.

Acquisition of a new cell sorting device in 2004 facilitated to extend our sorting routine to 8 colors. A continuous increase in the number of flow cytometric applications was observed: In 2005, 2800 cell-sorts und 44000 cell-analyses we performed, and the laboratory received the status of a core facility.

Molekulare Mikrobiologie und
Entwicklung Genetischer Diagnostik

Molecular Microbiology and
Development of Genetic Diagnostics

Gruppenleiter (Group Leader):	Univ.-Prof. DDr. Thomas LION (M.D., Ph.D., Professor)
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Abstracts:

Der Tätigkeitsschwerpunkt unserer Abteilung liegt seit einigen Jahren im Bereich der mikrobiologischen Forschung, Entwicklung und Diagnostik. Neben Forschungsprojekten, die sich mit der Assoziation verschiedener viraler Pathogene mit malignen Neoplasien befassen, beschäftigen wir uns intensiv mit infektiologischen Problemen bei allogen Knochenmark-transplantierten und onkologischen Patienten unter Chemotherapie. Bei schwer immungeschwächten Kindern stellen neben bakteriellen Infektionen vor allem Virus- und Pilzinfektionen eine sehr ernste Bedrohung dar. Eine frühzeitige und verlässliche Diagnostik ist eine wichtige Voraussetzung für erfolgreiche Therapie. Wir haben daher quantitative, molekulare Detektionsmethoden für mehr als 20 verschiedene pathogene Viren und für mehr als 50 klinisch relevante Pilzformen etabliert. Einige dieser rezenten methodischen Entwicklungen wurden patentiert (Patent Nr: A2119/2004) bzw. sind zur Patentierung eingereicht. Mit diesen, auf der real-time PCR basierenden Verfahren können wir unsere immunsupprimierten Patienten nunmehr auf das Vorliegen von invasiven Infektionen überwachen. Während auf dem Gebiet der molekularen Analyse invasiver Pilzinfektionen zurzeit die ersten Studien durchgeführt werden, konnten wir im Bereich der viralen Infektionen bereits zeigen, dass hochempfindliche, quantitative Analysen mit molekularen Methoden eine Früherkennung disseminierter Infektionen bis zu einigen Wochen vor dem Auftreten der ersten klinischen Symptome ermöglichen. Neue Erkenntnisse, die wir über Adenoviren gewinnen konnten, weisen darauf hin, dass die lebensgefährlichen disseminierten Erkrankungen zumeist von Darminfektionen ausgehen und dass die Überwachung der intestinalen Viruslast mit quantitativen PCR Methoden eine frühzeitige Erkennung von drohenden invasiven Infektionen ermöglicht. Die neuen diagnostischen Möglichkeiten leisten einen wichtigen Beitrag zur Verbesserung der Behandlungsstrategien von lebensbedrohlichen Infektionen bei schwer immunsupprimierten onkologischen Patienten.

Ein weiterer Schwerpunkt unserer Forschungs- und mittlerweile auch Diagnostikarbeit ist die molekulare Analyse von Spender- und Empfänger-Blutzellen (Chimärismus) nach allogener Knochenmarktransplantation (KMT) mit Hilfe von Mikrosatelliten (STR)-PCR Untersuchungen. Untersuchungen des Chimärismus im peripheren Blut und im Knochenmark ermöglichen die Erfassung biologisch und prognostisch wichtiger Parameter. Die konsequente Überwachung des Chimärismus stellt daher bei Patienten nach allogener KMT eine mittlerweile unverzichtbare Grundlage für den Einsatz und die Steuerung von Therapiemaßnahmen dar. Um bei diesem wichtigen diagnostischen Verfahren internationale Qualitätsstandards einzuführen, haben wir vor kurzem eine europäische Studie (EC-Projekt Nr: 2002-01485) abgeschlossen, die von unserer Gruppe koordiniert wurde. Im Rahmen dieser Studie wurde eine europaweit harmonisierte und standardisierte Methodik zur molekularen Detektion und Überwachung des Chimärismus entwickelt. Die Methodik, deren Patentierung derzeit vorbereitet wird, soll unter anderem als Grundlage für optimierte Diagnostik im Rahmen internationaler, multizentrischer KMT-Studien dienen.

Im Rahmen unseres Diagnostik-Programms stellen wir als Referenzlabor für molekulare Leukämiediagnostik Serviceleistungen für nationale und internationale Therapiestudien (ALL-BFM, AML-BFM, CML-Päd, CML-9, CML-11-CELSG) zur Verfügung.

Research, development and diagnostics in the field of microbiology have been a major area of activity in our division over the past years. In addition to research projects focusing on the association of various viral pathogens with malignant neoplastic diseases, a major part of our work is centered around infectious problems of oncological patients undergoing allogeneic bone marrow transplantation or chemotherapy. In addition to bacteria, viral and fungal pathogens are particularly frequent causes of life-threatening infections in severely immunocompromised children. Early and reliable diagnosis is an essential prerequisite for successful therapy. We have therefore developed quantitative molecular detection assays for more than 20 different pathogenic viruses and more than 50 clinically relevant fungus species. Some of the recent technical developments have been patented (Patent No A2119/2004) or are submitted for patenting. The tests, which are based on real-time PCR analysis, facilitate precise surveillance of immunosuppressed patients for the presence of invasive infections. While the first studies on the molecular analysis of invasive fungal infections are currently ongoing, we have already been able to show for viral infections that highly sensitive, quantitative monitoring with molecular methods permits detection of disseminated virus infections up to several weeks prior to the onset of clinical symptoms. Our recent data on adenoviruses indicate that life-threatening disseminated disease mostly emanates from intestinal infections, and that the monitoring of intestinal virus load by quantitative PCR approaches permits early detection of impending invasive infection. The new diagnostic approaches contribute to more efficient treatment strategies against life-threatening infections in severely immunocompromised cancer patients.

Another focus of our ongoing research and, meanwhile, also diagnostic work is the molecular investigation of donor and recipient origin of hematopoietic cells (chimerism) after allogeneic bone marrow transplantation, based on microsatellite (STR)-PCR analysis. The surveillance of hematopoietic chimerism in peripheral blood and bone marrow permits the assessment of biologically and prognostically important parameters. Careful surveillance of chimerism in patients after allogeneic BMT is therefore an indispensable diagnostic tool providing a basis for timely onset and management of treatment. In order to establish international quality standards for this important diagnostic approach, we have recently completed a European study (EC-grant No 2002-01485), which had been coordinated by our group. In this study, a European harmonized and standardized methodology for molecular detection and monitoring of chimerism has been developed. This methodology, which is currently in preparation for patenting, is expected to provide a basis for improved diagnostic support of international, multicenter BMT studies.

As part of our diagnostic program, we serve as a reference laboratory for molecular diagnosis in leukemia, providing services for various national and international therapy studies (ALL-BFM, AML-BFM, CML-Ped, CML-9, CML-11-CELSG).

Dokumentation und Statistik

Documentation and Statistics

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Abstacts:

Die Erfassung von Patienten mit hämato-onkologischen Erkrankungen im Kindes- und Jugendalter in den diversen nationalen und internationalen Therapiestudien ist die Hauptaufgabe dieser Abteilung. Sie legt die Basis, um neue verbesserte Therapiestrategien zur Anwendungen zu bringen und damit die Überlebensraten langfristig zu verbessern. Die Dokumentation reicht von den individuellen Patientendaten und dem jeweiligen Krankheitsprofil bei Diagnosestellung, über die Beschreibung des Behandlungsverlaufes bis zur Erfassung des Therapieerfolges sowie etwaiger Therapie-Nebenwirkungen. Die Behandlungsergebnisse werden regelmäßig evaluiert, um einerseits die Qualität der laufenden Studien (Ansprechraten, Morbidität und Akutmortalität), als auch die Effektivität der Therapie quoad vitam in allen Studien (Erhebung des rezidivfreien Überlebens und der Langzeitüberlebensraten) zu überprüfen.

Die Betreuung der Studiendatenbanken umfasst eine Vielzahl von Aufgaben wie zum Beispiel Entwicklung oder Installation von Eingabeprogrammen und Datenbanksystemen, Erstellung und Verwaltung von Datensatzbeschreibungen, Datentransfer erfasster Daten, Plausibilitätskontrollen, Erstellung von Abfragen zur Unterstützung des Datenmanagements der Dokumentationsassistenten, aber auch die Erstellung von abgeleiteten Datenbanken für studienübergreifende Fragestellungen insbesondere auch die Verbindung klinischer Daten mit im Forschungsinstitut erhobenen, wissenschaftlichen Datensätzen sowie regelmäßiger Datentransfer in übergeordnete Studienzentralen bzw. Register. Die Dokumentationsabteilung fungiert auch als Studienzentrale prospektiver Therapieoptimierungsstudien für Österreich, sowie auch für internationale Kooperationen. Eine enge Zusammenarbeit zwischen analysierenden Statistikern, erfahrenen Klinikern als auch Basiswissenschaftlern ist für die Interpretation von Daten, und die Erarbeitung neuer Ansätze Voraussetzung. In der Dokumentationsabteilung findet sich die erforderliche Kompetenz zur Erstellung neuer Studienprotokolle, insbesondere in Hinblick auf zahlreiche zu lösende statistische Probleme (Stichprobenabschätzungen, Studienplanung mit Festlegung der Studienendpunkte und Analysemethoden, Überprüfung der statistischen Relevanz, Erstellung von Randomisierungslisten, Planung von Interimsanalysen und Endauswertung).

Therapieoptimierungsstudien (TOS) in Österreich waren durch die Neuauflagen der EU Direktive und des österreichischen Arzneimittelgesetzes in ihrer weiteren Durchführung ernsthaft gefährdet. Nach intensiven Verhandlungen mit dem Bundesministerium für Gesundheit und Frauen konnte, durch maßgebliche Mitwirkung von Frau Doz. Ladenstein, eine Unterstützung zum Abschluss eines Rahmenversicherungsvertrages erwirkt werden, der die Fortführung von TOS im Bereich der österreichischen pädiatrischen Hämatologie und Onkologie auch in Zukunft sicherstellt.

The major task of this department is the registration and follow up of haemato-oncological patients including children and young adults, treated on national and international therapeutic studies to establish the basis for improved treatment strategies and survival in the future. This process encompasses documentation of individual patient data and description of disease extension and associated characteristics at diagnosis, documentation of the treatment course and outcome including treatment associated side effects. Regular evaluation of patients in ongoing studies assures recording of response rates, morbidity and mortality to maintain quality controls and to evaluate the long term therapeutic benefit on the basis of relapse free and overall survival rates.

The administration of study banks involves a wide range of tasks: the installation of input programs and of new data bank systems, the development of new input systems or the adjustment of already existing data banks (additional masks/fields), the development and administration of data-set descriptions, the development of data bank systems for the administration of patients, plausibility checks (within the annual routine assessments of the clinical studies), the creation of questionnaires to support data management of the documentation assistants, as well as the development of derived data banks to be able to answer questions that touch on various studies at the same time including research data; the periodical mailing of data to the respective study centres or registries.

The department also acts as study centre for national and international prospective therapeutic studies. A tight interaction of experienced clinicians, statisticians and scientists is the backbone for adequate data interpretation and development of new strategies. These results serve as a basis to discuss new therapy approaches, and thus pave the way to the development of new and improved protocols. This department has a high competence level to develop new treatment protocols, in particular in view of statistical design and tasks (calculation of random checks, the planning of studies including the definition of study goals (primary and secondary study goals), methods of analyses, assessment of statistical relevance, development of randomisation lists, the planning of interim and final analyses).

After 2004 the continuation of 'Treatment Optimising Studies' (TOS) was endangered based on the strict new EC regulations and their implementation within the Austrian Drug Law. The Ministry for Health and Women affairs (under her Ministry M. Rauch- Kallert) has supported these cooperative, area wide treatment policy of the Austrian PediatricHaemato-Oncology Group and has supported the closing of an adjustable insurance contract for Austria. Thus also GCP regulations including role of sponsor, safety regulations and handling of trial management may be accomplished. The CCRI has taken a central role in these regulatory necessities.

Externe Projekte

External Projects

Das Forschungsinstitut unterstützte im Berichtszeitraum externe pädiatrisch-onkologische Forschungsprojekte an folgenden Institutionen:

During the reporting period the Children´s Cancer Research Institute supported external research projects in pediatric-oncology at the following institutions:

Universitätsklinik für Kinder und Jugendheilkunde der Medizinischen Universität Wien
Department of Pediatrics, Medical University of Vienna
Dr. Mariella GRUBER-OLIPITZ (M.D.), Univ. Prof. Dr. Irene SLAVC (M.D.)

Tiroler Krebsforschungsinstitut an der Medizinischen Universität Innsbruck
Tyrolean Cancer Research Institute at Innsbruck Medical University
Dr. Petra OBEXER (PhD)

Institut für Immunologie der Medizinischen Universität Wien
Institute of Immunology, Medical University of Vienna
Dr. Peter STEINBERGER (M.D.), Univ. Prof. Dr. Johannes STÖCKL (M.D.)

Abstracts:

1.5. Neurotrophin-receptors in medulloblastoma
M. Gruber-Olipitz, I. Slavc

2.7. Impact of survivin expression on apoptosis protection and malignant transformation in neuroblastoma
P. Obexer

3.4. Identification of DC-antigens recognized by antibodies in graft versus host sera by retroviral expression cloning
P. Steinberger

Part II

Projects

1. Assessment of genomic aberrations for treatment stratification and as potential targets of novel treatment approaches

1.1. FISH screening for leukemia-associated genetic abnormalities

S. Strehl, K. Nebral, M. König, B. Spitaler, A. Attarbaschi, O.A. Haas

We have continued and extended our FISH analyses for the detection of leukemia-associated genetic aberrations. In this context, our ongoing interest in *MLL* rearrangements [1,2] for diagnostic and research purposes resulted in the detection of novel *MLL* partners [3], and the description of new cases with rare *MLL* fusion genes [4]. Moreover, we have analyzed several cases with t(8;16)(p11;p13) and chimeric MOZ and CBP transcripts (Schmidt et al, 2004). The main focus of our studies was, however, placed on the detection of the TEL*/AML1* fusion gene and *NUP98* rearrangements.

The most common abnormality in childhood ALL is the *TEL/AML1* fusion. It results from a cryptic t(12;21), which is virtually impossible to detect with conventional cytogenetic means alone. Affected patients usually present with favorable features at diagnosis, but nevertheless up to 20% relapse. In a retrospective analysis, we screened 372 patients by interphase FISH to assess the frequency of this abnormality and to determine the potential clinical relevance of the most common secondary karyotype abnormalities. These include the deletion of the non-translocated *TEL* allele and the duplication of the normal and the derivative chromosome 21. We identified 94 (25%) *TEL/AML1*-positive cases and confirmed not only their excellent early response to therapy, but also their significantly better event-free survival, as compared to *TEL/AML1*-negative patients. Moreover, we found preliminary evidence that secondary abnormalities influence the biological and clinical behavior of this ALL subgroup (Attarbaschi et al, 2004).

Nucleoporin 98 kDa (*NUP98*) is a promiscuous gene that is involved in several leukemia-associated translocations and has been reported to be fused to 18 different partner genes in hematological malignancies with 11p15 aberrations. The most frequently observed fusion partners of *NUP98* belong to the homeobox family of transcription factors, whereas the non-*HOX* fusion partners comprise a heterogeneous group of genes. We developed a FISH assays for the detection of *NUP98* rearrangements and determined the incidence and types of *NUP98* rearrangements in 59 consecutive childhood AML cases as well as in 14 adult hematopoietic disorders with 11p15 aberrations (Nebral et al, 2005b). This approach revealed five novel cases with a *NUP98* rearrangement. In one of the cases, we succeeded to identify topoisomerase 2B (*TOP2B*) as new *NUP98* partner gene and to characterize the resulting fusion gene (Nebral et al, 2005a). Intriguingly, this is only the second description of a chromosomal rearrangement involving a topoisomerase and both *TOP1* and *TOP2B* are fused to *NUP98*. This suggests that the choice of partner genes for *NUP98* is not random, and that *NUP98-TOP* fusions may represent a distinct group, similar to the *NUP98-HOX* fusions.

[1] Strehl, S. et al. (2003) Oncogene, 22, 157-160.
[2] König, M. et al. (2002) Br J Haematol, 116, 758-764.
[3] Meyer, C. et al. (2005) Proc Natl Acad Sci U S A, 102, 449-454.
[4] Moore, S.D. et al. (2005) Cancer Genet Cytogenet, 157, 87-89.

This work was supported by the research program "Genome Research for Health" of the Austrian Ministry of Education, Science and Culture (GEN-AU Child).

1.2. Automatic telomere length measurements in interphase nuclei by IQ-FISH

R. Narath, T. Lörch, K.M. Greulich-Bode, P. Boukamp, P.F. Ambros

Background: To benefit from the fluorescence based automatic microscope (FLAME) , which we have already successfully used to quantify gene amplification in neuroblastoma (Narath et al, 2004), we have adapted a PNA FISH technique to automatically determine telomere length in interphase nuclei.

Method: The method relies on the simultaneous acquisition of pan-telomeric signals and reference probe signals. We compared the quantitative figures to those for existing methods, i.e. Southern blot analysis and Quantitative FISH (Q-FISH). Telomere length measurements were undertaken on 8 neuroblastoma cell lines, one cell line showing tumor cell senescence and 7 osteosarcoma tumors.

Results: The data obtained with IQ-FISH technique using a reference probe were reproducible and the parallel analyses of IQ-FISH, Q-FISH and Southern blot revealed highly correlating results. Telomere length measurements disclosed marked differences between the senescent and non-senescent neuroblastoma cell line. Furthermore, this technique seems to be suited to detect ALT (alternative lengthening of telomeres) cells.

Discussion: Quantitative-FISH on interphase nuclei (IQ-FISH) allows the exact quantification of telomere length in interphase nuclei. Thus, this technique enables us to obtain not only exact information on the telomere length, but also morphological and topological details. The automatic measurement of large cell numbers allows the measurement of statistically relevant cell populations (Narath et al, 2005).

1.3. Linkage of sequence data with chromosomal data

P.F. Ambros, A. Kowalska, T. Ramsauer, T. Lörch, Z. Trajanoski

As the visualization of genomic data on the chromosomal level and vice versa the genomic information on the chromosomal level become more attractive, we searched for common information inherent in both. Searching and querying the whole human DNA sequence information has become possible since the first human genome draft sequence was published in 2001. On the cytogenetic level, different techniques have been developed to achieve a lateral structuring (banding pattern) and mapping of chromosomes. However, until today no direct linkage of the sequence data and chromosomal features (bands) exists.

It is well known that the GC content, worldwide available in publicly accessible data banks like Ensembl, is not evenly distributed along the genome but cluster at distinct areas of the genome. On the other hand, base specific fluorochromes have been used for chromosome banding since decades. This prompted us to directly compare both sets of information. We stained human chromosomes with chromomycin A3 displaying the strongest binding to GGCC clusters. We measured the fluorescence intensity of the stained chromosomes and compared these data with the frequency of the GGCC binding motif in the DNA sequence. Landmarks were defined across the chromosome to enable a correlation of chromosomal with sequence data. To prevent differential elongation/stretching artefacts a warp tool was developed for the ISIS FISH imaging system (MetaSystems, Germany). To prove the accuracy of our mapping tool we hybridized different BAC clones on chromomycin stained slides. Assignment of the FISH signals to the GGCC density profile via the chromomycin fluorescence intensity profile allowed the precise mapping of the genes according to their Mb positions in the corresponding DNA sequence data.

Our results indicate that the correlation of sequence information and base specific fluorochromes allows us to define the exact Mb positions of FISH probes (BACs, cosmids etc.), CGH data, break points as well as a direct link of the expression profile along the chromosomes obtained by the

CESH (comparative expressed sequence hybridization) technique with the sequence data. Thus, not only the ISCN information will be available but also a description of every chromosomal position in mega base pair units. This tool allows, for the first time, a direct correlation of chromosomal and sequence data information.

1.4. Visualization of Episomal and Integrated Epstein - Barr Virus DNA by Fiber FISH

J. Reisinger, S. Rumpler, T. Lion and P.F. Ambros

For many Epstein Barr virus (EBV)-associated malignancies, it is still a matter of controversy whether infected cells harbor episomal or chromosomally integrated EBV genomes or both. It is well established that the expression of EBV genes per se carries oncogenic potential, but the discrimination between episomal and integrated forms is of great relevance because integration events can contribute to the oncogenic properties of EBV, whereas host cells that exclusively harbor viral episomes may not carry the risks mediated by chromosomal integration. This notion prompted us to establish a reliable technique that not only allows to unequivocally discriminate episomal from integrated EBV DNA, but also provides detailed insights into the genomic organization of the virus. Here we show that dynamic molecular combing of host cell DNA combined with fluorescence in situ hybridization (FISH) using EBV-specific DNA probes facilitates unambiguous discrimination of episomal from integrated viral DNA. Furthermore, the detection of highly elongated internal repeat 1 (IR1) sequences provides evidence that this method permits detection of major genomic alterations within the EBV genome. Thus, fiber FISH may also provide valuable insights into the genomic organization of viral genomes other than EBV (Reisinger et al, 2005).

1.5. Neurotrophin-receptors in medulloblastoma

M. Gruber-Olipitz, I. Slavc

Medulloblastoma (MB) is the most common malignant childhood brain tumor. Its high risk of leptomeningeal dissemination mandates that standard postoperative treatment for MB not only includes local, but also craniospinal radiotherapy as well as chemotherapy. Such treatment causes long term morbidity including growth disturbances as well as neurocognitive dysfunction, which is particularly severe in young children. These severe therapy-induced sequelae demand a more sophisticated therapeutic approach, which distinguishes patients at a relatively low risk from those at high risk of tumor recurrence. Currently, patient stratification into different therapy arms is based only on clinical parameters such as metastatic stage, residual tumor size and patient age. To optimize treatment, i.e. improve patient outcome while at the same time reducing long-term sequelae in a lower risk subgroup, additional biological prognostic indicators are needed.

Recent reports identified high neurotrophin receptor TrkC mRNA expression as a powerful independent predictor of a favorable survival outcome in MB patients. However, the role of TrkC receptor function in the development and biology of MB remains unclear. Neurotrophins and their cognate receptors TrkA, TrkB, and TrkC regulate proliferation, differentiation and death of neuronal progenitor cells, and may be implicated in the progression of MB. It is likely that individual (or combined) pathways of neurotrophins and their receptors play an important role in the biological and clinical behavior of MB.

To determine downstream effector proteins of TrkC signaling, the medulloblastoma cell line DAOY was stably transfected with a vector containing the full-length TrkC cDNA sequence or an empty vector control. Accounting for the complexity of ligand-induced changes in cellular pathways and effector proteins, we investigated proteomic changes at multiple time points for up to 48 hrs following TrkC receptor activation by its ligand neurotrophin-3. Using proteomics technology we

were able to identify 18 proteins that were differentially expressed over a time period of 48 hours upon NT-3 stimulation. The proteins affected play substantial roles in therapeutically important processes such as differentiation, migration, invasion, proliferation, apoptosis and drug resistance. Almost all of the proteins have been described as being essential in the pathogenesis of different solid tumors, but have not been related to medulloblastoma pathogenesis so far.
Based on the data obtained, we are now determining the role of these proteins in the pathogenesis of MB. This study will lead to a better understanding of the role of neurotrophin receptors in medulloblastoma biology. Characterization of the molecular mechanisms that influence growth and/or differentiation of medulloblastoma may eventually permit the design of novel anti-metastatic, differentiation- or apoptosis-inducing therapies for this common pediatric malignant brain tumor.

1.6. Immune receptor rearrangements and leukemia development

S. Fischer, U. Monschein, E. Csinadi, S. Hübner, M. Peham, ER. Panzer-Grümayer

Immunoglobulin (Ig) and T cell receptor (TCR) gene rearrangements occur during normal lymphoid development and represent a fingerprint for each individual cell and its progeny. Thus, they are widely used as clone-specific markers for clonality analysis of lymphoid cells. They are present in virtually all lymphoid leukemias and may exert specific features as a consequence of the i) age of the patients and/or ii) leukemia subgroup, even though they are not causally linked to the leukemia development. Furthermore, the leukemia clone-specific Ig/TCR rearrangements may provide information as to the time at which the target cell of the leukemia is transformed as well as to the differentiation stage of the precursor cell. We have therefore analyzed the Ig/TCR rearrangements in ETV6-RUNX1 positive ALL and demonstrated that this leukemia subgroup has a high number of rearrangements, an indicator of a high somatic recombination rate, and a typical pattern of rearrangements that is linked to the age of the patients at initial diagnosis (Hübner et al, 2004). By comparing the Ig/TCR rearrangements of leukemias obtained at initial diagnosis and at relapse we found a high incidence of incomplete clonal changes that are derived from clonal selection and/or clonal evolution and seem to be prevalent in younger children. Of interest, these changes do not correlate with the duration of first remission. Our data suggest that a high diversity of Ig/TCR rearrangements in young children in combination with the high recombination rate mirrors the clonal evolution while in older children the patterns of Ig/TCR rearrangements seem to reflect a burned out phenotype due to many more rounds of selection and evolution (Panzer et al, 2005) (Peham et al, 2004). We further propose that these changes occur predominantly during latency (the time between initiation and clinical manifestation of the leukemia) in a preleukemic clone, which is probably represented by a persistent *ETV6-RUNX1* fusion gene positive clone detected at the genomic level (manuscript submitted). Our data are also in support of earlier studies suggesting that the target cell of this type of leukemia is not a multipotent stem cell but rather a B-lymphoid or B-lymphoid/myeloid cell. Of interest, patients with a low-level persistent fusion gene positive clone appear not to develop an early relapse and thus, these cells are not considered fully leukemic. Further studies, however, are needed to define the dignity of these cells as well as to assess the clinical impact of these cells for long-term prognosis in a larger cohort of uniformly treated patients.

2. Factors related to tumor growth, death, differentiation, senescence and metastasis

2.1. Induction of tumor cell senescence in cell lines with gene amplification

R. Narath, I.M. Ambros, D. Printz, P. Boukamp, P.F. Ambros

Recent reports have shown that immortalization does not irreversibly override the natural program of senescence and that tumor cells can re-enter the senescence pathway. As oncogene amplification is one mechanism for overcoming the senescence barrier, we chose a model system where spontaneous down-regulation of extra-chromosomally amplified *MYCN* copies by micronuclei formation leads to revertance of malignancy and to senescence. We tested whether it is possible to augment the entry of neuroblastoma tumor cells in the senescence process by low concentrations of the micronuclei inducing drug hydroxyurea (HU). We exposed 12 neuroblastoma cell lines with extra- and intra-chromosomally amplified *MYCN* copies and cell lines without *MYCN* amplification to HU and followed the morphological and functional changes. The reaction pattern differed among the *MYCN* amplified cell lines but all cell lines displayed either distinct morphological changes (F-cell formation or neurite outgrowth) or a drastic decrease in the survival rate. Focusing on two extra-chromosomally amplified neuroblastoma cell lines, we observed a drastic reduction of the *MYCN* copy number after HU treatment. Up to 100% of the induced neuroblastoma cells displayed a CD44 and MHCI up-regulation. The induced cells showed a twofold reduction of the telomere length, shut down of the telomerase activity, a reduced proliferation rate, down-regulation of p16 and were positive for senescence-associated-beta-galactosidase (SA-β-gal). Thus, induction of the senescence process is possible by HU and could represent a new additional strategy for treatment of *MYCN* amplified tumors.

2.2. Functional genomics of the Ewing´s sarcoma translocation t(11;22) - Identification of target genes for oncogenic EWS-FLI1 in its authentic cellular milieu

J. Ban, C. Siligan, G. Jug, C. Hutter, H. Kovar

Cancer associated EWS-fusion proteins are considered to work as transcription factors deregulating a set of genes that mediate oncogenic transformation. *EWS-FLI1*, the most frequent gene fusion in ESFT has been studied upon ectopic expression in several model systems since the tissue of origin for this disease is still unknown. However, the biological endpoints of EWS-FLI1 expression range from induction of cell death to altered differentiation and transformation depending on the cellular context. We have chosen to define EWS-FLI1 target genes directly in ESFT cells which provide the authentic cellular context and EWS-FLI1 gene dosage, by two complementary approaches: i) Isolation and direct cloning of genomic DNA from chromatin complexes precipitating with EWS-FLI1 antibodies (ChIP) ii) Definition of consistent changes in the transcriptome upon RNA interference (RNAi) mediated knock-down of EWS-FLI1. Using the ChIP approach, we isolated 99 putative direct EWS-FLI1 targets, one fifth of them are known to be involved in either neural differentiation or function. Using the RNAi approach, one third of the genes were found to be deregulated in ESFT cells. For one of these genes, *MK-STYX*, a putative component of mitogen activated kinase signalling, the exact DNA sequence motif binding to EWS-FLI1 was characterized and shown to be sufficient to drive EWS-FLI1 mediated transcriptional activation (Siligan et al, 2005). When comparing differential gene expression profiles of three different ESFT cell lines upon silencing of EWS-FLI1 by RNAi, significant variation in the pattern of deregulated genes was observed. One gene, *HEY1*, which was isolated by the ChIP approach, was significantly induced in a wildtype p53 ESFT cell line upon EWS-FLI1 silencing. *HEY1* induction was accompanied by increased p53 expression and phosphorylation resulting in $p21^{WAF1}$ expression and cell cycle arrest. When testing

three further wildtype p53 expressing ESFT cell lines, p53 and $p21^{WAF1}$ induction was consistently observed when EWS-FLI1 was shut-down. $P21^{WAF1}$ expression was dependent on p53 and, at least in one of the studied cell lines, on *HEY1* induction. These results are consistent with a model in which EWS-FLI1 suppresses basal p53 expression in ESFT thus allowing for unrestricted tumor cell growth, and explains the unusual rarity of p53 mutations in this disease. Ongoing studies aim at confirming further putative direct EWS-FLI1 target genes and at mapping them on the functional pathways deduced from the differential gene expression patterns by RNAi.

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2.3. Structural aspects of germline and chimeric EWS and development of inhibitory tools

D. Aryee, R. Bachmaier, K. Mühlbacher, H. Kovar

The chimeric protein EWS-FLI1, arising from chromosomal translocation in ESFT, acts as an aberrant tumorigenic transcription factor. The transforming activity of EWS-FLI1 minimally requires an ETS DNA binding domain and the EWS NH2 terminus. Proteins interacting with the EWS portion differ between germline and chimeric EWS despite their sharing identical sequences in this domain. Since antibodies specific to distinct EWS and EWS-FLI1 regions would be of prime importance to study their functional properties, we explored the use of the phage-display technology to isolate anti-EWS-FLI1 specific single-chain antibody fragments (scFvs). Using recombinant EWS-FLI1 as bait, 16 independent specific antibody clones were isolated from combinatorial phage display libraries, of which six were characterized in detail. Despite differing in their CDR sequences, all 6 scFvs bound to the same epitope spanning residues 51-75 within the shared minimal transforming EWS domain. However, none of them recognized ESFT-expressed EWS-FLI1 while they bound efficiently to cellular EWS. One scFv, when expressed as an intrabody, efficiently suppressed EWS dependent co-activation of HNF4-mediated transcription in-vivo. These data suggest that a prominent EWS epitope exposed on recombinant EWS-FLI1 structurally differs between germline and chimeric EWS in mammalian cells and that this region is functionally involved in the transcriptional activity of EWS (Aryee et al, in revision).

So far, nothing is known about EWS-FLI1 modifications and how they differ from germline EWS. In a pilot project we have addressed this question by 2D protein electrophoresis and identified several distinct EWS-FLI1 isoforms with a pattern reminiscent of extensive glycosylation. Using specific antibodies we identified O-linked N-acetylglucosamine (O-GlcNAc) side chains associated with EWS-FLI1. This modification is known to be as dynamic as phosphorylation frequently modulating each other in response to various stimuli. We have established conditions to reduce or enforce the level of O-GlcNAc modification and are currently testing the consequences for antibody recognition, protein stability, DNA-binding and transactivation by EWS-FLI1. Mass spectroscopic analysis of gel purified protein is performed in collaboration with Karl Mechtler at the IMP to map the sites of post-translational modifications on EWS-FLI1. Together, these studies will contribute to a better understanding of the structure-based function of cancer-associated EWS-chimeric proteins as opposed to their normal counterparts.

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2.4. Approaches to CD99 function in Ewing´s sarcoma family tumors

M. Kreppel, R. Joas, H. Kovar

High CD99 expression levels and rearrangements of the EWS gene with ETS transcription factor genes characterize the Ewing's sarcoma family of tumors (ESFT). CD99 is a cell surface glycoprotein whose engagement has been implicated in cell proliferation as well as upregulation and transport of several transmembrane proteins in hematopoietic cells. In ESFT, antibody ligation of CD99 induces fast homotypic cell aggregation and cell death although its functional role in these processes remains largely unknown. Here, using an RNAi approach, we studied for the first time the consequences of modulated CD99 expression in six different ESFT cell lines, representing the most frequent variant forms of EWS gene rearrangement. CD99 suppression resulted in growth inhibition and reduced migration of ESFT cells. Among genes whose expression changes in response to CD99 modulation, the potassium-channel modulatory factor KCMF1 was consistently upregulated. In a series of 22 primary ESFT, KCMF1 expression levels inversely correlated with CD99 abundancy. Cells forced to express ectopic KCMF1 showed a similar reduction in migratory ability as CD99 silenced ESFT cells. Our results suggest that in ESFT, high CD99 expression levels contribute to the malignant properties of ESFT by promoting growth and migration of tumor cells and identify KCMF1 as a potential metastasis suppressor gene down-regulated by high constitutive CD99 expression in ESFT (Kreppel et al, 2005).

While the gene expression profiling study after CD99 silencing provided valuable information about the endpoints of the CD99 pathway in ESFT, it does not allow for any conclusions about the early steps of CD99 silencing. We have therefore performed a yeast two-hybrid screen to isolate proteins interacting with the CD99 intra-cellular domain. Several candidate interactors have been identified. Ongoing investigations aim at the confirmation of these protein interactions by independent methods. Since CD99 has been suggested to serve as a potential novel therapeutic target, our studies will help to define the mechanistic consequences of CD99 directed therapeutic intervention.

Supported by the EU FP6 STREP project "PROTHETS".

2.5. Functions of CD99 in neoplastic and normal B-lymphopoiesis

Z. Husak, M. Dworzak

The sialoglycoprotein CD99 is highly expressed on normal human hematopoietic precursors, on neuronal, and on endothelial cells. In malignancies, CD99 has long been recognized as a particularly useful marker of Ewing's tumors and peripheral primitive neuroendocrine tumors. However, further studies have revealed strong CD99 immunoreactivity in a much broader range of cancers, including in particular acute lymphoblastic leukemias (ALL), as has been shown by our research group [1].

Multiple and controversial functions of CD99 are now extensively investigated. Two types of CD99 mRNAs were shown to be differentially expressed in a cell type-specific manner in hematopoietic cells, and two surface isoforms of CD99 were shown to lead either to adhesion or to apoptosis. In summary, the antigen seems to be involved in intracellular transport of transmembrane proteins, in adhesion of lymphoid cells, in migration of leukocytes through endothelia, as well as in proliferation, differentiation and apoptosis of T cells at several different stages of development. Interestingly, the CD99 group of transmembrane proteins and their intracellular precursors do not belong to any known family of (adhesion) molecules.

Current knowledge about CD99 is mostly based on data obtained in studies with T cells, where the antigen is definitely related to the highly important process of immunological selection. Since we found phenotypic similarities between immature T and B cell precursors with respect to CD99, we were prompted to suspect similar importance in B-lymphopoiesis. We therefore investigated CD99 isoform expression, CD99-induced cytoadhesion and apoptosis, and related signalling pathways in the context of B cell precursor ALL and normal immature B cells. Until now, we have established a stroma-cell supported culture system which is essential for lymphoblast cultivation in vitro. Furthermore, assay systems for proliferation, apoptosis, isoform expression on the RNA level, as well as protein expression quantification have been developed and validated. Current in vitro investigations are based on CD99 blocking studies using an array of anti-CD99 monoclonal antibodies against different functional epitopes, as well as RNA interference using CD99-specific si-RNA. We expect novel insights into the functions of CD99 in normal and neoplastic immature B cell precursors, and to clarify the involved subcellular signalling pathways and processes. Since involving several aspects of cell fate in ALL, we consider the unravelling of CD99 pathways of interest also for future prognostic assessments or therapeutic interventions in ALL.

[1] Dworzak et al., Leukemia 2003

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2.6. Modeling critical events in childhood acute lymphoblastic leukemia

C. Diakos, A. Inthal, G. Krapf, D. Beck

The t(12;21)(p13;q22) translocation is present in up to 25% of children with B-cell precursor acute lymphoblastic leukemia (ALL). It results in the fusion of the *ETV6* with the *RUNX1* gene and generates a chimeric transcription factor [1]. The *ETV6-RUNX1* gene fusion is an early or even first event in leukemia development. It commonly occurs already during fetal development but it is considered insufficient to cause clinically overt leukemia by itself based on mouse models and screening of unselected human cord blood samples. The latter indicates a 100-fold excess of newborns with this translocation compared to the incidence of the fusion gene carrying ALL. Most importantly, the second *ETV6* allele is present in these B precursors cells, a finding that contrasts with the respective gene deletion in the majority of *ETV6-RUNX1* positive ALL. Together, these data support the current concept that the deletion of the second *ETV6* gene is a final hit in leukemogenesis. In line with this notion are studies on *ETV6* deletion and immunogenotyping, which lead to the hypothesis that at least part of the relapses do not derive from the dominant leukemic clone at diagnosis, but rather from a potentially therapy-resistant "preleukemic" clone that is still characterized by the *ETV6-RUNX1* fusion gene [2,3], (Peham et al, 2004), (Panzer et al, 2005). While the target cell for this translocation is not known, current data suggest that *ETV6-RUNX1* exerts its specific function in the B cell compartment by a differentiation arrest at the transition from the pro-B to the pre-B stage [4], (Panzer et al, 2005). Our main objectives are: 1) To understand how a cell progresses from an initial genetic hit (*ETV6-RUNX1* gene fusion) to clinically overt leukemia and to assess a causal relationship of proposed critical events in leukemogenesis. 2) To define a chronological order between such pivotal genetic events - from birth to the clinical manifestation(s) of the leukemia. Such backtracking studies are also performed for other subgroups of leukemias (in collaboration with K. Schmitt and colleagues, Linz). First experiments, aimed at elucidating the functional contribution of *ETV6-RUNX1* to the malignant phenotype by suppressing the expression of the fusion gene by small interfering RNAs (siRNA) indicate that *ETV6-RUNX1* is linked to an anti-apoptotic checkpoint, cell cycle regulation as well as

proliferation. Thus, cells that acquired an *ETV6-RUNX1* fusion may not only be arrested in their differentiation but have also an impaired apoptosis, which, in consequence, gives rise to the development of a pre-leukemic clone.

[1] Pui et al. NEJM, 2004, 350:1535-48
[2] Ford et al, Blood 2001, 98:558-564.
[3] Konrad et al, Blood 2003, 101:3635-40
[4] Tsuzuki et al, PNAS 2004, 101/22:8443-48

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2.7. Impact of survivin expression on apoptosis protection and malignant transformation in neuroblastoma

P. Obexer

Neuroblastoma (NB) is the most frequent extracranial solid tumor in childhood. The gain or distal translocation of 17q has been shown to be of prognostic importance. The gene of the apoptosis inhibiting protein survivin located at the mainly affected segment, shows increased expression in neuroblastoma tumors and has been correlated with a malignant phenotype.

Immunoblot and quantitative RT-PCR analysis revealed high survivin expression in the neuroblastoma cell lines SH-EP, LAN-1, IMR32, SH-SY5Y, SK-N-SH, STA-NB1, STA-NB3, STA-NB4, STA-NB8, STA-NB10, STA-NB12, STA-NB13, and STA-NB15. Transgenic expression of survivin strongly enhances the clonogenic survival of STA-NB1 cells and, to a lesser extent, of SH-EP cells. Furthermore, survivin overexpression reduced the sensitivity to the chemotherapeutic agents cisplatin, doxorubicin and etoposide, whereas no effect of transgenic survivin was observed on vinblastine-treated SH-EP and STA-NB1 cells. RNA interference experiments in SH-EP neuroblastoma cells demonstrated that knock-down of survivin significantly reduced viability and caused morphological changes of the cells.

To investigate whether survivin plays a role in the deregulation of cell cycle checkpoint control, we investigated its binding to CDKs and found that endogenous survivin co-immunoprecipitated with CDK4 in neuroblastoma cells. Immunofluorescence analysis and confocal microscopy demonstrated co-localization of survivin with CDK4 and CDK6 on the chromosomes in pro-, prometa- and metaphase cells. The subcellular localization studies in neuroblastoma cells suggest a possible function of survivin/CDK4/6 complexes during mitosis.

As an upstream regulator of survivin we identified the transcription factor FKHRL1 which causes repression of survivin on mRNA steady state and protein level. To study whether repression of survivin is critical for FKHRL1 induced apoptosis we retrovirally infected survivin into SH-EP cells with conditional FKHRL1 expression. We found that survivin strongly inhibits FKHRL1-induced apoptosis for up to 72 hours. Transgenic expression of FKHRL1 sensitizes neuroblastoma cells to doxorubicin- and etoposide-induced apoptosis as measured by FACS-analysis. This increased sensitivity to chemotherapeutic agents can be abrogated by transgenic expression of survivin. In conclusion the repression of the apoptosis inhibitor protein survivin by FKHRL1 is critical for FKHRL1-induced apoptosis and FKHRL1-mediated increase of chemotherapy-induced cell death in neuroblastoma cells.

3. Study and modulation of the immune system and immuno-competence in children and adolescents with cancer to fight infection, tumor growth, and graft versus host disease

3.1. Biology of tumor antigen presentation by dendritic cells

Generation of potent anti-tumor immunity in mice by interleukin-12-secreting dendritic cells

S. Chang-Rodriguez, M. Lindbauer, R. Luger, K. Hüttner, S. Breuer, T. Felzmann

To induce cytolytic immunity, dendritic cells (DCs) need to release bioactive interleukin-12 (IL-12) p70 heterodimeric molecules. To study the role of IL-12 for the generation of an anti-tumor immune response we generated 2 classes of DCs. (1) DCs were initiated to secrete IL-12 by exposure to LPS/IFN-γ for 2 hours resulting, as demonstrated in vitro, in continued IL-12 release for another 24 hours (termed active DCs). (2) DCs were exposed to LPS/IFN-γ for 24 hours and injected into mice at a time point, when IL-12 production had ceased (termed exhausted DCs). These two classes of DCs were probed for their capacity to induce a cytolytic anti-tumor immune response in vivo in a syngeneic mouse tumor model. The mouse tumor cell line K-Balb was engineered to express neomycin phosphotransferase (NPT) as a model tumor antigen. DCs were charged with various NPT-derived antigens, including recombinant NPT protein, whole tumor cell lysate, and NPT-derived synthetic peptides, and the induction of in vivo anti-tumor immunity was determined by measuring tumor growth. Only the injection of active DCs, i.e. cells that maintained the capacity to secrete IL-12, but not exhausted DCs that had lost the ability to produce IL-12, resulted in a measurable deceleration of growth of K-Balb-NPT tumors. This anti-tumor immune response was most pronounced when using recombinant protein as an antigen source, which was evident in a prophylactic as well as in a therapeutic setting. The absence of a response to parental K-Balb tumors confirmed the antigen specificity of the anti-tumor immune response. Together these data provide evidence for the unique capacity of actively IL-12 secreting DCs to trigger effective anti-tumor immunity using exogenous tumor antigens.

3.2. Biology of tumor antigen presentation by dendritic cells

Semi-mature IL-12 secreting dendritic cells present exogenous antigen to trigger cytolytic immune responses

A. Dohnal, P. Paul, P. Kinross, T. Felzmann

Dendritic cells (DC) are candidates for antigen-presenting cells that present exogenous antigen on MHC class I molecules to cytotoxic T-lymphocytes (CTL), a process referred to as cross-priming. We triggered interleukin (IL)-12 release from DC, which was limited to the first day after maturation induction, by a combination of lipopolysaccharide (LPS) and interferon (IFN)-γ. To stimulate T-lymphocytes we used soluble protein derived from lysis of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCL) or ovalbumin loaded onto DC. Co-culture was initiated 2-6 or 48 hours after maturation corresponding to "semi-mature" actively IL-12 secreting type 1 DC (sm-DC1) or a "fully mature" DC1 that had lost the ability to release IL-12 (fm-DC1), respectively. IL-12 secreting sm-DC1 but not fm-DC1 efficiently triggered cytolytic activity in autologous T-lymphocytes. The combination of IL-1β, IL-6, TNF-α, and prostaglandin E2 generated type 2 DC that did not secret IL-12 (DC2) and could not prime T cell cytolytic activity. However, supplementation of cultures using DC2 with IL-12 resulted in CTL activity while the presence of anti-IL-12 monoclonal antibodies in cultures using IL-12 secreting sm-DC1 suppressed CTL activity.

Thus, actively IL-12 secreting sm-DC1 are necessary and sufficient for the antigen-specific expansion of CTL in response to exogenously provided soluble antigen.

3.3. Cancer vaccination using IL-12 secreting dendritic cells

T. Felzmann, A. Dohnal, H. Hügel, D. Wimmer, C. Eichstill, D. Wagner, G. Ressmann

Extensive in vitro and in vivo evidence suggests that a type 1 immune response guided by interleukin (IL) 12 secreting mature type 1 dendritic cells (mDC1) may be more beneficial in cancer immune therapy because it promotes cytotoxic immunity, compared to a type 2 response induced by mDC2, which tend to promote antibody production. We conducted clinical pilot trials for the anti-tumor immune therapy of patients suffering from solid pediatric malignancies, patients with metastatic renal cell carcinoma, and patients suffering from hormone refractory prostate cancer, with tumor antigen loaded DCs. In contrast to previous anti-tumor immune therapy trials we used mDC1 generated by exposure of immature DCs (iDC) to LPS and IFN-γ. Such mDC1 had a typical DC immune phenotype (CD14-, CD83+, CD80+, CD86+, MHC I/II high), released large amounts of IL-12 (between 1-5 ng/106 DCs/ml), and strongly supported a mixed leukocyte reaction. So far, we have treated approximately forty patients with our cancer vaccine. No major toxic side effects of this treatment were observed. In delayed type hypersensitivity test we observed in the majority of patients a positive immune response against the tracer antigen keyhole limpet hemocyanine and some patients also responded to intracutaneously inoculated irradiated tumor cells. In addition, we obtained clear in vitro evidence for the induction of an anti-tumor immune response by showing that T-lymphocytes collected after cancer vaccination responded to a higher extend to tumor antigens as compared to T-lymphocytes collected before the treatment. Because of the advanced disease in the patients included in these trials we did not obtain clear evidence of tumor regression. However, selected patients had signs of tumor regression and an extended relapse free survival. We conclude that the anti-tumor immune therapy with mDC1 is safe and feasible. Follow up studies designed to provide efficacy data for cancer vaccine therapy are under way.

3.4. Identification of DC-antigens recognized by antibodies in graft versus host sera by retroviral expression cloning

P. Steinberger

Allogenic stem cell transplantation (SCT) is used for an increasing number of individuals afflicted with malignant and non-malignant diseases. However, Graft versus-Host Disease (GvHD) continues to be a significant problem in many of these patients leading to decreased quality of life with impaired functional status.

Dendritic cells (DC) are key players in the regulation of immune responses and centrally involved in the development of long lasting and effective immunological processes. A number of studies point to a role of these cells in GvHD. The main goal of this project is to investigate DC-function in this pathology focussing on the significance of DC-reactive autoantibodies in patients suffering from GvHD.

Screening a large panel of GvHD sera that we have obtained from our clinical collaborators at the St Anna Childrens' Hospital and from other centers where stem cell transplantation is performed, we found that about 25% of these patients have strong reactivity with DC. In contrast, elevated reactivity to these cells was not found in any of the sera from healthy individuals. To exclude that this DC-reactivity might stem from alloreactive antibodies we have generated mdDC from several GvHD patients. We found that these sera also strongly reacted with autologous DC and thus indeed contain autoreactive antibodies directed against DC surface antigens.

To analyze DC biology in GvHD we have generated mdDC from a large collective of patients who have undergone allogenic stem cell transplantation and from healthy individuals. To assess the effects of autoreactive sera on their differentiation and function, DC were generated in autologous serum or for control purposes in a serum pool from healthy donors. Mature and immature DC were subsequently analysed regarding their marker profile, cytokine production and allostimulatory capacity. To assess effects of autoreactive sera in DC function allogenic mixed lymphocyte reactions were performed in autologous serum. We are currently evaluating the large body of data generated during these experiments together with our clinical collaborators.
A main goal of this project is the identification of DC antigens recognized by autoreactive GvHD sera. We have generated a representative cDNA expression library from human DC. Within a fruitful collaboration with the group of Prof. Fritsch from the CCRI, we are currently trying to isolate DC antigens recognized by such sera.
We expect that knowledge regarding the antigens involved will aid our understanding of DC autoreactivity and furthermore also increase our understanding of mechanisms underlying GvHD.

3.5. Anti leukemia-specific immune response in children with T-lineage acute lymphoblastic leukemia

A. Dohnal, S. Hübner, E.R. Panzer-Grümayer

For more than a decade, clinical and immunological studies have addressed the elimination of leukemic cells by the immune system. While donor lymphocyte infusions, a special form of immune therapy that can be applied after stem cell transplantation, have induced sustained remissions in patients with a relapse of chronic myeloid leukemias, they have resulted in complete remission only in a small percentage of patients with ALL [1]. It is however not known whether the fast progression of the acute disease outpaces the development of an immune response or if an intrinsic resistance prevents the latter. Studies in B-cell precursor ALL have demonstrated that these leukemias are poor antigen presenting cells and induce T cell tolerance due to the absence of efficient B7 family-mediated costimulation. Thus, repairing the antigen presenting capacity of tumor cells as well as the reversal of tolerance seems to be necessary to elicit a specific immune response. T-ALLs have not yet been analyzed for their immunogenicity. We thus addressed the potential immunogenicity of T-ALL, a small subgroup of childhood leukemia with increased risk for treatment failure and early relapse, by serological identification of leukemia derived antigens by recombinant expression cloning (SEREX) [2]. Thirteen antigens of known genes including four novel isoforms were detected by this approach. The functions of these proteins are associated with mitotis, chromosome condensation as well as with DNA replication, transcriptional and post-transcriptional regulation and finally also apoptosis. Characterization of the novel isoforms revealed a restricted mRNA expression for HECTD1, CX-ORF-15 and hCAP-E in T-ALL cell lines (n=6) and over 60% of primary T-ALLs (n=16). Moreover, specific antibodies against these novel isoforms were detected in 15% to 30% of patients with the highest frequency for HECTD1. The significance of these data was emphasized by the finding that the alternatively spliced HECTD1 protein was present at high levels in T-ALL but not in normal hematopoietic tissues. Our data provide the first evidence that T-ALL may induce an immune response in children thereby supporting new approaches for adoptive immune therapy (manuscript in revision).

[1] Kolb et al, Blood 2004;103:767-776

[2] Sahin et al, Proc Natl Acad Sci USA. 1995; 92:11810-11813

3.6. Cytomegalovirus (CMV)-specific immunotherapy by pp65-loaded semi mature DC1 (sm-DC-pp65) after allogeneic stem cell transplantation (SCT) from CMV seronegative donors

J. Pichler, T. Felzmann, J. Stemberger, G. Fritsch

After SCT, CMV seropositive patients with a CMV IgG negative donor are, despite pre-emptive therapy, at high risk for CMV disease and CMV-related mortality. These patients may profit from CMV-specific immunotherapy.

The matrix phosphoprotein CMV pp65 is targeted by 70%-90% of CMV-specific CD8+ cytotoxic T lymphocytes (CTL). DC are potent antigen presenting cells that are required for the induction of immune responses. IL-12p70-secreting DC1 appear to be important for the initiation of cytotoxic immune responses. The potential of DC to produce bioactive IL-12p70 is limited to the first 24 hours after exposure to a DC1 maturation stimulus. These IL-12 secreting DC are here termed semi-mature DC.

Injection of mature DC pulsed with pp65-derived peptides was well tolerated and induced peptide-specific CTL in CMV seropositive patients that had received a stem cell graft from a seropositive donor. However, this technique is limited by its HLA restriction. The respective pp65-peptides are not known for all HLA types and the known peptides induce only CMV-specific CTL. Long-lasting virus (CMV)-specific immunity seems to be dependent on both CD4+ and CD8+ virus-specific T cells in the recipient. We are now using a promising alternative and load sm-DC1 for immunotherapy with recombinant full-length pp65 protein. This offers the advantage that helper- and cytotoxic cell epitopes may extend the CMV-specific immune response. The phase I-II study includes CMV seropositive patients with CMV infection following SCT from a CMV seronegative stem cell donor. Using IL-4 and GM-CSF stimulation, monocyte-derived sm-DC1pp65 are generated from blood or from an apheresis product of the donor. Thereafter, cells are loaded with recombinant pp65 for 2 hours and matured with LPS/IFN-γ for another 6 hours. Cells are cryopreserved in aliquots of 1 million cells and thawed on clinical request for intranodal injection. The complete cell culture process is performed under GMP-conditions. The generation of DC is confirmed by surface expression of co-stimulatory molecules (FACS), by IL-12p70 secretion (ELISA), and by measuring the T cell stimulatory properties (mixed lymphocyte reaction). The induction of CMV-specific immune response in the recipient is monitored for up to 180 days after sm-DC-pp65 application. The proportion of CMV pp65-specific CD4+ and CD8+ T cells is detected by measuring cytokine secretion of T cells (FACS) and by CMV-specific T cell receptor analysis (HLA tetramer technology). So far, we generated sm-DC-pp65 for four patients, and one patient received the cells.

3.7. The tolerogeneic potential of indoleamine 2,3-dioxygenase (IDO) in antigen presenting cells

U. Hainz, B. Jürgens, A. Heitger

The exploration of the immunomodulatory effects of IDO, the rate limiting enzyme for metabolizing tryptophan in mammals, has become the main focus of research in the field of transplantation immunology in the past years. Starting from our previous observation that the frequently observed impaired T-cell function after hematopoietic stem cell transplantation (HSCT) is based on a suppressor activity of a non-T-cell population [1], we now have demonstrated that IDO activity, mediated by monocytes, substantially contributed to post-HSCT T-cell dysfunction (Hainz et al, 2005). Post-HSCT monocytes were identified to be highly sensitive to be turned into a suppressor population upon stimulation and to mediate their suppressor activity by up-regulating IDO activity (Hainz et al, 2005).

The conceptual view of the immunoregulatory activity of IDO implicates that it is antigen specific [2]. The final result of an IDO mediated effect in the microenvironment, namely (i) depletion of tryptophan (which is essential to complete the activation and proliferation cascade once T cells are stimulated) (ii) accumulation of tryptophan metabolites (such as the kynurenines) that mediate apoptosis, is specific inhibition and apoptotic elimination of T cells. According to the hypothesis this effect should be restricted to those T cells that are activated by the particular antigen which is presented by the antigen-presenting cells (APCs). Building on this concept, we began to investigate the potential of dendritic cells (DC), the most potent subpopulation of APCs, to induce tolerance We do this in the context of our ultimate research goal that is to specifically prevent graft-versus-host disease (GVHD) in HSCT while preserving protective immunity against environmental pathogens, i.e. generate specific non-reactivity of donor T cells against recipient cells. Our most significant findings thus far are: (i) maturation of monocyte-derived DCs with lipopolysaccharide (LPS) and IFN-γ in vitro induced maximum IDO expression and activity after 48 hours of culture; (ii) these IDO^+ DCs, when compared to DCs without IDO activity (DC exposed to LPS/ IFN-γ for 6 hours only, IDO^-), have an impaired capacity to stimulate allogeneic T cells; (iii) this impaired T cell stimulation was overcome by inhibiting IDO activity; (iv) T cell apoptosis in cultures with IDO^+DCs occurs predominantly in activated T cell populations. Together, these observations support the concept that IDO^+ DCs regulate T cell function in an antigen specific fashion. Current approaches assessing the induction of "true tolerance", i.e. the absence of allogeneic responses while preserving e.g. antiviral responses in secondary cultures as well as the set-up of a murine transplantation model, are underway.

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[2] A. Mellor and D. Munn. IDO expression by dendritic cells: Tolerance and tryptophan catabolism; Nature Reviews Immunol 2004; 4:762-774

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3.8. Studies on a possible cooperation of interleukin-10 and rapamycin on the induction of tolerance in a human mixed lymphocyte reaction

B. Jürgens, M. Battaglia, M.G. Roncarolo, A. Heitger

A further approach recently probed to induce alloantigen specific tolerance is to expose donor T cells ex vivo to host cells in the presence of IL-10 [1,2]. The exposure to IL-10 has been suggested to induce a particular T cell population, named type 1 regulatory T cells that suppress naïve T cell responses in an antigen-specific fashion. In a collaborative effort with the laboratory of M.G. Roncarolo (San Raffaele Telethon Institute for Gene Therapy, HSR-TIGET, Milano, Italy) B. Jürgens, building on preliminary findings of U. Ernst, who spent some months in M Roncarolo's lab, investigated a possible cooperative effect of IL-10 with rapamycin [3]. To test the induction of allo-antigen specific tolerance we again used the model of the in vitro mixed lymphocyte reaction (MLR), which is most relevant to human graft-versus-host disease (GVHD). Several important observations were made: (i) In a primary MLR, the combination of IL-10/rapamycin resulted in a higher inhibition of allo-specific proliferative responses as compared to that of IL-10 or rapamycin alone; (ii) Exposure to exogenous IL-10 with/without rapamycin in a primary MLR prevented the development of a memory phenotype in T cells; (iii) Withdrawal of inhibitory IL-10 or IL-10

combined with rapamycin in a second MLR allowed for full recovery of proliferation by time; (iv) Only cultures that were pre-exposed to IL-10 alone, but not when exposed to a combination of IL10/rapamycin or rapamycin alone, in primary MLR were able to release IL-10 after re-challenge with the same antigen. From these experiments we conclude that rapamycin acts as an immunosuppressant augmenting the primary T cell suppressive effect of IL-10. However, rapamycin appeared to also interfere with the release of the regulatory cytokine IL-10 and thus may be of limited value in the generation of definite type 1 regulatory T cells. Both, IL-10 alone as well as IL-10/rapamycin are unable to induce sustained unresponsiveness, i.e. anergy, in bulk T cell populations. These findings have been transferred to and will be published in collaboration with M. Roncarolo's lab. Their current experiments address the issue whether repeated stimulations of allogeneic cultures in the presence of IL-10 with/without rapamycin are necessary to induce true anergy specific to allo-antigens.

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[3] Battaglia, M. and Roncarolo, M. G., The role of cytokines (and not only) in inducing and expanding T regulatory type 1 cells. Transplantation 2004. 77: S16-18.

3.9. $CD4^+CD25^+$ T cells after pediatric hematopoietic stem cell transplantation express the putative regulatory T cell marker FOXP3

M. Seidel, U. Ernst, A. Heitger

In this project we studied the CD4+CD25+ T cell population in pediatric patients after hematopoietic stem cell transplantation (HSCT). The role of CD4+CD25+ T cells after HSCT, whether they represent effector cells mediating graft-versus-host disease (GvHD) or regulatory cells (Treg) suppressing GvHD[1], is unclear. This topic is particularly relevant as some therapies aiming to suppress GvHD suggest eliminating CD4+CD25+ T cells by use of anti-CD25 monoclonal antibodies. Recent evidence proposed the expression of FOXP3 in CD4+CD25+ T cells to specifically identify regulatory T cells. In the current study we monitored the frequency of CD4+CD25+ T cells by flow cytometry in pediatric patients of the transplantation unit of the St. Anna Children's Hospital who had received allogeneic HSCT. Patients with and without GvHD were observed for one year after HSCT. In addition, we examined the amount of FOXP3-mRNA expression and proliferative and suppressor capacities in vitro in highly enriched CD4+CD25+ populations. Our findings first showed that, as compared to controls, the CD4+CD25+ cell frequency was increased after HSCT but was similar in patients with and without GvHD. Strikingly, CD4+CD25+ T cells uniformly expressed high levels of FOXP3-mRNA (~ 2log > CD4+CD25– cells) suggesting that FOXP3 expression and the frequency of CD4+CD25+ T cells are strictly correlated. Post-HSCT CD4+CD25+ FOXP3+ T cells failed to proliferate upon polyclonal stimulation, thus resembling Treg cells. However, even CD4+CD25– cells, when stimulated, were induced to express FOXP3 as they converted to a CD4+CD25+ phenotype. These "induced" CD4+CD25+FOXP+ T cells readily proliferated upon stimulation and failed to suppress CD4+CD25– T cells. Thus, FOXP3 expression appears to generally reflect the frequency of CD4+CD25+ T cells irrespective of the presence of GvHD. CD4+CD25+FOXP3+ populations may contain recently activated T cells that lack typical Treg characteristics. Thus, we clearly demonstrate that FOXP3 expression, in contrast to some recent

literature [2], is not a useful biomarker for indicating GvHD. To clearly define the role of CD4+CD25+ T cells in GvHD, a more extensive approach than simply quantifying FOXP3 expression is required.

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[2] Miura Y, Thoburn CJ, Bright EC, Phelps ML, Shin T, Matsui EC, et al. Association of Foxp3 regulatory gene expression with graft-versus-host disease. Blood 2004; 104(7):2187-93.

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4. Assessment of minimal residual disease for treatment stratification

4.1. Analysis of minimal residual diseases in children with acute lymphoblastic leukemia

S. Fischer, U. Monschein, E. Csinadi, M. Konrad, S. Hübner, M. Peham, E.R. Panzer-Grümayer

In collaboration with the Austrian ALL and SCT Study Groups (G. Mann, C. Peters, H. Gadner), the German and International BFM-ALL and SCT Study Groups and the European Study Group for the detection of MRD

Current cytotoxic treatment protocols induce complete remission according to cytomorphological criteria after induction therapy in 95-98% of children with acute lymphoblastic leukemia (ALL). However, about 20% of children with ALL suffer a relapse, implying that not all leukemic cells are eradicated. Cytomorphological criteria are not sufficient for adequate assessment of the remission status because the threshold of detection is 1-5% of leukemic cells. More sensitive techniques like molecular analyses or flow cytometry have shown recently that the amount of minimal residual disease (MRD) is an independent prognostic parameter. In a European collaboration we have demonstrated that patients can be divided into three different groups based on the combined information of MRD in the bone marrow during the first 3 months of treatment (BFM-90/AIEOP-91 protocols) that distinguish patients with good prognosis from those with poor and intermediate prognosis [1].

In the current BFM-ALL 2000 protocol we use the information of the MRD-based risk group stratification to test whether 1) we can reproduce these data on clinical demands (feasibility study), 2) it is possible to reduce treatment in the MRD-based low risk group without compromising the excellent results and 3) children in the MRD-based intermediate risk and high risk groups will benefit from treatment intensification.

Further, MRD will be prospectively analyzed during the course of the disease in high-risk patients, in children with a relapsed ALL and/or before undergoing stem cell transplantation. Accompanying research is aimed at identifying patients with an extraordinarily rapid response to treatment as identified by day 15 MRD [2] as well as evaluating the prognostic relevance of an additional MRD analysis during consolidation (day 52 of treatment) for the MRD-based intermediate risk group. To separate the patients who are going to be cured from those who will relapse is an important task in the intermediate risk group, since this group comprises about 40% of ALL patients with a relapse rate of about 25% [3].

Methods: Rearrangements of antigen receptor genes result in junctional regions that can be regarded as fingerprint-like clone-specific sequences due to deletion and random insertion of nucleotides. They are used as MRD targets. PCR-based MRD detection by clone-specific junctional regions generally reaches sensitivities of 10e-4 to 10e-5 [4]. Real time quantification of the amount of residual disease is performed by Taqman technology.
Quality control rounds and meetings are regularly performed within the European (ESG) and I-BFM MRD Group to maintain a high standard of the methods and to provide a uniform assessment and report of the data.

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[2] Panzer et al, Blood 95(3):790-794, 2000

[3] Biondi et al, Leukemia 14:1939– 1943, 2000

[4] Pongers-Willemse et al, Leukemia 13;110-118, 1999

4.2. Immunological detection of minimal residual disease in childhood acute leukemia

A. Schumich, M. Dworzak

Acute lymphoblastic leukemia (ALL), the most frequent pediatric cancer, has become a curable disease. However, 20% of children with ALL still relapse. To optimize and economize treatment further, therapy must be tailored upon individual factors of relapse risk, of which the most relevant is response to treatment. This response is estimated by assessing minimal residual disease (MRD) in bone marrow. Flow cytometry (FCM) and polymerase chain-reaction methods (PCR) can both be used in this context, but the latter approach is considered as gold standard. PCR-MRD is now in the phase of prospective validation within current treatment protocols of several of the most renowned childhood leukemia societies, including the "BFM"-association. However, for future broad clinical application, further efforts seem warranted to optimize the cost-effect ratio of MRD assessment. In this respect, FCM, which is technically easier and less time-consuming, may bear several advantages over PCR methods. In a previous investigation with FCM, we have already elaborated risk-determining cut-off values of MRD for children with ALL treated according to BFM protocols [1]. Hence, time has come to assess the exact correlation of PCR and FCM [2].
On the basis of a multicentric international collaboration we have been investigating MRD using our highly standardized 4-color FCM approach in a large series of consecutively recruited pediatric ALL patients (aim n= 600) treated according to the AIEOP-BFM ALL 2000 protocol in Austria, Germany, and Italy. Recruiting of patients to this large study started in 2000, and has been continued until now (anticipated end of recruitment by end of 2006).
The aims and end-points of the study are:
1) Prospective validation of previous FCM-MRD results based on a limited series of n=108 patients (incl. retrospectively elaborated risk-determining cut-off values); refinement of risk grouping according to FCM results from various early treatment time-points.
2) Correlation of FCM with concomitantly applied PCR for MRD detection regarding applicability to patients, concordance in risk estimates, and costs. Comparison of mononuclear versus total leukocyte preparations for MRD assessment.
3) Investigation of options for increasing the reliability and accuracy of FCM-MRD testing, for technical simplification, and for a further reduction of costs:

- Use of new time-points during non-hemoregenerative periods of treatment
- Use of blood as diagnostic material for MRD assessment and risk estimation
- Values of absolute MRD quantification compared to relative estimates.

4) Standardization and quality control of multicentric MRD-assessment in ALL
By the end of this translational study, it should be possible to define whether FCM (as compared to PCR) is the method of choice for future clinical application, regarding prognostic reliability, applicability, and costs of MRD assessment in pediatric ALL.

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4.3. Detection of tumor cells in the bone marrow and aphaeretic product

D. Modritz, R. Ladenstein, A. Luegmayr, I.M. Ambros, U. Pötschger, H. Gadner, P.F. Ambros

This study has been conducted to determine whether accurate monitoring of dynamics of bone marrow (BM) clearing could identify subgroups of neuroblastoma patients with different prognoses. Patients and Methods: Two hundred and nineteen BM specimens of 44 stage 4 patients treated according to Austrian multi-center protocols were tested at diagnosis and given time points during treatment by computer-assisted fluorescence scanning and repositioning system. The automatic immunofluorescence plus FISH (AIPF) device, combining GD2-based immunocytology and subsequent molecular-cytogenetic analysis of the same cells, enables unambiguous detection and exact quantification of the tumor cell infiltrate. Results: The median age of patients was 2.4 years. Median observation time was 5 years; the 5-year overall survival (OS) was 59%±8%. When the kinetics of tumor cell clearing in the BM of *MYCN* amplified and/or 1p36-deleted patients (n=29) was evaluated, a correlation was observed between the speed of clearance and outcome. The 5-year progression-free survival (PFS) was 71%±9% in patients showing rapid BM-clearing within 4 cycles of induction treatment while it was 25%±15% in those with delayed clearing (p=0.018). However, in patients with tumors without *MYCN* amplification and/or del1p36 (n=15) the BM-clearing time did not have the same predictive value. Conclusion: Based on the genetic make-up the prognostic impact of residual BM disease in stage 4 patients appears to differ. The rapidity of BM response in stage 4 neuroblastoma patients with unfavorable genetic features may provide an important tool to predict prognosis and accordingly may imply different therapeutic strategies in the future.

5. Tumor predisposing conditions: contribution of inherited and prenatal mutations, parental origin and parental exposure

5.1. Diagnostic evaluation of the Beckwith Wiedemann Syndrome with a semi-quantitative methylation-sensitive PCR assay

P. Zeitlhofer, A. Weinhäusel, O.A. Haas

The Beckwith–Wiedemann syndrome (BWS, OMIM#130650) is a clinically and genetically heterogeneous intrauterine overgrowth syndrome. It occurs in 1:12.000 - 1:15.000 newborns. Approximately 85% of cases are sporadic and 15% are familial forms. Affected children have a significant risk (4% to 21%) to develop various types of embryonal tumors [1,2]. BWS is caused by genetic or epigenetic alterations in two imprinted domains at 11p15. They contain the growth regulatory genes *IGF2*, *H19*, *KCNQ1OT1* and *CDKN1C*, which are differentially methylated and

mono-allelically expressed in a parent of origin specific manner [3]. Based on the respective methylation abnormality patterns the following sub-forms are distinguished:

- Type I (45-55%) is characterized by the demethylation of the maternal *KCNQ1OT1* allele. These individuals have a low risk for embryonal tumors, but never develop Wilms tumors.
- Type II (5-10%) is characterized by the methylation of the maternal *H19* allele. These individuals have a significant Wilms tumor risk (33-50%).
- Type III (20%) is characterized by a paternal uniparental disomy (UPD) of 11p, most often in mosaic form. These individuals have a significant Wilms tumor risk (30%).
- Type IV (10% of sporadic and 40% of familial cases) is characterized by *CDKN1C* gene mutations that mainly occur in patients of Asian origin. Their tumor risk is very low.
- In addition, rare cases (1%) are found with translocations and micro-deletions that affect the maternal chromosome 11. The tumor risk in these individuals is very low.

This biological and clinical diversity not only underlines the necessity to unequivocally identify individuals with BWS, but also to accurately define the causative epigenetic or genetic defect. We therefore conceived a diagnostic strategy that is based on a methylation-sensitive PCR (MS-PCR) approach. It enables the simultaneous semi-quantitative comparison of all relevant methylated and unmethylated regions in three duplex reactions. Following the validation with previously defined material, we screened samples from 62 potential BWS cases and confirmed the clinical suspicion in 15 (24%) of them: 12 were classified as type I, another one had an UPD (type III) and the remaining two had an unusual pattern that nevertheless was consistent with a BWS.
Our novel MS-PCR assay significantly simplifies and speeds up the previously cumbersome diagnostic evaluation of this complex childhood tumor predisposition syndrome. It helps to identify and classify all currently known BWS forms that concur with methylation disturbances and thereby provides invaluable information for the further clinical management of the affected individuals.

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5.2. X nondisjunction and inactivation patterns in hyperdiploid acute lymphoblastic leukemia (ALL)

P. Zeitlhofer, A. Weinhäusel, M. König, S. Strehl, O.A. Haas

The most common numerical chromosome aberration in childhood ALL is the gain of an extra X chromosome in both male and female patients (Haas, 2004), [1]. We therefore investigated whether this non-disjunction event affects the active and inactive X chromosomes in a random or non-random fashion. In female cases both the active or inactive X may be duplicated in a random or non-random fashion, whereas in male patients only the solitary active homologue can be duplicated. Theoretically, a duplicated active X might subsequently also be subjected to de novo inactivation in either sex. The inactivation status of acquired X chromosomes is usually evaluated by methylation-specific PCR (MS-PCR), which allows the simultaneous quantification of various

differentially methylated polymorphic DNA sequences, such as those in the *HUMARA* or *FMR1* genes. Previous evidence from such analyses in NHL patients suggested that the acquired X chromosomes are and remain always active in male patients, whereas in females both the active and inactive X are duplicated in a random fashion [2]. In childhood ALL this issue has not yet been studied. However, quantification with MS-PCR has its limitations, especially in cases with low blast cells numbers. To overcome this problem, we have developed a simultaneous dual-color DNA/RNA FISH assay that enables the enumeration of active and inactive X chromosomes on a single cell level. FISH was performed with probes specific for the X centromere and the *XIST* RNA, which is exclusively expressed from and covers vast parts of the inactive X in human interphase cells. Following the successful evaluation of the assay on methanol/acetic acid-fixed cells from healthy individuals and 23 cases with various constitutional X chromosome aneuploidies, we analyzed 54 methanol/acetic acid-fixed samples from hyperdiploid cases of childhood ALL. They consisted of 24 males with two X, 23 females with three X and seven females with four X. In contrast to all constitutional control samples, which as expected contained only one active X, two of the three X in leukemic cell samples from both male and female patients were active. In contrast, all female patients with four X had duplicated both the active and inactive X chromosome. These findings prove that the active X is exclusively duplicated. The consistent gain of both the active and inactive X in female cases with four X, on the other hand, further corroborates previously established evidence that a single non-disjunction event leads to the maldistribution of chromosomes [3,4].

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[3] Paulsson K. et al. (2003) Blood, 102, 3010-3015

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6. Diagnostic and Clinical Research and Development

6.1. European harmonized and standardized methodology for molecular detection and monitoring of chimerism

F. Watzinger, S. Preuner, T. Lion (on behalf of the Eurochimerism Concerted Action Consortium)

Transplantation of hematopoietic stem cells from related or unrelated donors is becoming an increasingly important approach to treatment of different malignant and non-malignant disorders. Thus there is growing demand for clinical diagnostic methodologies permitting the surveillance of donor- and recipient-derived hemopoiesis (=chimerism) during the post-transplant period. The techniques currently used are very heterogeneous, rendering uniform evaluation and comparison of diagnostic results between centers difficult. Leading laboratories from ten European countries have therefore performed a collaborative study supported by a grant from the European Commission, the *Eurochimerism Concerted Action*. The aim of this concerted action was the development of a standardized diagnostic methodology for the detection and monitoring of chimerism in patients undergoing allogeneic stem cell transplantation (SCT). A panel of in-house microsatellite markers, selected on the basis of their excellent performance in chimerism analysis at experienced centers, has been carefully evaluated and optimized for quantitative chimerism testing under standardized

experimental conditions. The best markers that had been shown to optimally meet the specific requirements of quantitative chimerism analysis were compiled to the *Eurochimerism panel*. The ability of the Eurochimerism panel to provide informative markers for the monitoring of chimerism within the European population, particularly in the related transplantation setting, was shown to be excellent. In addition to the outstanding informativeness of the Eurochimerism panel, the standardized chimerism assay permits highly sensitive detection and accurate quantification of hematopoietic cells of patient or donor origin. Wide use of the standardized technique for chimerism analysis and the implementation of international consensus criteria for appropriate investigation of chimerism after allogeneic SCT proposed by the Eurochimerism consortium will provide a basis for improved diagnostic support for the clinical management of allogeneic stem cell transplant recipients (Lion et al, 2005b). (Lion et al, in press), (Lion, in press).

6.2. Intestinal Adenovirus infections and risk of disseminated disease in children undergoing allogeneic stem cell transplantation

K. Ebner, S. Matthes-Martin, M. Suda, S. Preuner, M. Rauch, D. Jugovic, F. Watzinger, N. Reisinger, A. Lawitschka, C. Peters, G. Fritsch, H. Gadner, T. Lion

Disseminated adenovirus (AdV) disease is associated with high mortality in allogeneic stem cell transplant (SCT) recipients. We have recently demonstrated that molecular detection of AdV in peripheral blood (PB) precedes the onset of life-threatening virus disease [1]. In most instances, detection of AdV in stool preceded the onset of AdV viremia, thus raising the possibility that intestinal infections represent a potential source of virus dissemination. To address this question, we have monitored more than 130 consecutive pediatric patients transplanted at our center for the presence and the load of AdV in stool and in PB by a real-time PCR approach covering the entire spectrum of currently known human AdV serotypes (Ebner et al, 2005b; Patent pending). Nearly one third of the patients tested positive in serial stool samples, revealing adenoviruses of the subgenera A, B, C, D and F, with strong predominance of subgenus C. Some of the patients had diarrhea, but its occurrence did not seem to correlate with the AdV load detected. None of the patients who revealed maximum levels of AdV positivity below 1x10E6 copies/g of stool have shown viremia during the posttransplant course, while the incidence of viremia in individuals with peak virus levels above this threshold was nearly 70%. The observation of AdV viremia was followed by fatal disseminated AdV disease in several instances, despite treatment with antiviral agents administered upon detection of AdV in PB. By contrast, immune therapy with donor-derived AdV-reactive T cells resulted in elimination of the virus and complete recovery from the infection in most patients who had been offered this type of treatment. Our observations indicate a high risk of progression of intestinal to invasive AdV infection in patients with viral load in stool above 1x10E6 copies/g, particularly in the presence of rapidly expanding virus copy numbers. The median time span between detection of the first log increase of AdV copies in stool above this threshold and first observation of viremia was 11 days. Quantitative monitoring of AdV in stool and early onset of antiviral treatment based on virus proliferation kinetics may therefore be warranted in attempts to prevent progression to systemic infection by timely application of antiviral treatment (Lion et al., Bone Marrow Transplant., 2005).

[1] Lion et al, Blood 102(3):1114-20, 2003

6.3. Molecular analysis of Adenoviruses: phylogenetic, taxonomic and clinical implications

K. Ebner, W. Pinsker, T. Lion

The adenovirus (AdV) hexon constitutes the major virus capsid protein. The epitopes located on the hexon protein are targets of neutralizing antibodies in vivo, serve in the recognition by cytotoxic T cells, and provide the basis for the classification of adenoviruses into the 51 serotypes known to date. We have sequenced the entire hexon gene from human serotypes with incomplete or no sequence information available (n=34) and performed comparative analysis of all sequences. The overall sequence similarity between the 51 human serotypes ranged from 0.7 to 25.4% at the protein level. The sequence information has been exploited to assess the phylogeny of the adenovirus family, and protein distances between the six AdV species (A-F) and among individual serotypes within each species were calculated. The analysis revealed that the differences among serotypes within individual species range from 0.3-5.4% in the conserved regions (765 aa) and from 1.5-59.6% in the variable regions (154-221 aa). Serotypes of different species showed an expectedly greater divergence both in the conserved (5.9-12.3%) and variable (49.0-74.7%) regions. Construction of a phylogenetic tree revealed three major clades comprising the species B+D+E, A+F, and C, respectively. For serotypes 50 and 51 the original assignment to species B and D, respectively, is not in accordance with the hexon DNA and protein sequence data, which placed serotype 50 within species D and serotype 51 within species B. Moreover, the hexon gene of serotype 16, a member of species B, was identified as the product of a recombination between sequences of species B and E. In addition to providing a basis for improved molecular diagnostics and classification, the elucidation of the complete hexon gene sequence in all AdV serotypes yields information on putative epitopes for virus recognition, which may have important implications for future treatment strategies permitting efficient targeting of any AdV serotype (Ebner et al, 2005b), (Ebner et al, 2005a).

6.4. Molecular diagnosis of invasive fungal infections: species identification and quantitative analysis by broad-spectrum PCR assays

L. Baskova, S. Preuner, T. Lion

Invasive fungal infections (IFI) play an increasingly important role as life-threatening complications in severely immunocompromised patients. Early application of antimycotic agents is an essential prerequisite for successful therapy. However, standardized diagnostic techniques permitting rapid, sensitive and, no less importantly, economic screening for the clinically relevant fungi have been lacking. We have developed two different real-time PCR systems for quantitative analysis of pathogenic fungi. The Pan-AC assay (patenting in progress) permits in a single reaction the detection of all important Aspergillus and Candida species, which are responsible for the great majority of IFI in immunosuppressed individuals. In view of the increasing incidence of invasive infections caused by hitherto uncommon fungal species, new diagnostic tests with very broad specificity are required. We have therefore developed an additional two-reaction Pan-fungus assay (patenting in progress), which facilitates quantitative detection of a wide spectrum of fungal species (n>50), including also the newly emerging pathogenic fungi. The assays display high sensitivity and show no cross-reactivity with non-fungal pathogens or human DNA sequences. We have established an additional rapid molecular assay based on PCR fragment length analysis of a variable region in the fungal genome permitting rapid identification of the fungal species present. In a current trial, the new technical approach has been applied to the monitoring of pediatric patients with febrile neutropenia during chemotherapy after allogeneic stem cell transplantation. In view of

the hitherto unclear clinical impact of molecular screening of IFI, serial blood specimens were collected during febrile episodes and investigated in a double-blind fashion. Fungal pathogens were reproducibly identified in peripheral blood of more than 20% of the patients screened, revealing predominantly mold (Aspergillus spp, Fusarium spp) infections. The new assays are readily applicable to routine clinical diagnosis and provide a rapid and economic approach to the screening and monitoring of invasive fungal infections. The study is ongoing to assess the clinical benefit of screening for pathogenic fungi by the highly sensitive and quantitative broad-spectrum real-time PCR techniques introduced.

6.5. Investigations into the quantitative analysis of antigen-specific lymphocytes by flow cytometry

C. Schermann, G. Fritsch

Latent viruses like Cytomegalovirus (CMV) and Adenovirus (AdV) cause significant morbidity and mortality in immune-compromised individuals, particularly in those undergoing allogeneic bone marrow transplantation (BMT). Viral infection requires antiviral treatment despite considerable side effects of these drugs. A technique enabling detection of virus-specific effector lymphocytes in patient blood might prevent such treatment schemes, if specific lymphocytes were detectable.

To address this, we combined the commercially available "cytokine secretion assay" (CSA) with multi parameter flow cytometric (FACS) analysis using 7-8 colors. We examined the cellular secretion of IL2, IL4, IL10 and IFN-γ, by T cell subtypes and NK cells, after non-specific and specific stimulation with staphylococcus enterotoxin B (SEB, control), and with CMV or AdV antigens. Aim of the work was to compare the cytokine profiles of lymphocytes obtained from healthy donors with those of pediatric patients 3 to 12 months after bone marrow transplantation.

IFN-γ was shown to be the dominant cytokine produced by all lymphocytes in both groups after stimulation with SEB and CMV-antigen. In addition, the majority of cytokine-secreting lymphocytes co-expressed CD69. We also observed a considerable non-specific stimulatory effect caused by the source of CMV-antigen initially used for *in vitro* stimulation. Therefore, we focused on the question, whether or not it is possible to use the CSA to detect antigen-specific T cell subtypes against CMV via their IFN-γ secretion. As a kind of control, we implemented a novel and highly sensitive test system, the tetramer technology, which employs HLA-specific tetramers for flow cytometric detection of CMV-specific $CD8^+$ T cells. Comparing the results of both techniques, we found a respectable difference in the amount of CMV-specific $CD8^+$ IFN-γ^+ and $CD8^+$ tet^+ T cells. In addition, non-specific stimulation was observed, even when using a new and more specific stimulus, pp65 recombinant protein, for *in vitro* stimulation. Therefore we propose the combination of CSA and tetramer technology to correctly identify CMV-specific effector T cells.

6.6. European Neuroblastoma Study Reference Centre

R. Ladenstein

SIOPEN-R-NET aims to build a European Research Network to optimise the use of pre-existing European clinical and research infrastructures in the individual countries to improve consistency and complementarity. These activities aim to ultimately improve survival in children suffering from high-risk neuroblastoma and will support risk adopted treatment and quality of life in low and intermediate risk cases. To suffice the multiple aspects of this disease and to achieve the multiple goals, 28 partners are participating in 19 European countries and are interacting with 11 subcommittees working on the various tasks of their particular field and specification.

The whole organisation co-ordinates multi-national European infrastructures of about 200 university clinics, cancer centres and hospitals, research institutes and laboratories in a disease too rare to be advanced on a national level of any of the participating countries. An Internet and Web based centralised data bank and communication system is developed under this thematic neuroblastoma network. The European WEB portal is established (http://www.siopen-r-net.org) and interacting features are currently under development and will go online in 2004. The SIOPEN-R-NET project will build and manage a research network for joint data gathering and/or experiments to improve Europe-wide quality by using adequate preparation, exchange and circulation of reference material, built up material resources and repositories needed for research work. SIOP-R-NET will establish the first European Neuroblastoma tumour bank allowing basic research being competitive world-wide. SIOPEN-R-NET will thus help identifying and describing biological risk factors as initiated by the ENQUA project (European Neuroblastoma Quality Assessment project). By improving disease evaluation, it will establish new diagnostic tests and procedures aiming at detecting early markers and weak signals in pathology, including nucleic acid diagnostic tests, and in vivo imaging. Europe-wide reference assessment and central quality controls will allow new insight into disease response dynamics and a better understanding of minimal metastatic residual disease causing fatal disease recurrence. Guided drug dosing will be introduced to increase drug efficacy to lower toxicity seeking risk adapted treatment intensity, which is directly related to life quality. Immunresponse will be monitored and correlated with clinical outcome. SIOPEN-R-NET will help to improve the quality and user-orientation of these research services to the European research community but also to parent association of families with children suffering from neuroblastoma.
The SIOPEN-R-NET has now reached the third project year successfully with the majority of deliverables and milestones in place. Yearly reports to the EC and amendments have achieved EC authorities approval. A major highlight was the integration and activation of image transfer tools within the RDE system to support the clinical neuroblastoma trials.
The European ESIOP Neuroblastoma group has developed the High Risk Neuroblastoma Study HR-NBL1/ESIOP Study which started accrual on 02/02/2002. The trial is chaired by Ass. Prof. R. Ladenstein and the CCRI is the official European study office. The study is managed through the WEB based Remote Data Entry System supporting online registration and randomisation and which allows direct link and communication to more than 200 European clinics and hospitals. This study tool has been developed by the ARC Seibersdorf Research Gmbh (IT service centre: hrsnbl@telbiomed.at) and is now integrated into the SIOPEN-R-NET project. So far, 546 patients have been recruited to the trial. 63 stage 2/3 MYCN amplified patients and 483 stage 4 patients have been included. The study group has achieved one major study aim in completing accrual to R0 randomisation testing the use of G-CSF (filgastrim) within induction chemotherapy producing profound myelosuppression (236 randomisations so far). The main endpoint of the study is the mean number of febrile episodes. The comparison of the primary endpoint showed a significant reduction of the mean number of febrile episodes ($p=0.015$). The use of G-CSF was also able to significantly lower days in hospital/days with fever and days with antibiotics, haematological toxicity and GUT toxicity on the bases of CTC scores. Overall results are in favour of G-CSF and thus its use is recommended now for the induction treatment in this European trial.

Following the successful recloning of the chimeric antibody ch 14.18/CHO antibody in February 2004 to achieve better production yields, further sub-cloning, productivity and binding testing confirmed a fourth generation sub-clone with superior productivity and resulted in a new master

cell bank. A new large scale production was terminated January 2005. The confidential CMC (Chemistry, Manufacturing and Controls) Report is available for National Coordinators submitting to Ethics and Competent Authorities including safety and stability testing .

The Phase I BRIDGING STUDY using ch14.18/CHO antibody in children with refractory neuroblastoma (an ESIOP Neuroblastoma Protocol: Final Protocol Version as of 15.03.2005, EudraCT Number: 2005-001267-63, Sponsor's Protocol Code Number: SIOPENRNET001 had achieved Ethical Committees approval in three European Countries (Austria, Germany and Italy) and was closed in December 2005. This limited phase I clinical study for equivalence data was activated in June 2005 in three centres to confirm the toxicity profile and pharmacokinetics of the new antibody production. The original protocol as well as a detailed report of these data is available confidentially on request: no SUSARS occurred and the toxicity profile was very much in line with previously published data thus allowing to proceed to activate the second randomisation within the high risk study asking a immunotherapy question after two randomised myeloablative treatments (R1) and stem cell reinfusion.

Supported by the European Commission EC Grant No. QLRI-CT-2002-01768 (project coordinator: Ass. Prof. Ladenstein)

6.7. Langerhans Cell Histiocytosis Study Reference Centre

H. Gadner, N. Grois, M. MinkovPatient Registry:

By the end of 2004 3146 patients with Langerhans cell histiocytosis (LCH) were registered. 2562 of these are enrolled onto the clinical trials.

Ongoing Trials:

LCH III Study

The LCH III study opened in April 2001. After a study period of 53 months, 737 patients were enrolled onto the study. 315 patients were multisystem patients. 157 (50%) patients were "RISK" patients, and 158 patients were "LOW RISK" patients. 422 patients were single system patients. The primary study endpoint for Low Risk patients is the frequency of reactivations after 6 months of therapy comparing the 2 treatment arms with 6 months versus 12 months continuation therapy duration. In 154/158 patients of the "Low Risk Group" information at week 6 was available. Overall 94 Low Risk patients (61%) were randomized and this rate is still lower than the expected randomization rate of 72% . The time of randomization was at a median of 6.4 weeks after treatment start. Considering the current patient accrual with 20-30 randomizations done per year the needed final sample size of 148 patients will be reached by the end of 2008. The primary study endpoint for risk patients is the frequency of nonresponse in risk organs at week 12 comparing the 2 treatment arms with 2 drugs (vinblastine + prednisone) versus 3-drugs (vinblastine, prednisone+methotrexate). 157 risk patients were reported and 134 randomizations were performed (85%). The response in risk organs at week 6/12 is available in 92/124 (74%) evaluable patients. In 32 patients (26%) no information was reported. 9 deaths were reported. This is in keeping with the stopping rules of the study that require a 12-week death rate < 10%. Considering the current patient accrual with 30-35 randomizations done per year the needed final sample size of 228 patients will be reached by the end of 2008.

LCH CNS 2003 Study

The LCH CNS 2003 protocol was released in June 2003 and constitutes a continuation of the LCH CNS 2000 study in a condensed version. It is the goal of the study to implement a uniform diagnostic program, to collect information on the natural history and pathophysiology of the disease, and on the outcome of patients treated with various therapeutic approaches. The recommendations for the diagnostic program include magnetic resonance imaging (MRI) to be done once/year (more frequently in case of tumorous lesions), motor efficiency tests and neurophysiological tests (brain stem evoked potential, visual and event related potentials) to be done once/year, psychometric tests every 2nd year, endocrine evaluation as clinically indicated. No guidelines for specific therapy are included in the protocol at this point.

By August 2005, 99 patients with neurodegenerative CNS disease were registered at the Study Center, however in only a minority of these patients complete diagnostic evaluation according to the protocol is reported: Neurological examination (EDSS, ARS) in 31 patients, psychological tests 27 patients, electrophysiological tests 17 patients.

Diagnostic Programme

● Molecular and immunological leukemia MRD diagnostics (Panzer, Dworzak)

● Detection of disseminated neuroblastoma cells in blood, bone marrow and apheresis products using the Automatic Immunofluorescence Plus FISH (AIPF) method (RCDetect, MetaCyte, MetaSystems, Germany) (Ambros)

● Classical cytogenetic diagnostics of hematological neoplasias and of constitutional chromosome aberrations with various banding techniques (Haas)

● Diagnostics of chromosome breakage syndromes (i.e. Fanconi anemia) (Haas)

● Fluorescence in-situ hybridisation diagnostics (FISH) of:

Chromosome aberrations in neuroblastoma, Ewing´s sarcoma and rhabdomyosarcoma (Ambros):

MYCN amplification, deletion of 1p36, gain on 17q in neuroblastoma; EWS gene rearrangemets (including the cryptic EWS/ERG translocation) and chromosome 8 and 12 gains in Ewing´s sarcomas; FKHR gene rearrangements in alveolar rhabdomyosarcomas

All microdeletion and microduplication syndromes (Haas):

1p36 microdeletion, Wolf-Hirschhorn, Cri-Du-Chat, Williams-Beuren, Prader-Willi, Angelman, Charcot-Marie-Tooth, Miller-Dieker, Smith-Magenis, DiGeorge (CATCH22), and
Kallmann syndromes

Leukemia-associated deletions (selected) (Haas):

del(5q), del(7q), del(11q), del(6q), del(13q), del(17p)

Spectrum of CLL-specific chromosome abnormalities (Haas)

Leukemia- and Lymphoma-specific rearrangements (selected) (Haas):

SIL/TAL - deletion of SIL, t(8;14) - IGH/CMYC, t(8;21) - AML1/ETO, t(9;22) - BCR/ABL, MLL rearrangements, t(12;21) - TEL/AML1, t(11;14) - IGH/CCND1, IGH rearrangements, t(14;18) - BCL2/IGH, t(15;17) - PML/RARA, inv(16) - MYH11/CBFB, E2A rearrangements, t(16;18) – MOZ/CBP

Numerical chromosome changes by whole chromosome painting and 24 color FISH (Haas)

- Molecular genetic analysis and mutation screening of various diseases and syndromes (Haas):

Albright hereditary osteodystrophy / pseudohypoparathyroidism, Angelman syndrome, Apert syndrome / Apert-Crouzon disease, Beckwith-Wiedemann syndrome, Blackfan-Diamond anemia, Carney complex, Denys-Drash syndrome, X-linked lissencephaly / double cortex syndrome / heterotopia, factor V deficiency / coagulation factor V, familial mediterranean fever, fragile X syndrome / marker X syndrome, Frasier syndrome, hereditary pancreatitis, Holt-Oram syndrome, hyper-IgM immunodeficiency, hyperimmunoglobulinemia D and periodic fever syndrome, lymphoproliferative syndrome, X-linked, Gilbert syndrome / Meulengracht syndrome, hyperbilirubinemia I, multiple endocrine neplasia types I, II and IIb (MEN I, MEN II, IIb), Nijmegen breakage syndrome / Ataxia-telangiectasia variant V1, Noonan syndrome, osteopetrosis, paraganglioma, familial nonchromaffin, 1 / PGL1, glomus tumor, carotid body tumors, paraganglioma, familial nonchromaffin, 2 / PGL4, glomus tumor, carotid body tumors and multiple extraadrenal phaeochromocytomas, paraganglioma, familial nonchromaffin, 3 / PGL3, glomus tumors, familial periodic fever, persistent Mullerian duct syndrome / pseudohermaphroditism, Prader-Willi syndrome, primary hypolactasia, progressive myoclone epilepsy / Unverricht and Lundborg disease, pseudoaldosteronismus typ1, Rett syndrome, Silver-Russel syndrome, Torsion dystonia 1 / dystonia musculorum deformans 1, Von Hipple-Lindau syndrome (VHL), WHIM syndrome, Wilms tumor / nephroblastoma / WT1 gene, Wiskott Aldrich syndrome / eczema-thrombocytopenia-immunodeficiency syndrome

- Flow cytometry (Fritsch):

Quantification (4-color, single-platform) of CD34+ cells, of T lymphocytes (for DLI) and of residual leukocytes, erythrocytes or platelets in blood components;

Monitoring of engraftment (4-color, dual-platform);

Enumeration of leukocyte subtypes (4- to 8-color, single- or dual-platform) in blood, bone marrow, and apheresis products;

Cell sorting (up to 8-color) of cells from different sources like blood, bone marrow, or cell culture.

● Cell manipulations (Fritsch):
All cell manipulations are performed under good manufacturing practice (GMP) conditions and include quality control (QC) measurements like flow cytometric analysis and sterility testing:

CD34 positive selection prior to bone marrow transplantation from HLA-mismatched donors; CD3/CD19 depletion prior to bone marrow transplantation in case of defined donor/recipient HLA mismatches, or instead of CD34 positive selection;

Plasma depletion from bone marrow or apheresis products prior to infusion of components obtained from AB0 incompatible donors;

Cryopreservation and storage logistics of autologous or allogeneic blood products (stem cells, DLI);

In vitro generation of dendritic cells presenting pp65-derived MHC1 epitopes, to generate anti-CMV-specific immunity (Pichler).

VARIA

Abgeschlossene Diplomarbeiten/Dissertationen / Completed M.Sc. Diplomas/Ph.D. Theses

D.I. Alexander M. Dohnal (betreut von/supervised by Renate Panzer-Grümayer)
"Anti leukemia-specific humoral immune response in children with T-lineage acute lymphoblastic leukemia"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

D.I. Karin Ebner (betreut von/supervised by Thomas Lion)
"Rolle von Adenoviren bei malignen Tumoren"
(Dissertation zur Erlangung des akademischen Grades Dr.rer.nat. / Ph.D. thesis)

Ursula Hainz (betreut von/supervised by Andreas Heitger)
"The role of tryptophan catabolism in T-cell regulation after hematopoietic stem cell transplantation"
(Dissertation zur Erlangung des Doktor der Naturwissenschaften / Ph.D. thesis)

Ruth Joas (betreut von/supervised by Heinrich Kovar)
"Identification of cellular proteins interacting with the CD99/MIC2 transmembrane protein using the Yeast Two-Hybrid System"
(Diplomarbeit zur Erlangung des akademischen Grades Magister der Naturwissenschaften / M.S. Diploma thesis)

Birgit Jürgens (betreut von/supervised by Andreas Heitger)
"Studies on a possible cooperation of interleukin-10 and rapamycin on the induction of tolerance in a human mixed lymphocyte reaction"
(Diplomarbeit zur Erlangung des akademischen Grades Magistra der Naturwissenschaften / M.S. Diploma thesis)

Rita Narath (betreut von/supervised by Peter Ambros)
"Quantification of revertant tumor cells in MYCN amplified neuroblastoma cell lines"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Karin Nebral (betreut von/supervised by Sabine Strehl und/and Oskar A. Haas)
"Detection and characterization of *NUP98* rearrangements in hematopoietic malignancies"
(Diplomarbeit zur Erlangung des akademischen Grades Magistra der Naturwissenschaften / M.S. Diploma thesis)

Heide Niederleitner (betreut von/supervised by Renate Panzer-Grümayer)
"Detection of a proposed preleukemic clone in patients with TEL-AML1 positive ALL"
(Diplomarbeit zur Erlangung des akademischen Grades Mag.rer.nat. / M.S. Diploma thesis)

Claus Schermann (betreut von/supervised by Gerhard Fritsch)
"Investigations into the quantitative analysis of antigen-specific lymphocytes by flow cytometry"
(Diplomarbeit zur Erlangung des akademischen Grades Magister der Naturwissenschaften / M.S. Diploma thesis)

Christine Siligan (betreut von/supervised by Heinrich Kovar)
"Isolation of EWS-FLI1 target genes in Ewing´s sarcoma"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Manuel Steiner (betreut von/supervisor by Milen Minkov und/and Helmut Gadner)
"Reactivations in Langerhans cell histiocytosis"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Edda Unger (betreut von/supervisor by Nicole Grois und/and Helmut Gadner)
"Permanent Consequences in LCH patients with focus on neuropsychological sequelae – a single center study"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Martha Wnorowski (betreut von/supervisor by Nicole Grois und/and Helmut Gadner)
"Pattern and Course of neurodegenerative Langerhans cell Histiocytosis"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Petra Zeitlhofer (betreut von/supervised by Andreas Weinhäusel und/and Oskar A. Haas)
"X Chromosome Nondisjunction and Inactivation Mechanisms in Childhood Acute Lymphoblastic Leukemia (ALL) and Non Hodgkin Lymphoma (NHL)"
(Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften / Ph.D. thesis)

Alexander Zimmerhackl (betreut von/supervised by Thomas Lion)
"Rolle von Polyoma-Viren bei malignen Tumoren"
(Diplomarbeit zur Erlangung des akademischen Grades Mag.rer.nat. / M.S. Diploma thesis)

Fremdgeförderte Projekte / External Grants

EU Projekt SIOPEN-R-NET Biology substudy and Bone marrow substudy
EC grant QLRI-CT-2002-01768; (11/02-04/06); (Peter Ambros / Ruth Ladenstein)

"Antitumorimmuntherapie"
Forschungsförderungsfond für die Gewerbliche Wirtschaft (FFF) / Austrian Industrial Research Promotion Fund
Projekt Nr./Project No Zl. 804445 (since 05/2001); (Thomas Felzmann)

"Klinische Entwicklung eines Immuntherapeutikums zur Krebsbehandlung basierend auf Interleukin-12 freisetzenden dendritischen Zellen zur direkten Aktivierung von Killerzellen"
Wiener Wirtschaftsförderungsfonds (WWFF) / Vienna Business Agency Projekt Nr./Project No GZ 16884/02 (since 12/2002); (Thomas Felzmann)

"Therapeutic Cellular Vaccines"
6. Rahmenprogramm der Europäischen Union/6th Framework Program of the European Union (EU-FP6)
Projekt Nr./Project No 503583; (since 8/2003); (Thomas Felzmann)

The LCH study reference center
is supported by the family organizations "Histiozytosehilfe e.V." (Germany),"Histiocytosis Association of America / Canada or Belgium", "The Histiocytosis Research Trust" (GB), "Shelta Australia" (Nicole Grois)

"Analysis of X Chromosome Nondisjunction and Inactivation Mechanisms in Childhood acute lymphoblastic leukemia"
Fonds zur Förderung der wissenschaftlichen Forschung (FWF) / Austrian Science Foundation
Projekt Nr./Project No. P1515; (10.2001-03.2004); (Andreas Weinhäusel / Oskar A. Haas)

"European Leukemia-Net"
Network of excellence Projekt: Strengthen and develop scientific and technological excellence in research and therapy of leukemia (CML, AML, ALL, CLL, MDS, CMPD) by integration of the leading national leukemia networks and their interdisciplinary partner groups in Europe (European LeukemiaNet LSH-2002-2.2.0-3)
(01.2004-01.2009); (Oskar A. Haas)

"Evaluation and quantification of epigenetic alterations in the Beckwith-Wiedemann syndrome with methylation-sensitive and real-time PCR"
Jubiläumsfond der Österreichischen Nationalbank / Research Foundation of the Austrian National Bank
Projekt Nr./Project No. 11010; (10.2004-03.2006); (Andreas Weinhäusel / Oskar A. Haas)

"FISH und Klonalitätsuntersuchungen bei aggressiver Fibromatose"
Krebshilfe Steiermark
(10.2003-01.2004); (Andreas Leithner / Reinhard Windhager / Andreas Weinhäusel / Oskar A. Haas)

"Functional Genomics of Childhood Malignancies Part II"
Forschungsprogramm "Genome Research for Health" des Österreichischen Bundesministeriums für Unterricht, Wissenschaft und Kultur / Austrian Ministry of Education, Science and Culture (GEN-AU Child, GZ 200.071/3-VI/2a/2002); (12.2002-11.2005); (Heinrich Kovar / Oskar A. Haas / Renate Panzer-Grümayer)

"Molecular cytogenetics of solid tumors"
European Co-operation in the Field of Scientific and Technical Research
Projekt Nr./Project No. COST B19; Co-ordinator of working group 5 (WG5) "Genetic predisposition and susceptibility to cancer"
(09.2000-09.2006); (Oskar A. Haas)

"Molekulargenetische Analyse von Knochen und Weichteiltumoren"
Österreichischen Gesellschaft für Orthopädie und orthopädische Chirurgie
(10.2003-10.2005); (Andreas Leithner / Reinhard Windhager / Andreas Weinhäusel / Oskar A. Haas)

"Exploration of the tolerogenic potential of indoleamine 2,3-dioxygenase expressed in antigen presenting cells", Fonds zur Förderung der wissenschaftlichen Forschung (FWF)/ Austrian Science Foundation. Projekt Nr./Project No P 16764-B13 (01.2004-12.2006) (Andreas Heitger)

„Dissection of the EWS-FLI1 oncogenic pathway by RNA interference"
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)/Austrian Science Foundation
Projekt Nr./Project No. P16067-B04; (12.2002-11.2005); (Heinrich Kovar)

„Functional confirmation of EWS-FLI1 target genes"
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)/Austrian Science Foundation
Projekt Nr./Project No. P16067-B04; (04.2005-03.2008); (Heinrich Kovar)

„Functional Genomics of Childhood Malignancies Part II"
Forschungsprogramm "Genome Research for Health I" des Österr. Bundesministeriums für Unterricht, Wissenschaft und Kultur / Austrian Federal Ministry of Education, Science and Culture (GEN-AU Child, GZ 200.071/3-VI/2a/2002); (12.2002-05.2006); (Heinrich. Kovar)

„Genome Plasticity and Childhood Cancer"
Forschungsprogramm "Genome Research for Health II" des Österr. Bundesministeriums für Unterricht, Wissenschaft und Kultur / Austrian Federal Ministry of Education, Science and Culture; (GEN-AU-Ch.I.L.D., GZ 200.136/1-VI/1/2005); (01.2006-12.2008); (Heinrich Kovar)

„In-situ identification of target genes for oncogenic EWS-FLI1in its authentic cellular milieu."
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)/Austrian Science Foundation
Projekt Nr./Project No. P14299GEN; (09.2000-04.2005); (Heinrich Kovar, Sabine Strehl, Renate Panzer)

„Mechanisms of escape from oncogenic stress-induced p53 dependent apoptosis in cancer cells"
Jubiläumsfonds der Österreichischen Nationalbank
Projekt Nr./Project No. 10488; (01.2004-12.2005); (Heinrich Kovar)

„Prognosis and Therapeutic Targets in the Ewing Family of Tumors - PROTHETS"
6. Forschungsrahmenprogramm der Europäischen Kommission / 6th framework program of the European Commission, (STREP "PROTHETS" contract LSHC-CT-2004-503036); (01.2005-12.2007); (Coordinator Piero Picci / Bologna; partner Heinrich Kovar)

"Diagnostic approaches to chimerism testing after allogeneic stem cell transplantation for early detection of graft rejection and relapse: Technical development, standardization, and European coordinated clinical implementation "
European Commission
Grant No QLRT-2001-01485; (09/2002-04/2005); (Thomas Lion)

"Investigation of Polyomavirus and Adenovirus Involvement in childhood Cancer"
Jubiläumsfonds der Österreichischen Nationalbank
Projekt Nr./Project No. 11168; (01/2005-01/2006); (Thomas Lion)

"Molekulares Monitoring des BCR/ABL Genrearrangements im Rahmen des CELSG Trial: CML-11"
Novartis
(01/2005-01/2007); (Thomas Lion)

"Molekulares Verfahren zum Nachweis von Pilzinfektionen: Von klinischer Testung zur Vermarktung"
Zentrum für Innovation und Technologie (ZIT)
(12/2005-12/2008); (Thomas Lion)

"Optimierung der Diagnostik und der klinischen Therapie invasiver Pilzinfektionen bei Kindern mit akuter myeloischer Leukämie"
Fonds der Stadt Wien für innovative interdisziplinäre Krebsforschung
(01/2005-01/2006); (Thomas Lion)

"Towards Improved Diagnostics and Clinical Management of Invasive Fungal Infections in Immunocompromised Patients: Molecular Detection and Quantitative Monitoring by Real-time PCR"
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)
Projekt Nr./Project No. P16929-B13; (01/2004-06/2006); (Thomas Lion)

„Backtracking T-ALL-associated oncogenic rearrangements to birth "
OÖKKF
(Panzer, Oberösterreichische Kinderkrebsforschung fördert ein gemeinsames Projekt mit Prof. Dr. K. Schmidt, Linz)

„Molekulare Mechanismen der TEL-AML1 Fusion in Leukemien"
FWF
Projekt Nr. / Project No. P17551-B14; (10.2004-10.2005); (Eva Renate Panzer-Grümayer)

"RNAi supported approach elucidating the TEL-AML1 function in leukemia"
ÖNB
Projekt Nr. / Project No. 10720; (Eva Renate Panzer-Grümayer)

"FOXP3, an essential transcription factor in regulatory T cells, as tool to determine immune tolerance in pediatric patients after hematopoietic stem cell transplantation" Jubiläumsfonds der Österreichischen Nationalbank/Research Foundation of the Austrian National Bank Projekt Nr./Project No. 10839 (06.2003 – 12.2005) (Markus Seidel)

Organisation von Tagungen / Organisation of Meetings

18. Tumorzytogenetische Arbeitstagung, 19.-21. Mai 2005, Semmering, Austria.
Oskar A. Haas / Sabine Strehl / Nicole-Monika Reisinger

8th international childhood acute lymphoblastic leukemia workshop („Ponte di Legno") report,
27.-28. April 2005, Vienna, Austria
Helmut Gadner

Preise / Awards

Peter Ambros: 1. Preis, Otto-Kraupp-Preis, für die beste ausgewählte medizinische Habilitation in Österreich

Inge und Peter Ambros: Großer zentraleuropäischer Preis auf dem Gebiet der innovativen interdisziplinären Krebsforschung

Lenka Baskova: SIOP 2005 - Best Poster Award

Andreas Weinhäusel: Wissenschaftspreis 2003 (verliehen 2004) der Österreichischen Gesellschaft für Kinder- und Jugendheilkunde für die beste experimentelle Arbeit

Andishe Attarbaschi: Wissenschaftspreis 2004 der Österreichischen Gesellschaft für Kinder- und Jugendheilkunde für die beste Publikation aus pädiatrischer Hämatologie und Onkologie

Publikationen / Publications

Attarbaschi,A., Mann,G., Konig,M., Dworzak,M.N., Trebo,M.M., Muhlegger,N., Gadner,H., and Haas,O.A. (2004). Incidence and relevance of secondary chromosome abnormalities in childhood TEL/AML1+ acute lymphoblastic leukemia: an interphase FISH analysis. Leukemia *18*, 1611-1616.

Bernstein,M., Kovar,H., Paulussen,M., Randall,R.L., Schuck,A., Teot,L.A., and Jurgens,H. (2005). Ewing Sarcoma Family of Tumors: Ewing Sarcoma of Bone and Soft Tissue and the Peripheral Primitive Neuroectodermal Tumors. In Principles and Practice of Pediatric Oncology, P.A.Pizzo and D.G.Poplack, eds. Lippincott Williams & Wilkins.

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Ebner,K., Pisker,W., and Lion,T. (2005a). Comparative sequence analysis of the hexon gene in the entire spectrum of human adenovirus serotypes: phylogenetic, taxonomic and clinical implications. Journal of Virology *79*, 12635-12642.

Ebner,K., Suda,M., Watzinger,F., and Lion,T. (2005b). Molecular detection and quantitative analysis of the entire spectrum of human adenoviruses by a two-reaction RQ-PCR assay. Journal of Clinical Microbiology *43*, 3049-3053.

Felzmann,T., Huttner,K., Breuer,S., Wimmer,D., Ressmann,G., Wagner,D., Paul,P., Lehner,M., Heitger,A., and Holter,W. (2005). Semi-mature IL-12 secreting dendritic cells present exogenous antigen to trigger cytolytic immune responses. Cancer Immunol Immunother *54*, 769-780.

Fritsch,G. and Pichler,J. (2004). The lower limit of residual white blood cell counting: how low is low? Transfusion *44*, 1257-1258.

Gadner,H. and Haas,O.A. (2005). Anfälligkeit für maligne Erkrankungen bei Kindern und Jugendlichen. Pädiatrie und Pädologie *1*, 12-20.

Grois,N., Pötschger,U., Prosch,H., Minkov,M., Arico,M., Braier,J., Henter,J.I., Janka-Schaub,G., Ladisch,S., Ritter,J., Steiner,M., Unger,E., and Gadner,H. (2005a). Risk factors for diabetes insipidus in langerhans cell histiocytosis. Pediatr Blood Cancer *Epub ahead of print*.

Grois,N., Prayer,D., Prosch,H., Lassmann,H., and CNS LCH Co-operative Group (2005b). Neuropathology of CNS disease in Langerhans cell histiocytosis. Brain *128*, 829-838.

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