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The Relevance of the BCL-2 Gene and Wilm's Tumor Gene for the Long-Term Prognosis of Acute Myeloid Leukemia

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Various prognostic scores based on clinical findings, immunophenotyping, cytogenetics or molecular characteristics have been established in AML so far. Based on the present study and previous reports, we postulated a new highly relevant prognostic score in AML based on the coexpression of two genes, the protooncogene bcl-2 and the tumor supressor gene wt1. Both genes are expressed in the majority of AMLs (75-80%). Bcl-2 is a gene involved in the inhibition of apoptosis and wt1 in the differentiation and proliferation of hematopoietic precursor cells and possibly in that of acute leukemias. Additionally, we could demonstrate that transfection of leukemia cell lines with the wt1 gene delayed apoptosis during cell differentiation, indicating an interaction of wt1 with the prevention of cells to DNA damage possibly. Bcl-2 and wt1 were shown to be relevant factors for the long-term outcome. The intensity of the expression of both genes, bcl-2 and wt1, is significantly correlated in AMLs ($p<0.005$). As both genes implicate prognostic significance, we investigated the prognostic relevance of the coexpresssion of both genes. For this, leukemic blast cells obtained from bone marrow of 111 patients (age 17-82, median 49 years) with newly diagnosed AML (97 pts. de novo, 14 secondary AML) were analysed for bcl-2 and wt1 mRNA using RT-PCR. The levels of the transcripts were visually quantitated and confirmed by a competitive PCR. Based on the expression of bcl-2 and wt1 mRNA, it is possible to establish a prognostic index with ***low risk*** (no or low expression of bcl-2 and wt1), ***intermediate*** risk (high expression of bcl-2 or wt1) and ***high risk*** (high expression of bcl-1 and wt1) with differences in the CR rate (74% vs. 60% vs. 62%) and significant differences in the 1- and 3-year-overall survival (55% and 51% vs. 44% and 28% vs. 39% and 0%, $p=0.025$). Concerning pts. under 60 years the overall survival was 76% and 70% vs. 68% and 50% vs. 53% and 0%, $p=0.01$). The prognostic score is independent from cytogenetic findings. In conclusion, the coexpression of bcl-2 and wt1 mRNA is a significant prognostic factor for the long-term outcome in the therapy of AML.

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MECHANISMS OF STEM CELL MOBILIZATION

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Previous experiments have indicated that Interleukin-8 (IL-8) induces a rapid mobilization of hematopoietic progenitor cells with colony forming capacity, radioprotective and long term lymphomyeloid repopulating ability (Blood 85: 2269, 1995). Since ß-2 integrins are essential for adhesion and transendothelial migration of progenitor cells, we studied the involvement of LFA-1 in the IL-8 induced mobilization. Balb-C mice were treated with an intraperitoneal injection of anti-LFA-1 antibody (H154.163). Antibody administered as a single injection induced no mobilization of colony forming cells within a time frame of 24 hours. Mice treated with anti-LFA-1 antibody exhibited trombocytopenia (anti-LFA-1 296±65, saline 749±115x10^9/ml), while no effect on the white blood cell counts was observed. In other experiments, mice were pretreated with anti-LFA-1 antibody prior to injection with IL-8 (30 μg i.p.). IL-8 induced mobilization of hematopoietic progenitor cells was completely blocked by pretreatment with the anti-LFA-1 monoclonal antibody (anti-LFA-1 + IL-8 42±28 versus saline + IL-8 590±771 CFU-GM/ml blood, n=17). In contrast, pretreatment with anti-LFA-1 antibody did not block the IL-1 induced mobilization of HPC or G-CSF induced mobilization. Addition of anti-LFA-1 antibody to colony cultures in semi-solid media had no inhibitory activity. Finally, it was considered that the IL-8 induced mobilization of primitive HPC was not blocked by treatment with anti-LFA-1 antibody, since LFA-1 is reported to be expressed on relatively mature HPC. Transplantation of 5x10^5 blood derived mononuclear cells derived from IL-8 mobilized animals pretreated with anti-LFA-1 or saline controls protected 95% and 19% of lethally irradiated recipient mice respectively. The survival of recipients transplanted with an equal number of MNC derived from IL-8 mobilized animals was 86%. Our results therefore indicate that anti-LFA-1 antibody completely prevents the rapid mobilization of colony forming cells and of cells with radioprotective capacity induced by IL-8. These experiments indicate a major role for the ß2-integrin LFA-1 in the mechanism of IL-8 induced mobilization.

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Effect of PEG-rHu MGDF in the induction treatment of acute myeloid leukemia.

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In a multicentre study, adult patients with de novo acute myeloid leukemia (AML) were randomized to receive blinded study drug (MGDF at 2.5 or 5 µg/kg/day or placebo) for up to 21 doses following induction chemotherapy with daunorubicin, ara-c and etoposide. Data on the first course of induction chemotherapy are available on 70 patients (MGDF 2.5 µg n=24, MGDF 5 µg n=24, placebo n=22). Median recovery time to an unsupported platelet count of 20x10^9 /l was 21 days for all groups. A prolonged elevation of platelet count was seen in both groups of patients receiving MGDF. The effects of MGDF appeared to be specific to the platelet lineage, as the median time to recovery of ANC>0.5x10^9 /l (26 days) and the red cell blood cell transfusion requirements were similar in the MGDF and placebo groups. Platelet counts of >1000x10^9 /l were noted in approximately half of the patients receiving MGDF, occuring 6-8 days after the completion of study drug. The incidence of thrombotic events was not different between the treatment groups, and MGDF was well tolerated. The complete remission rate was 77% for the MGDF group, and 86% for the placebo group. These data indicate that MGDF is biologically active and well tolerated in patients receiving induction chemotherapy for AML. At present studies with alternative schedules of MGDF are ongoing. Thus for earlier platelet recovery a pretreatment with MGDF is studied as well as priming has to be considered.

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HEMOPOIETIC GROWTH FACTOR REGULATION IN MALIGNANT HEMOPOIESIS

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A heirarchy of progenitor cells exists both in normal hemopoiesis and among the leukemic cells in acute myelogenous leukemia (AML) which includes both directly clonogenic cells (CFC) and more primitive cells which are detected by their ability to give rise to CFC progeny after ≥ 5wks in longterm culture (LTC) with fibroblast feeder layers, LTC-initiating cells (LTC-IC). Recently it has been possible to establish multilineage normal human hemopoiesis and dissemination of AML cells in NOD/SCID mice. Both the LTC system and NOD/SCID mice provide assays for determining the progenitor content of populations of normal and malignant human hemopoietic cells and are being used to study factors which regulate their development. Although the growth of many AML samples can be supported by conditions optimized for normal hemopoietic progenitors some differences between normal and AML LTC-IC are begining to emerge. For examples, murine fibroblast feeders engineered to produce human IL-3, Steel factor (SF) and high concentrations of G-CSF have been shown to enhance detection of normal LTC-IC and their output of CFC progeny. However this effect is not seen with AML LTC-IC which are usually more responsive to high concentrations of SF, IL-3 and/or flt-3 ligand (FL). In NOD/SCID mice longterm multilineage engraftment of normal human marrow cells can be enhanced 10-fold with i.p. administration of human cytokines. Although the growth of some AML cells in these animals also appears to be enhanced by human growth factor supplements many engraft and disseminate widely without the need for human cytokines. In further contrast to normal hemopoietic cells, AML cells which engraft in NOD/SCID mice lack expression of Thy-1, an antigen found on many normal LTC-IC and on cells which reconstitute hemopoiesis in myeloablated animals. Thus, both the LTC and the NOD/SCID mouse assays provide systems in which to explore fundamental differences in the characteristics of normal and malignant hemopoietic stem cells that may ultimately be exploited for therapeutic purposes.