4.10 Progress report of subproject 10

Project title: Long term effects of myeloablative radiochemotherapy with subsequent autologous peripheral blood stem cell transplantation in patients with malignant lymphomas - incidence and early diagnosis of secondary haematological neoplasias

Project leaders: Prof. Dr. Wolfgang Hiddemann  
PD Dr. Martin Dreyling  
Dr. Georg Lenz  
Klinikum Großhadern/LMU  
Medizinische Klinik III  
Marchioninistr. 15  
D-81377 München  
Email: martin.dreyling@med3.med.uni-muenchen.de

4.10.1 Summary

Within the last few years, high dose therapy with autologous stem cell rescue has been proven to result in a superior progression-free survival in different subentities of malignant lymphomas. However, this positive effect may be hampered by the cumulative occurrence of secondary malignancies in up to 20% of patients after a 5-year follow-up.

The main focus of the project is the determination of incidence and early diagnosis of secondary haematological neoplasias in different lymphoma subtypes. All patients with follicular and mantle cell lymphoma who received a high dose regime with autologous stem cell transplantation within the therapy studies of the German Low Grade Lymphoma Study Group (GLSG) will be followed for up to 5 years, and bone marrow specimens will be systematically screened for morphological and molecular alterations. These investigations will also be extended to patients with multiple myeloma, chronic lymphocytic lymphoma and aggressive lymphomas to define the disease-related predisposition. The central infrastructure for the reception and distribution of patient samples has been established. The project was coordinated with the second participating scientific centre in Goettingen. In this context a formalistic sample collection and asservation concept for patient samples has been developed.

4.10.2 Results and ongoing activities

High dose chemotherapy with autologous stem cell transplantation represents a well-established therapeutic option in different subtypes of malignant lymphomas. In relapsed aggressive lymphomas, higher rates of long-term remissions can be achieved. Therefore, an international consensus committee recommended the high dose approach as standard procedure in relapsed aggressive lymphoma.
Similarly, a French randomised study proved the superiority of high dose therapy in multiple myeloma. In indolent lymphoma, the GLSG recently confirmed the beneficial effect of high dose therapy on the progression-free survival. The frequency of long-term effects of high dose therapy, particularly secondary haematological neoplasias that may occur several years after initial treatment is not well defined. In previous smaller studies, secondary haematological neoplasias were detected in up to 20% of lymphoma patients within 5 years after high dose therapy. However, the exact incidence is not known. Similarly, it is well known that extensive conventional chemotherapy (especially the cumulative dose of alkylating drugs) and the addition of radiotherapy strongly influences the rate of secondary neoplasias. In addition, especially in Hodgkin’s disease, it has been speculated that a disease-related T-cell defect may result in a higher predisposition for secondary neoplasias. Previously, three subgroups of secondary myeloid neoplasias have been defined based on exposure to different chemotherapeutic drugs (alkylating drugs, anthracyclines or topoisomerase II-inhibitors), characteristic chromosomal alterations (monosomy 5 and 7, balanced translocations, 11q23 (i.e. MLL-rearrangements), and clinical course. In addition, different pathogenetic pathways (including polymorphisms of detoxifying enzymes) have been correlated to secondary haematological neoplasias. Interestingly, some studies detected temporary clonal chromosomal aberrations, which resolved spontaneously.

To systematically define the incidence and elucidate the molecular pathogenesis of secondary haematological neoplasias after high dose chemotherapy, a clinical screening program with a multimodal scientific working program has been established:

1. Bone marrow morphology including cytochemistry (T. Haferlach, Munich and F. Griesinger Göttingen)
2. Cytogenetic analysis and hybridisation with probes of distinct genomic regions (C. Schoch, Munich and D. Haase, Göttingen)
3. Immunophenotyping to detect aberrant expression of surface cell markers (W. Kern, Munich and F. Griesinger, Göttingen)
4. RT-PCR of the MLL gene (S. Schnittger, Munich and F. Griesinger, Göttingen)
5. Analysis of polymorphism of detoxifying enzymes: GSTM-1, GSTT-1 (T.G. Schultz, Göttingen)
6. Analysis of DNA repair:
   - Microsatellite instability (G. Herzog, Munich)
   - Promoter methylation and Analysis of DNA repair enzymes (G. Lenz and G. Hutter, Munich)
Current status of project:

In cooperation with the second participating scientific centre at the University of Göttingen, the infrastructural prerequisites and an internal network structure have been established. As a successor of the former clinical coordinator (G. Herzog), G. Lenz has been installed as “assistant coordinator” with extensive scientific experience. The multimodal scientific working program described above, was applied on the first 10 patients' samples, independently of clinical or morphological abnormalities. However, on the basis of the estimated frequency of secondary haematological neoplasias the subsequent patients samples were investigated initially only by morphology of the bone marrow and analysis of the differential blood count. The molecular techniques were subsequently applied in cases with morphological abnormalities.

In a pilot study of 72 patients with acute myeloid leukaemia (AML), leukemic cells were investigated by morphology, cytogenetic analysis, immunophenotyping, determination of the microsatellite instability (MSI) and molecular analysis of the cell cycle regulatory genes p53 and p16. Interestingly, the frequency of microsatellite instability was significantly different in de novo and secondary AML cases (26 % vs. 50 %; p = 0.05). The detected MSI was independent of the p53 mutation status. Thus, these 2 alterations are independent pathways of genomic instability, playing a mayor role in the development of secondary haematological malignancies. Currently, the manuscript of these analyses is being finalized for publication.

On the basis of these results, a “snap shot” analysis of 430 patients recruited within the recent GLSG study was carried out, investigating the frequency of secondary haematological neoplasias after high-dose chemotherapy (160 patients) in comparison to conventional interferon therapy (270 patients). Questionnaires with a special focus on the clinical presentation of secondary malignancies were sent out to all clinical institutions involved. In addition, the databases of the study centre were hand searched for completion of missing data. After a median follow-up of 3.5 years, approximately 5% of the patients receiving high-dose therapy developed a secondary haematological neoplasia in comparison to only 1% of the patient cohort in the interferon maintenance arm. However, mainly myelodysplastic syndromes were observed. Therefore a longer median follow-up of these patients is essential to determine the frequency of secondary transformation into AML.

4.10.3 Cooperation within the network

The special focus of this project to determine the frequency of haematological malignancies after high-dose therapy has been presented to the different study groups (GLSG, German CLL Study Group, the Study Group Multiple Myeloma).
Especially, the cooperation with the German High Grade Lymphoma Study Group is of great importance, as in early 2003 a randomised trial investigating the role of high-dose therapy as first-line therapy in diffuse large cell lymphoma has been initiated. The follow-up of these patients will enable the analysis of incidence and pathogenesis of secondary haematological malignancies in comparison to an etoposide containing chemotherapy regime in a second lymphoma entity.

Within the Competence Network, there is a close interaction with the previously funded subproject 11 (coordination: N. Schmitz), which focuses on the clinical aspects of high-dose therapy in malignant lymphomas. Scientifically, our project is closely connected to the research program on secondary neoplasias in Hodgkin's disease (coordination: V. Diehl). In addition, there is an intensive scientific exchange with the recently established European MCL Research Network (coordination: M. Dreyling, W. Hiddemann), which investigates different aspects of molecular lymphoma genesis in close collaboration of basic researchers, pathologists and clinical investigators.

4.10.4 Publications


4.10.5 Objectives for the current funding period

A manuscript including the detailed results of the “snap shot” analysis will be prepared for publication within the next few months. However, as the rate of transformation into secondary AMCL is the clinically most relevant question, an extended follow-up of more than 5 years is urgently warranted (end of 2005).

To provide a reliable clinical follow-up of the patients after high dose therapy, the local database will be transformed to a web-based information platform of up to 300 patients’ samples.

The systematic screening for secondary haematological neoplasias will also be extended to patients with aggressive lymphomas, CLL, and multiple myelomas, based on the data of the
different study centres. Thus, especially the cooperation with the German High Grade Lymphoma Study Group (DSHGNHL) will be intensified.