Satellite Symposium V

Modern Diagnostics in Chronic Myeloproliferative Diseases (CMPDs)

T. HAFERLACH, W. KERN, S. SCHNITTGER, and C. SCHOCH
Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, University Hospital Grosshadern, Ludwig-Maximilians-University, Marchioninistreet 15, 81377 Munich, Germany

Abstract

According to the new WHO classification a group of chronic myeloproliferative diseases (CMPDs) were defined: chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia and hypereosinophilic syndrome (CEL/HES), polycythemia vera (PV), chronic idiopathic myelofibrosis (with extramedullary hematopoiesis, CIMF), essential thrombocythemia (ET), and so called CMPD/unclassifiable. As clinical features and laboratory findings differ widely between these diseases several diagnostic approaches are mandatory at diagnosis for classification and are needed also for follow up studies, especially for the measurement of minimal residual disease (MRD). We here outline the laboratory set up at diagnosis and during follow up in CMPDs with specific focus on the respective therapeutical consequences. Only by using a comprehensive diagnostic panel including cytomorphology, cytogenetics, and molecular genetic methods establishing the correct diagnosis, optimizing treatment as well as evaluating treatment response is possible in CMPDs today.

Introduction

The chronic myeloproliferative diseases (CMPDs) are clonal stem cell disorders and are characterized by proliferation in the bone marrow and peripheral blood of 1-3 of the hematopoietic cell lineages[1]. In contrast to acute leukemias the proliferation is effective and results in increased number of leukocytes, and/or erythrocytes and/or platelets. Although the clinical onset is often insidious all CMPDs can undergo clonal evolution and may transform to blast crisis or to myelofibrosis, both with ineffective hematopoiesis and several clinical complications. Therefore, not only at diagnosis but also in defined time intervals during the course of the disease a comprehensive diagnostic work-up is necessary to measure therapeutical success or progression despite therapy[2].

Accoring to the new WHO proposal[3] chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia and hypereosinophilic syndrome (CEL/HES), polycythemia vera (PV), chronic idiopathic myelofibrosis (with extramedullary hematopoie-sis) (CIMF), essential thrombocythemia (ET), and so called CMPD/unclassifiable are classified as CMPDs. As new techniques and markers evolved within the last decade all but the last "entity" can more or less be clearly discriminated at diagnosis and need specific laboratory follow-up investigations[1,4–7].

This review will focus mainly on the clarification of markers for diagnosis and will comment on a comprehensive approach for treatment control in CMPDs.

The methods applied today for the diagnosis of CMPDs are a combination of cytomorphology, histomorphology, cytogenetics, fluorescence in situ hybridization and some specific molecular marker studies[2,8]. These techniques are complementary. Although a stepwise approach seems appropriate, in many instances they have to be performed parallel. For an adequate management and understanding of the CMPDs all laboratory results have to be discussed also with respect to the patients history and the clinical performance.

Material and methods for diagnosis and follow-up studies in CMPDs

As the differential diagnosis in CMPDs is not always as easy as in acute leukemias the investigation of peripheral blood smears, bone marrow aspirates, and trephine biopsies are necessary. Only in PV same classification systems[9] do not exclusively rely on bone marrow and the diagnosis can be based also on peripheral blood smears and some other clinical parameters alone[9–11]. In addition to the May Grünwald Giemsa staining the myeloperoxidase reaction (in CML blast crisis), and iron staining (in PV) may lead to further information with respect to differential diagnosis and may therefore be a part of the diagnostic work-flow. Especially for CIMF and ET, but also in cases of CML and PV the bone marrow histomorphology included Giemsa, PAS, chloroacetate-esterase and Gomori silver impregnation for reticulin fibers to assess the marrow connective tissue is mandatory. This does not only lead to parameters for classification but can also add some prognostic markers[12, 13].

The implementation of classical cytogenetic analyses using metaphases is necessary in all patients with CML at diagnosis and represents so far the goldstandard for therapy control and definiton of cytogenetic response[8]. This is true especially for patients receiving interferon or imatinib treatment[14]. However, during the last years several studies demonstrated the importance of cytogenetic data especially for prognosis in PV and CIMF. Therefore, many investigators suggest also a cytogenetic analysis at diagnosis for all other CMPDs in addition to the CML patients[1].

In the last decade newer molecular techniques such as interphase fluorescence in situ hybridization (IP-FISH) and hyper-metaphase FISH (HM-FISH) have been investigated in CML and were supplemented by further molecular

Table 1. Laboratory methods in CMPDs with relevance for classification and prognosis that should be investigated at diagnosis

	Cytomorphology on pB/BM smears	Histomorphology	Cytogenetics	Molecular-genetics
CML	+	+	+	+
CNL	+	+	+	–
CEL/HES	+	+	+	+
PV	+/?	?	+	+
CIMF	+	++	+	–
ET	+	+	?	–
CMPD/unclassifiable	+	+	?	–

pB=peripheral blood; BM=bone marrow; CML=chronic myeloid leukemia; CNL=chronic neutrophilic leukemia; HES=chronic eosinophilic leukemia and hypereosinophilic syndrome; PV=polycythemia vera; CIMF=Chronic idiopathic myelofibrosis; ET=essential thrombocythemia; CMPD/unclassifiable;
+ should be performed; – is not recommended; ? is under debate

Table 2. Chromosomal and molecular markers in CMPDs, according[1, 3, 4, 17, 18]

	Cytogenetic results FISH markers	Moleculargenetic markers	For follow-up studies
CML	t(9;22)(q34;q11) *BCR/ABL*	*BCR/ABL*	+
CNL	in some cases: +8, +9, del(20q)	–	–
CEL/HES	in some cases: +8, i(17q) 8p11 translocations in CEL	*PDGFRA/FIP1L1* in HES	+?
PV	in some cases: +8, +9, del(20q), del(13q), del(1p)	*PRV-1*	+
CIMF	in some cases: del(13q), +8, +9, del(20q), partial trisomy 1q	–	–
ET	in rare cases del(13q), +8, +9	–	–
CMPD/ unclassifiable	?	–	–

For further specific diagnosis and classification in CMPDs the following classification systems and guidelines can be applied and are outlined in detail in tables 3–8.

Table 3. Cytomorphological criteria for phases in CML according to different classification systems as measured on peripheral blood smears or bone marrow samples

	WHO[3]	IBMTR (www.ibmtr.org)	German CML Study Group (www.kompetenznetz-leukaemie.de)
CP	blasts <10% (bone marrow or (peripheral blood)	blasts <10% (bone marrow and/or peripheral blood)	blasts and metamyelocytes <15% peripheral blood)
AP	blasts 10–19% (bone marrow or peripheral blood)	blasts >10%, or blasts plus promyelocytes >20% (bone marrow and/	blasts plus promyelocytes >10% (bone marrow or peripheral blood) or peripheral blood)
	>20% basophils or eosinophils (in peripheral blood)	>20% basophils or or eosinophils (in peripheral blood)	>20% basophils or eosinophils (in peripheral blood)
BP	blasts ≥20% (bone marrow or peripheral blood)	blasts plus promyelocytes >30% (in bone marrow or peripheral blood)	blasts plus promyelocytes ≥50% (in bone marrow) ≥30% (in peripheral blood)

CP: chronic phase; AP: accelerated phase; BP: blast phase

Table 4. Diagnostic criteria for chronic neutrophilic leukemia (CNL) as measured on peripheral blood smears or in bone marrow samples[3, 22, 23]

- Leukocytosis in pB ≥25×10^9/l
- Segmented neutrophils and bands >80% of WBC
- Immature WBC <10%
- Myeloblasts <1%
- No genetic markers that would be predictive
- for a specific other CMPD

Table 5. Diagnostic criteria for chronic eosinophilic leukemia (CEL) and hypereosinophilic syndrome (HES) (according to WHO[3] and Cools[18, 19])

- Persistant eosinophilia in pB ≥1.5×10^9/l and increased number of bone marrow eosinophils; myeloblasts <20% in blood and/or bone marrow
- Exclude all other causes for eosinophilia
- No genetic markers that would be predictive for a specific other CMPD
- *PDGFRA/FIP1L1* detectable in HES

Table 6. Some diagnostic parameters used for the diagnosis of polycythemia vera measurable on blood and bone marrow samples (according to WHO[3]). Specific thresholds and marker combinations as well as other parameters necessary (erythropoietin level, spleen size, CRP etc.) should be looked up in the respective publications[9–11]

- Increased hemoglobin and hematocrit in pB
- Increased platelet count in pB ≥400×10^9/l
- Granulocytes in pB ≥ 12 x 10^9/l
- No genetic markers that would be predictive for a specific other CMPD

If bone marrow biopsy was performed:

- Increased bone marrow cellularity with trilineage proliferation
- Absence of stainable iron in bone marrow

Table 7. Blood and bone marrow findings in chronic idiopathic myelofibrosis at the fibrotic stage[3, 24, 25]

Blood:

- Leukoerythroblastosis
- Prominent red blood cell poikilocytosis with dacrocytes

Bone marrow:

- Reticulin and/or collagen fibrosis
- Decreased cellularity
- Dilated marrow sinuses with intralumial hematopoiesis
- Atypical and prominent megakaryocytic proliferation
- New bone formation, i.e. osteosclerosis

Table 8. Morphological diagnostic criteria for essential thrombocythemia[3, 26]

- Sustained platelet count in pB ≥ 600×10^9/l
- In bone marrow mainly proliferation of the megakaryocytic lineage with clusters of increased numbers of enlarged but mostly mature megakaryocytes
- No morphological criteria or genetic markers that would be predictive for a specific other CMPD

investigations using polymerase chain reaction (PCR) for the detection of the *BCR/ABL* fusion transcript in CML[8, 14] or by other PCR assays in the other CMPDs. It is recommended to measure the expression of the *PRV-1* gene in cases with suspected PV as it has been described that a high *PRV-1* expression correlates with this CMPD[15–17]. In HES rearrangements of *PDGFRA* and *FIP1L1* genes have been described which lead to an increased tyrosine kinase activity which can be specifically inhibited using imatinib treatment. Furthermore elevated serum tryptase level seem to identify a subset of patients with HES[18–20].

A short overview of techniques and material in the respective CMPDs is given in table 1 and will not be discussed in detail here.

Table 2 reviews very briefly the most important cytogenetic and molecular markers relevant in CMPDs. These markers can be evaluated at diagnosis but may also be investigated for follow-up studies and therapy control including minimal residual disease in some cases.

In CML a new score has been proposed at diagnosis especially useful for the definition of prognosis[21]. In addition to the age of the patient and size of the spleen the following parameters from the peripheral blood count are included: percentages of blasts, eosinophils, basophils and number of platelets. No parameters from bone marrow analyses are included.

Conclusions

According to the new WHO classification[3] a group of chronic myeloproliferative diseases (CMPDs) was defined: chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL) and hypereosinophilic syndrome (HES), polycythemia vera (PV), chronic idiopathic myelofibrosis (with extramedullary hematopoiesis, CIMF), essential thrombocythemia (ET), and so called CMPD/unclassifiable. Several different clinical features and an increasing number of laboratory parameters are necessary for the classification of CMPDs. A combination of diagnostic approaches including peripheral blood smears and bone marrow biopsy, and more and more also cytogenetic and molecular genetic investigations are mandatory at diagnosis[1, 4–7]. The respective results allow the differential diagnosis and are the basis for follow up studies measuring minimal residual disease (MRD) to evaluate treatment response on an individual basis. This is daily routine in CML[8, 14], newly tested in PV[15, 17] and may also be recommended for HES in the near future.

Some further investigations, however, are warranted with respect to molecular background in CNL[22, 27], in CIMF[25], and in ET[26, 28].

As far as known and identified all these data need a combination of different specimens of blood and bone marrow samples and very experienced hematologists, cytogeneticists and molecular biologists. The need for investigating every specific method in a CMPD patient therefore has to be newly defined in prospective settings. Although in CML already an algorithm seems quite clear for diagnosis and follow-up studies[2, 8], new guidelines may be necessary after new treatment approaches such as imatinib are implemented. With respect to all other CMPDs the diagnostic set-up at this time is emerging as more investigations are performed and especially when new molecular markers are defined. This was demonstrated for PV and HES very recently[17, 18].

Thus, in all cases of CMPDs these specific sources and parameters should be investigated to lead to a more reliable and biologically orientated classification of CMPDs in the near future. This will also enhance our understanding of pathogenetic mechanisms and will allow to control the success of therapy on molecular levels. New techniques such as gene expression profiling may furthermore revolutionize our understanding in CMPDs as it is in progress for the acute leukemias[29]. In conclusion, a comprehensive diagnostic panel including cytomorphology, cytogenetics, and molecular genetic methods is necessary for the diagnosis in CMPDs and can give optimal markers for treatment evaluation.

References

(1) Bench AJ, Cross NC, Huntly BJ, Nacheva EP, Green AR. Myeloproliferative disorders. Best Pract Res Clin Haematol. 2001;14:531-551.
(2) Haferlach T, Schoch C. Modern techniques in leukemia diagnosis. Internist. 2002;43:1190-1202.
(3) Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. 2001. Lyon, IARC Press.
(4) Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera, and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol. 2002;76:133-145.
(5) Kralovics R, Buser AS, Teo SS et al. Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. Blood. 2003;102:1869-1871.
(6) Thiele J, Kvasnicka HM. Chronic myeloproliferative disorders with thrombocythemia: a comparative study of two classification systems (PVSG, WHO) on 839 patients. Ann Hematol. 2003;82:148-152.
(7) Spivak JL. Diagnosis of the myeloproliferative disorders: Resolving phenotypic mimicry. Semin Hematol. 2003;40:1-5.
(8) Schoch C, Schnittger S, Bursch S et al. Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia. 2002;16:53-59.
(9) Wasserman LR. The management of polycythaemia vera. Br J Haematol. 1971;21:371-376.
(10) Streiff MB, Smith B, Spivak JL. The diagnosis and management of polycythemia vera in the era since the Polycythemia Vera Study Group: a survey of American Society of Hematology members' practice patterns. Blood. 2002;99:1144-1149.
(11) Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Semin Thromb Hemost. 1997;23:339-347.
(12) Kvasnicka HM, Thiele J, Schmitt-Graeff A et al. Impact of bone marrow morphology on multivariate risk classification in chronic myelogenous leukemia. Acta Haematol. 2003;109:53-56.

(13) Buesche G, Hehlmann R, Hecker H et al. Marrow fibrosis, indicator of therapy failure in chronic myeloid leukemia - prospective long-term results from a randomized-controlled trial. Leukemia. prepub 2003.
(14) Schoch C, Haferlach T, Kern W et al. Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia. 2003;17:461-463.
(15) Klippel S, Strunck E, Temerinac S et al. Quantification of PRV-1 mRNA distinguishes Polycythemia vera from secondary erythrocytosis. Blood. 2003;102:3569-3574.
(16) Liu E, Jelinek J, Pastore YD et al. Discrimination of polycythemias and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. Blood. 2003;101:3294-3301.
(17) Pahl HL. PRV-1 mRNA expression and other molecular markers in polycythemia rubra vera. Curr Hematol Rep. 2003;2:231-236.
(18) Cools J, DeAngelo DJ, Gotlib J et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003;348:1201-1214.
(19) Cools J, Stover EH, Boulton CL et al. PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFRalpha-induced myeloproliferative disease. Cancer Cell. 2003;3:459-469.
(20) Klion AD, Noel P, Akin C et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. Blood. 2003;101:4660-4666.
(21) Hasford J, Pfirrmann M, Hehlmann R et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst. 1998;90:850-858.
(22) Bohm J, Kock S, Schaefer HE, Fisch P. Evidence of clonality in chronic neutrophilic leukaemia. J Clin Pathol. 2003;56:292-295.
(23) Bohm J, Schaefer HE. Chronic neutrophilic leukaemia: 14 new cases of an uncommon myeloproliferative disease. J Clin Pathol. 2002;55:862-864.
(24) Tefferi A. The forgotten myeloproliferative disorder: myeloid metaplasia. Oncologist. 2003;8:225-231.
(25) Reilly JT. Cytogenetic and molecular genetic aspects of idiopathic myelofibrosis. Acta Haematol. 2002;108:113-119.
(26) Steensma DP, Tefferi A. Cytogenetic and molecular genetic aspects of essential thrombocythemia. Acta Haematol. 2002;108:55-65.
(27) Hasegawa T, Suzuki K, Sakamoto C et al. Expression of the inhibitor of apoptosis (IAP) family members in human neutrophils: up-regulation of cIAP2 by granulocyte colony-stimulating factor and overexpression of cIAP2 in chronic neutrophilic leukemia. Blood. 2003;101:1164-1171.
(28) Gale RE. Pathogenic markers in essential thrombocythemia. Curr Hematol Rep. 2003;2:242-247.
(29) Haferlach T, Kohlmann A, Kern W et al. Gene expression profiling as a tool for the diagnosis of acute leukemias. Semin Hematol. 2003;40:281-295.

Imatinib Mesylate Selectively Influences the Cellular Metabolism of Cytarabine in BCR/ABL Negative Leukemia Cell Lines and Normal CD34+ Progenitor Cells

M. BORNHÄUSER[1], T. ILLMER[1], P. LE COUTRE[2], J. PURSCHE[1], M VON BONIN[1], FREIBERG-RICHTER, J., M. SCHAICH[1], U. PLATZBECKER[1], C. THIEDE[1], O.G. OTTMANN[3], CH. Köhne[1], J. Braess[4], G. Ehninger[1] and E. SCHLEYER[1]
1. Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus, Dresden, Germany; 2. Medizinische Klinik für Hämatologie und Onkologie, Campus Virchow, Charité, Humboldt Universität Berlin, Germany; 3. Medizinische Klinik III, Johann Wolfgang Goethe Universität, Frankfurt a.M., Germany; 4. Medizinische Klinik III, Klinikum Grosshadern, Ludwig-Maximilians University, Munich, Germany

Abstract

STI-571 (Imatinib/Glivec®) has been shown to have synergism with various chemotherapeutic agents including cytosine arabinoside (Ara-C) in BCR/ABL positive leukemia cells. The antiproliferative and proapopotic effects of STI-571 in these experiments are mainly explained by its ability to specifically block the fusion-protein BCR/ABL which has a constitutively active tyrosine kinase activity. We investigated the effects of STI-571 in combination with Ara-C on BCR/ABL negative leukemia cell lines and CD34+ hematopoietic progenitor cells in-vitro. Raji, HL-60, K562, Kasumi and KG1a leukemia cells and CD34+ cells from healthy donors were incubated with 5-20 µg/ml Ara-C for 5 h alone or in combination with 10 µg/ml STI-571. Intracellular levels of Ara-CTP measured by HPLC were increased 1.5-3 fold in leukemia cells with most promiment effects in HL-60, Kasumi and Raji cells. In HL-60 cells a linear correlation between the concentration of STI-571 (1-10 µg/ml) and the subsequent levels of Ara-CTP was observed. A linear increase of Ara-CTP could be induced by increasing the incubation time with STI-571 from 2-6 h with a ceiling effect after 8 h. In contrast coincubation of mononuclear cells or purified CD34+ cells with STI-571 at therapeutic concentrations lead to decreased intracellular levels of Ara-CTP. The synergism between Ara-C and STI-571 was even more pronounced in Raji and HL-60 cells when 300 ng/ml G-CSF were added at the beginning of the culture period. Intracellular measurements of STI-571 revealed no decreased or increased levels of the compound when increasing Ara-C concentrations were used.

Our findings indicate that STI-571 can have significant impact on nucleoside metabolism in malignant and non-malignant hematopoietic cells. Further investigations will have to show whether theses effects can lead to increased cytotoxicity in primary blasts of patients with acute leukemia.

Introduction

STI-571 (Imatinib mesylate, Glivec) has become a successful treatment option for patients with Ph+ CML or ALL because of its specific mode of action which is associated with the interaction at the ATP binding moieity of the BCR/ABL fusion protein[1]. The inhibition of the tyrosine kinase function of BCR/ABL has been shown to be associated with major clinical effectiveness at all stages of chronic myeloid leukemia (CML)[2, 3]. The specific interaction between STI-571 and its target protein is strongly supported by the finding that mutations of the BCR/ABL gene which negatively influence the ability of STI-571 to bind to its target region lead to clinical resistance in patients with Philadelphia chromosome (Ph+) positive CML and Ph+ ALL[4]. To further increase the efficacy of STI-571 several investigators tried to combine the compound with other chemotherapeutic agents which had shown clinical activity in Ph+ leukemia. Ara-C proved to have relevant synergistic effects in cell lines and was therefore incorporated into clinical protocols focussing on the optimised treatment of patients with newly diagnosed or resistant CML. Most authors have shown that the susceptibility of Ph+ cells towards Ara-C induced apoptosis is increased by the concurrent blockade of BCR/ABL[5]. Only few data are available on the effects of STI-571 on Ph- leukemia cells. Although some effects were observed in acute myeloid leukemia, these observations could not be linked to any known chemical or biological phenomenon, so far[6].

No comparable advances have been made in the treatment of Ph- negative leukemia in which resistance to chemotherapy could not be overcome with convincing long-term results. In acute myeloid leukemia (AML) several efforts have been undertaken to positively influence the intracellular metabolism of the most active compound cytarabine by either increasing the fraction of cells proliferating with G-CSF or by preincubation with fludarabine which has been shown to increase Ara-CTP levels two-fold[7, 8].

The aim of this study was to investigate the effects of STI-571 on Ara-CTP levels in leukemia cells. In this report we show that intracellular Ara-CTP levels can be increased up to 3-fold in different Ph- leukemia cells by pre-incubation with STI-571 whereas Ara-CTP levels were decreased in CD34+ non-malignant cells.

Material and Methods

Informed consent was obtained from healthy donors whose cells were used for subsequent CD34+ selection and in-vitro experiments. The study concept had been approved by the local institutional review board.

Drugs. STI-571 was provided by Novartis Pharma GmbH (Basel, Switzerland, with courtesy of H. Gschaidmeier). STI-571 stock solutions for HPLC stan-

dards were dissolved in 100% methanol and stored at –20°C at a concentration of 1000 µg/ml. Since N-DesM-STI is not commercially available it was separated and purified by liquid – liquid extraction from patient urine and subsequently purified by the HPLC method described below. The resulting N-DesM-STI solution showed a purity of 99% as tested by analytical HPLC. N-DesM-STI authenticity was proved by the similarity of UV-spectra, existence solely in STI-571 treated patient plasma or urine and finally by magnet-resonance spectroscopy (data not shown). N-DesM-STI stock solutions for HPLC standards were dissolved like STI-571 in 100% methanol and stored at –20°C at a concentration of 1000 µg/ml. Cytarabine was delivered from sigma and initially dissolved in PBS. Fresh working solutions in PBS were prepared before each experiment.

Cell Lines. Cell lines (HL-60, K562, Raji, Kasumi and KG1a) were purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Braunschweig, Germany). Cells were grown in RPMI-1640 medium (Biochrom AG, Berlin, Germany) supplemented with penicillin, streptomycin and 10% fetal calf serum (FCS) under standard conditions. Cell cultures were performed at 37°C and 5% CO_2 atmosphere in easy-flasks, 25 cm^2 (Nalge-Nunc, Denmark, Roskilde).

CD34+ and mononuclear cells. Mononuclear cells were freshly isolated by density gradient centrifugation on Ficoll solution (Pharmacia, Germany). These cells were then cultured in RPMI and 10% FCS. CD34+ cells were isolated by immunomagnetic selection (Miltenyi Biotec, Bergisch Gladbach, Germany) from G-CSF mobilised leukapheresis samples. Antibody incubation, cell washing and magnetic enrichment were performed according to the manufacturer's instructions.

Ara-CTP formation. After incubation, cells were washed twice in 0.9% NaCl and then lysed in a buffer [0.1 m KH_2PO_4, 0.5% acetonitrile, 0.005 m PIC A (tetrabytylammonium phosphate), pH 2.6]. AraCTP was measured by HPLC analysis as previously published[9]. HPLC analysis involved a 250/5-mm C18 reversed phase column as the stationary phase and the aforementioned buffer as the mobile phase. Detection was performed by UV detection at 280 nm. Quantification was performed by the external standard method.

Intracellular STI-571 levels. Cellular STI-571 was measured by HPLC and will be described in detail in a separate publication[10]. In brief, as analytical column a ZirChrom HPLC column, 3µm, PDB-ZrO2, 3% carbon, 50×4×6 mm with a precolumn of the same solid phase specificity was used. The system was designed as online-enrichment system with another PDB-ZrO2 precolumn as enrichment column. Flow was set on 0,4ml/min at room temperature in the analytical part and on 2 ml/min at room temperature in the enrichment part. The analytical eluent consisted of 600 ml 0,01M KH_2PO_4 / 0,09M K_2HPO_4+400 ml Methanol / liter (v/v), while the enrichment eluent was prepared with 450 ml 0,1M KH_2PO_4+350 ml H_2O+200 ml CH_3OH (v/v). For quantitation the external standard method was used.

Results

Ara-CTP levels in leukemia cell lines. As shown in Figure 1 (left part), a significant increase of intracellular Ara-CTP levels could be observed for the cell lines Raji, K562, HI-60, KG1a and Kasumi. The effect was less pronounced in immature KG1A cells. Ara-CTP was increased 1.5 to 3 fold after a 5 h incubation with STI-571. The data points represent triplicate measurements in all incubations shown in multiple experiments over a time period of 3 month.

Ara-CTP in CD34+ hematopoietic stem cells. When performing the same set of experiments with purified CD34+ cells we observed a decrease of Ara-CTP levels after pre-incubation with STI-571. This effect could be confirmed in three independent experiments using CD34+ cells selected from leukapheresis samples of G-CSF mobilized healthy donors (Figure 1, left part). Moreover, similar results were detected under the same conditions in peripheral mononuclear cells from healthy donors after fiqoll separation (2 independent experiments) (Figure 1, left part).

Dose effects. Using increasing concentrations of STI-571 we could show a linear correlation between the concentration of STI-571 used for preincubation and the subsequent intracellular Ara-CTP levels measured in HL-60 cells. The Ara-C concentration used in the experiments shown in Figure 2 was 20 µg/ml. Additionally we analysed the cellular cytidine-triphosphate levels (CTP) and found no significant change under STI-571 coincubation (Figure 2).

Time effects. In HL-60 cells we observed a linear correlation between the duration of the pre-incubation with STI-571 and the Ara-CTP levels measured thereafter. Using 10 µg/ml STI 571, the Ara-CTP levels increased in a almost linear fashion up to 6 hours and ceiled off after 8 hours while the incubation with AraC alone reached steady-state levels after 2 hour on an 5 times lower plateau. For the AraC+STI-571 curve we calculated a correlation coefficient of 0,9828 based on a polynomal function of second order. Results are summarized in Figure 3.

Intracellular levels of STI-571. To exclude the possibility that the uptake of STI-571 is blocked by Ara-C at higher dose levels, we performed sequential analyses of intracellular STI-571 concentration after AraC coincubation. We observed no significant change of the intracellular STI-571 levels induced by the coincubation with Ara-C as depicted in one example in Figure 2.

Effect of N-desmethylimatinib on AraCTP formation. Similar observations as found under STI-571 coincubation with AraC were made for N-desmethylimatinib, the active main metabolite of STI-571. In detail, in Rajii cells the cellular AraCTP concentration was 3.5 fold higher after coincubation with 10 µg/ml N-desmtehylimatinib for 5 hours compared to AraC alone. However, only a limited amount of N-desmethylimatinib was available and therefore we could not perform the same number of experiments as with the combination of STI-571 and AraC. These data should therefore be validated in further experiments.

Discussion

High-dose Ara-C has been shown to be the most active component of induction and post-remission treatment of patients with acute myeloid leukemia (AML)[11, 12]. The use of Ara-C in cumulative doses of 18 g and more is an independent prognostic factor for leukemia-free survival of patients with de-novo AML[13]. Early studies had shown that intracellular Ara-CTP levels can be increased by various pharmacological interventions. Pretreatment of leukemia cells with granulocyte-colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) leads to increased levels of intracellular Ara-CTP and more cytotoxicity[14, 15]. Subsequent clinical studies have shown the feasibility and efficacy of so called 'G-CSF priming' strategies[16]. Recently, results of a

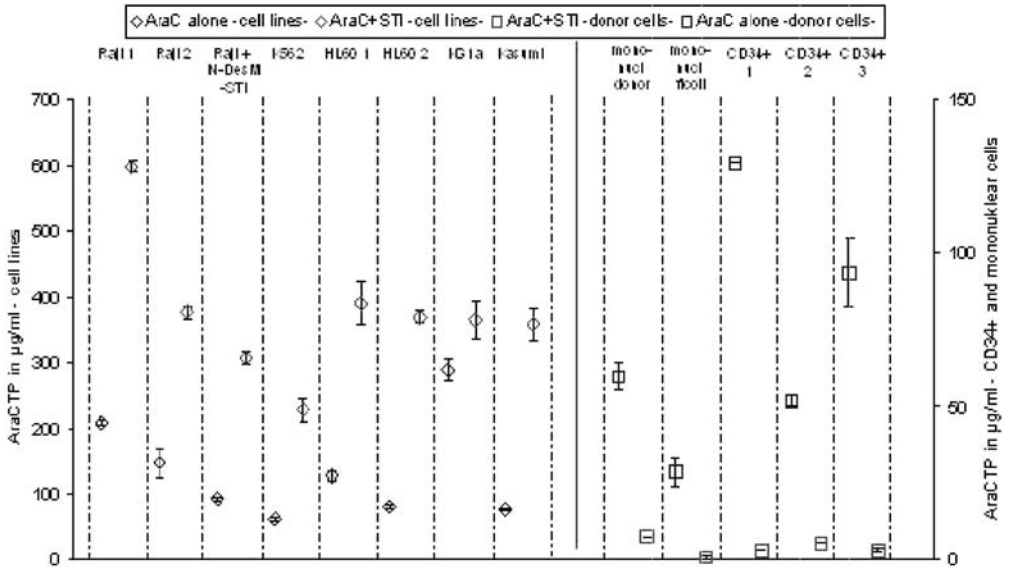

Figure 1: STI-571 interaction on AraCTP formation in different cell lines (5h incubation of 5 µg/ml AraC +/– 10 µg/ml STI-experiments were done in triplicate except om CD34+ 1 cells – experiment time period 3 month)

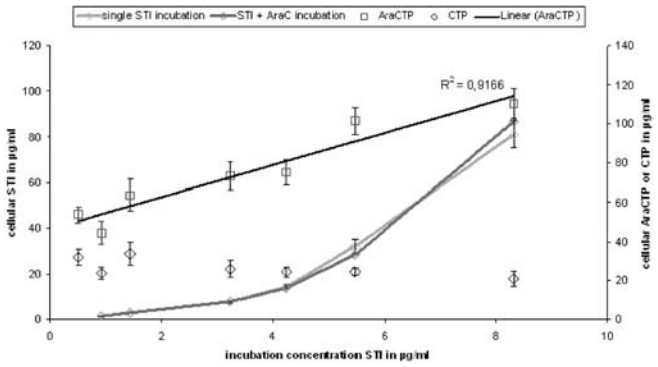

Figure 2: AraCTP and CTP formation under increasing STI-571 concentrations, AraC interaction on cellular STI-571 uptake (5h incubation of 20 µg/ml AraC+increasing STI-571 in HL-60 cells)

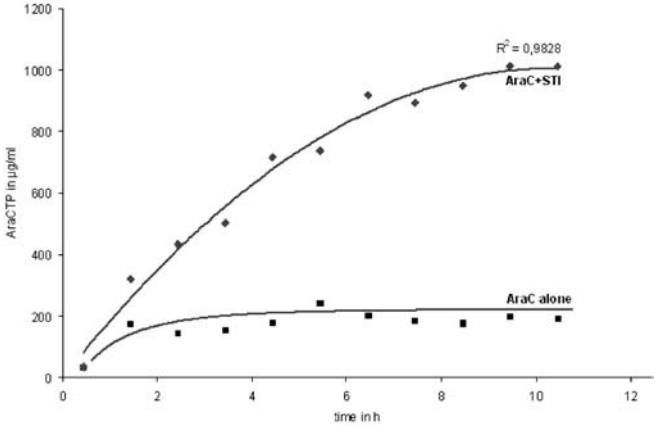

Figure 3: AraCTP formation in relation to the incubation time +/– STI-571 (5 µg/ml AraC +/– 10µg/ml STI-571 in Raji cells – 5E10 per time point)

prospective randomised trial have demonstrated the superiority of G-CSF priming in patients with AML and standard risk cytogenetics[17].

In order to further increase the intracellular levels of Ara-CTP, investigators at the MD Anderson Cancer Centre added the purine analogue fludarabine to the standard combination of anthracyclines and Ara-C[18]. After preclincial studies had shown synergistic effects associated with increased intracellular Ara-CTP levels, phase II studies in patients with relapsed and refractory AML confirmed the expected efficacy[19, 20].

STI-571 (Imatinib mesylate) has been introduced as the first representative of a new generation of small molecules allowing for specific, so called targeted therapies. Although the specific effects of STI-571 are mainly explained by the blockade of the tyrosine kinase activity of the BCR/ABL fusion-protein, the compound is known to suppress additional receptor tyrosine kinases in hematopoietic and leukemic stem cells[21]. Limited activity had been shown for STI-571 in AML cells but moderate synergism of STI-571 and Ara-C was described for certain concentration levels[6].

Nevertheless, case reports have demonstrated that STI-571 might have activity in BCR/ABL- AML[22]. Although the blockade of constitutively active c-kit was supposed to be responsible for these anti-leukemic effects, not all patients with AML who responded so far, were shown to have activating mutations of c-kit[23].

Our results suppose that STI-571 influences the metabolism of Ara-C leading to increased Ara-CTP levels in several leukemia cell lines. The exact molecular reason for this phenomenon has not been elucidated yet. One of the possible explanations might be that during the preincubation period at higher doses STI-571 blocks intracellular kinases in proliferating leukemia cell lines, which is compensated by a subsequent activation of nucleotide metabolism and proliferation. This might explain the increase of Ara-CTP measured in-vitro.

The opposite effect observed in CD34+ stem cells could be a consequence of the more quiescent status these cells have after mobilisation with G-CSF[24]. This fact and a different nucleotide metabolism makes them less susceptible to the effects of Ara-C[15]. In addition, some of the cell lines like HL-60 do not express c-kit or CD117 whereas CD34+ hematopoietic stem cells are CD117 positive as well. This could explain why the additional blockade of c-kit by STI-571 leads to a significant decrease of nucleotide metabolism. A cell cycle specific mechanism could be postulated for these observations.

A further hypothesis is that STI-571 may interact with enzymes responsible for nucleotide metabolism and may thereby lead to increased levels of Ara-CTP in leukemia cells[25, 26]. A good candidates for such an interaction are phosphatases like the 5-nucleotidase.

The concentration of STI-571 used in the current experiments is higher than trough plasma levels achieved in patients receiving 600–800 mg STI-571[27]. Therefore we have to consider that the changes of Ara-CTP observed in these experiments may not be reproducible at lower concentrations and may have limited clinical relevance.

As described above, the speculations about the reasons for the increase of Ara-CTP in several leukemia cell lines after incubation with STI-571 have to be replaced by functional analyses of different molecular pathways. Additional assays measuring cytotoxicity, induction of apoptosis and cell cycle status are currently incorporated into our investigations. If these preliminary results can be confirmed with primary leukemia cells, clinical studies combining STI-571 and Ara-C may become warranted in patients with relapsed or refractory AML.

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References

1. O'Dwyer ME, Druker BJ (2000) STI571: an inhibitor of the BCR-ABL tyrosine kinase for the treatment of chronic myelogenous leukaemia. Lancet Oncol 1:207-211
2. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038-1042
3. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348:994-1004
4. Hochhaus A, Kreil S, Corbin A, La Rosee P, Lahaye T, Berger U, Cross NC, Linkesch W, Druker BJ, Hehlmann R, Passerini C, Corneo G, D'Incalci M (2001) Roots of clinical resistance to STI-571 cancer therapy. Science 239:2163
5. Thiesing JT, Ohno-Jones S, Kolibaba KS, Druker BJ (2000) Efficacy of STI571, an abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against bcr-abl-positive cells. Blood 96:3195-3199
6. Scappini B, Onida F, Kantarjian HM, Dong L, Verstovsek S, Keating MJ, Beran M (2001) Effects of signal transduction inhibitor 571 in acute myelogenous leukemia cells. Clin Cancer Res 7:3884-3893
7. Gandhi V, Estey E, Keating MJ, Plunkett W (1993) Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 11:116-124
8. Hiddemann W, Wormann B, Reuter C, Schleyer E, Zuhlsdorf M, Bockmann A, Buchner T (1994) New perspectives in the treatment of acute myeloid leukemia by hematopoietic growth factors. Semin Oncol 21:33-38
9. Schleyer E, Ehninger G, Zuhlsdorf M, Proksch B, Hiddemann W (1989) Detection and separation of intracellular 1-beta-D-arabinofuranosylcytosine-5-triphosphate by ion-pair high-performance liquid chromatography. J Chromatogr 497:109-120
10. Schleyer, E., Pursche, S, Kohne, CH., Schuler, U., Renner, U., Gschaidmeier, H., Freiberg-Richter, J., Leopold, T., Jenke, A., Bonin, M., Bergemann, T., le Coutre, P., Ottmann, OG., Gruner, M., Bornhauser, M., and Ehninger, G. A simple and sensitive HPLC method for detection and quantitation of STI-571 and its main metabolite N-desmethyl-STI in plasma, urine, cerebrospinal fluid, culture medium and cell preparations. J.Chromatogr. 2003. In Press
11. Kern W, Schleyer E, Unterhalt M, Wormann B, Buchner T, Hiddemann W (1997) High antileukemic activity of sequential high dose cytosine arabinoside and mitoxantrone in patients with refractory acute leukemias. Results of a clinical phase II study. Cancer 79:59-68
12. Kern W, Aul C, Maschmeyer G, Schonrock-Nabulsi R, Ludwig WD, Bartholomaus A, Bettelheim P, Wormann B, Buchner T, Hiddemann W (1998) Superiority of high-dose over intermediate-dose cytosine arabinoside in the treatment of patients with high-risk acute myeloid leukemia: results of an age-adjusted prospective randomized comparison. Leukemia 12:1049-1055
13. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, Omura GA, Moore JO, McIntyre OR, Frei E, III (1994) Intensive postremis-

sion chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med 331:896-903
14. Gandhi V, Estey E, Du M, Nowak B, Keating MJ, Plunkett W (1995) Modulation of the cellular metabolism of cytarabine and fludarabine by granulocyte-colony-stimulating factor during therapy of acute myelogenous leukemia. Clin Cancer Res 1:169-178
15. Reuter C, Schleyer E, Rolf C, Wormann B, Büchner T, Hiddemann W (1997) Differential effect of GM-CSF pretreatment on intracellular Ara-C metabolism in normal bone marrow mononuclear cells vs acute myeloid leukemia (AML) blasts. Leukemia 11:561-571
16. Jahns-Streubel G, Reuter C, Unterhalt M, Schleyer E, Wormann B, Buchner T, Hiddemann W (1995) Blast cell proliferative activity and sensitivity to GM-CSF in vitro are associated with early response to TAD-9 induction therapy in acute myeloid leukemia. Leukemia 9:1857-1863
17. Lowenberg B, van Putten W, Theobald M, Gmur J, Verdonck L, Sonneveld P, Fey M, Schouten H, de Greef G, Ferrant A, Kovacsovics T, Gratwohl A, Daenen S, Huijgens P, Boogaerts M (2003) Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. N Engl J Med 349:743-752
18. Gandhi V, Estey E, Du M, Keating MJ, Plunkett W (1997) Minimum dose of fludarabine for the maximal modulation of 1-beta-D-arabinofuranosylcytosine triphosphate in human leukemia blasts during therapy. Clin Cancer Res 3:1539-1545
19. Estey EH, Kantarjian HM, O'Brien S, Kornblau S, Andreeff M, Beran M, Pierce S, Keating M (1995) High remission rate, short remission duration in patients with refractory anemia with excess blasts (RAEB) in transformation (RAEB-t) given acute myelogenous leukemia (AML)-type chemotherapy in combination with granulocyte-CSF (G-CSF). Cytokines Mol Ther 1:21-28
20. Estey E, Thall P, Andreeff M, Beran M, Kantarjian H, O'Brien S, Escudier S, Robertson LE, Koller C, Kornblau S, . (1994) Use of granulocyte colony-stimulating factor before, during, and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colony-stimulating factor. J Clin Oncol 12:671-678
21. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, Lydon NB (2000) Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther 295:139-145
22. Kindler T, Breitenbuecher F, Marx A, Hess G, Gschaidmeier H, Gamm H, Kirkpatrick CJ, Huber C, Fischer T (2003) Sustained complete hematologic remission after administration of the tyrosine kinase inhibitor imatinib mesylate in a patient with refractory, secondary AML. Blood 101:2960-2962
23. Schittenhelm M, Aichele O, Krober SM, Brummendorf T, Kanz L, Denzlinger C (2003) Complete remission of third recurrence of acute myeloid leukemia after treatment with imatinib (STI-571). Leuk Lymphoma 44:1251-1253
24. Uchida N, He D, Friera AM, Reitsma M, Sasaki D, Chen B, Tsukamoto A (1997) The unexpected G0/G1 cell cycle status of mobilized hematopoietic stem cells from peripheral blood. Blood 89:465-472
25. Galmarini CM, Graham K, Thomas X, Calvo F, Rousselot P, El Jafaari A, Cros E, Mackey JR, Dumontet C (2001) Expression of high Km 5'-nucleotidase in leukemic blasts is an independent prognostic factor in adults with acute myeloid leukemia. Blood 98:1922-1926
26. Galmarini CM, Thomas X, Calvo F, Rousselot P, Rabilloud M, El Jaffari A, Cros E, Dumontet C (2002) In vivo mechanisms of resistance to cytarabine in acute myeloid leukaemia. Br J Haematol 117:860-868
27. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344:1031-1037

Gleevec in the Treatment of AML

T.KINDLER[1*], F. BREITENBUECHER[1*], A. MARX[2*], B. WEINKAUF[3*], J. DUYSTER[4*], C. PESCHEL[4*], C. J. KIRKPATRICK[2*], M. THEOBALD, H. GSCHAIDMEIER[3*], C. HUBER[1*], and T. FISCHER[1]
[1]Johannes Gutenberg-University, Department of Hematology/Oncology, Mainz, Germany; [2]Johannes Gutenberg-University, Department of Pathology, Mainz, Germany; [3]Novartis Pharma AG, Nuernberg, Germany; [4]III.Medical Department, Technical University Munich, Munich, Germany

Acute myeloid leukemia (AML) is a clonal stem cell disorder that increases in incidence with age. Although induction chemotherapy results in high rates of complete remission, older patients and those who are refractory to or ineligible for standard chemotherapy generally have poor treatment outcomes. More than 70% of AML patients have blast cells that express c-kit, a receptor tyrosine kinase for the ligand stem cell factor (SCF). SCF stimulation results in proliferation of AML blasts. Further, constitutive c-kit tyrosine phosphorylation and activating mutations have been described in a subset of AML patients. Here, we present the update of a phase II pilot study investigating the efficacy and safety of imatinib mesylate, a tyrosine kinase inhibitor targeting Bcr-Abl, platelet-derived growth factor receptor (PDGFR) and c-Kit, in patients with c-kit positive AML refractory to or not eligible for chemotherapy. In addition, we present *in vitro* and *in vivo* data in primary AML cells investigating the molecular mechanisms of imatinib induced effects. 21 patients were enrolled and received imatinib 600 mg orally once daily. The overall hematologic response rate was 24%: two patients who started imatinib in a refractory situation with persistent blasts in BM and PB after chemotherapy experienced a complete hematologic remission; one patient met the criteria for no evidence of leukemia and two patients achieved a minor response. Treatment with imatinib demonstrated a good safety profile and was well tolerated. Western blot analysis and immunohistochemistry demonstrated c-Kit activation in primary AML cells. Further, imatinib treatment of primary AML cells inhibited c-Kit tyrosine-phosphorylation and SCF-induced phosphorylation of Akt. Genomic DNA-sequencing of c-KIT showed no mutations in exons 2, 8, 10, 11, 12, and 17. However, cDNA-sequencing of exon 9 revealed predominance of a differentially spliced isoform of c-KIT featuring deletion of a 12 bp segment encoding amino acids 510–513 (GNNK). Consecutive FACS-analyses performed during the course of imatinib treatment indicated that c-kit positive blasts were preferentially sensitive to the effects of imatinib. Overall, this report suggests that imatinib has clinical activity in a subset of c-kit positive AML patients.

Imatinib Mesylate (Glivec®, Gleevec™) in the Treatment of Chronic Myelogenous Leukemia (CML) and Gastrointestinal Stromal Tumors (GIST)

A. HOCHHAUS
III. Medizinische Klinik, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, Mannheim, Germany

Selective inhibition of deregulated tyrosine kinases by imatinib mesylate (Glivec® or Gleevec™, Novartis, Basel, Switzerland) is a promising therapeutic strategy in patients with chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). The results of imatinib therapy have continued to improve during the last years. From phase I to phase II trials of chronic phase CML patients who failed *interferon α therapy,* the complete cytogenetic response (CCR) rate increased from 13 to 41%, and in phase III studies, 76% of newly diagnosed chronic phase patients achieved CCR.[1, 2]

Because of the high CCR rate observed with imatinib, the appropriate measure for response in CML has now become molecular remission using PCR detection of residual BCR-ABL transcripts. Molecular analyses have demonstrated a rapid decrease of BCR-ABL transcript levels in newly diagnosed CML patients treated with imatinib[3]. Nested PCR may reveal residual BCR-ABL transcripts in samples which are negative by real time PCR. BCR-ABL transcript levels correlate with cytogenetic response, but continue to decrease even after CCR has been achieved. Imatinib is superior to IFN/Ara-C in terms of speed and degree of molecular responses, but residual disease is rarely eliminated[4]. 'Complete molecular remission', i.e. undetectable BCR-ABL transcripts by real time and nested PCR, has been observed in a small minority of <5% of patients. However, the achievement of a 'major molecular remission', i.e. of a 3-log reduction of the tumor load or of ratios BCR-ABL/ABL <0.1%, is associated with continuous remission in chronic phase CML patients[3, 4, 5]. Molecular response is clearly emerging as a key endpoint for clinical trials. Guidelines for molecular monitoring will help to make molecular data comparable and interpretable in a standardized fashion.

Significant responses have been observed even in advanced phase CML, these responses, however, are not durable in most patients[6, 7]. Hematologic relapses have been observed even after the reaching CCR or low BCR-ABL transcript levels. In an attempt to model resistance, several groups have generated imatinib-resistant cell lines using BCR-ABL-transformed murine hematopoietic cells and BCR-ABL-positive human cell lines. Mechanisms of imatinib resistance identified from these in vitro studies include several-fold increase in the amount of BCR-ABL protein, amplification of the BCR-ABL gene, and overexpression of the multidrug resistance P-glycoprotein. Sensitivity to imatinib may be restored by withdrawal of imatinib from the cultures. Increased levels of alpha$_1$ acid glycoprotein (AGP) have been suggested to cause binding of imatinib with consecutive inactivation. Treatment of mice with drugs binding AGP, e.g. erythromycin, was able to overcome such resistance. However, the clinical relevance of this observation is still controversial. The contribution of AGP to the development of resistance in clinical settings has to be determined[8].

Clinical studies have demonstrated responses in chronic phase (CP) patients lasting more than two years whereas most responding patients in blast crisis (BC) may relapse early despite continued therapy. Drug resistance has been observed, particularly in advanced phase but also in CP CML. In AP, 24% of patients failed to reach hematologic remission and 51% have relapsed after an initial response to imatinib monotherapy. In myeloid BC, 66% failed to achieve hematologic remission and 88% relapsed after initial response. In contrast, in CP the proportion of relapsing patients within 24 months was 13% in patients after failure to interferon α and 4% in newly diagnosed CML patients, respectively.

Clinical mechanisms of imatinib resistance are heterogeneous. Two groups have been described, one of which involves reactivation of BCR-ABL, with continuing dependence on BCR-ABL-signalling, whereas in the other group BCR-ABL remains inhibited but is bypassed by alternative signalling pathways. BCR-ABL amplification at the genomic and transcript levels occurs in some patients. This may confer a growth advantage of cells that overexpress BCR-ABL. Sequencing of the region encoding the amino acids that bind ATP and imatinib revealed acquired mutations which lead to substitutions of amino acids which are important for specific binding of imatinib. In vitro analysis of the capacity of imatinib to inhibit mutated ABL protein demonstrated variable biological consequences of these mutations. In other resistant patients, CML cells achieve the ability to bypass the BCR-ABL inhibition exerted by imatinib, suggesting that in such cases neither BCR-ABL overexpression nor ABL kinase domain mutations are responsible for resistance. In these cases, resistance may be due to evolution of the disease with occurrence of novel numeric or structural cytogenetic aberrations, e.g. trisomy 8, iso(17q) which lead to BCR-ABL independent proliferation of leukemic cells[9].

The occasional development of new chromosomal abnormalities demonstrating clonal growth of Ph negative hematopoiesis is of uncertain clinical significance. In most cases, they are likely due to a selection of prexisting clones lacking BCR-ABL and therefore not responding to imatinib.

In order to circumvent resistance, the use of subtherapeutic dosages of imatinib (<300 mg/day) should be avoided. The optimal therapy of resistant patients is still unclear. Options are dose increases up to 2×400 mg/day, combination with synergistically acting drugs, i.e. low dose ara-C, or switch to an alternative cytostatic therapy. The potential impact of combination therapies with low dose ara-C or interferon α to avoid the occurrence of resistance is being tested in prospective randomized trials.

Although the development of imatinib resistance presents new therapeutic challenges, the fact that BCR-ABL is active in many imatinib resistant patients suggests that the chimeric oncoprotein remains a rational drug target. Since mutations are heterogeneous, it may be unlikely to find a common new inhibitor with broad utility to overcome resistance. Knowledge of the mutations should permit the development of assays to detect drug-resistant clones before clinical relapse. Physicians treating CML with imatinib should be aware of the potential for resistance development in their patients. Approaches to prevent or handle resistance include dose escalation and combination therapy, both of which are being evaluated clinically.

In addition to various oncogenic forms of the BCR-ABL tyrosine kinase, imatinib also inhibits the receptor for stem-cell factor (SCF) – c-kit, a member of the type III group of receptor kinases. Preclinical studies have established that the drug blocks c-kit autophosphorylation, as well as SCF-stimulated downstream signalling events.

Gastrointestinal stromal tumors (GISTs) represent a subset of soft-tissue sarcomas that involve the gastrointestinal tract and are thought to have a common anchestor with the interstitial cells of Cajal. Somatic gain-of-function mutations in the c-kit gene are present in up to 92% of GISTs and serve as the scientific rational for the use of imatinib in GIST. Oncogenic c-kit mutations in GIST habe been localized to the extracellular domain, kinase domains 1 and 2 and predominantly in the juxtamembrane domain of the c-kit protein. Phase II studies have demonstrated a high level of efficacy of imatinib with 71% complete and partial responses[10, 11]. However, recent data shows that a minority of GIST patients lacking c-kit mutations and/or overexpression may also benefit from imatinib on the basis of alternative activation of *PDGFR α.*

Paracrine and/or autocrine activation of the PDGFR kinases α and β has been postulated in numerous malignancies, and the presence of PDGF autocrine loops is most well documented in gliomas. PDGF overexpression is also well documented in dermatofibrosarcoma protumerans, a highly recurrent, infiltrative skin tumor that is characterized by a chromosomal rearragement involving chromosomes 17 and 22. Rearrangements of *PDGFR α* and *β* have been described in chronic myeloproliferative disorders associated with eosinophilia, e.g. chronic myelomonocytic leukemia[12], hypereosinophilic syndrome[13], and atypical CML[14]. Such patients have been successfully treated with imatinib and phase II studies are ongoing.

Imatinib represents a significant medical advance in the treatment of neoplastic disease, and as the first molecularly targeted, rationally designed drug therapy for CML, imatinib establishes a new paradigm for future drug development. Clinical phase-I- to III trial data have provided proof of its clinical benefit, and with its specific mechanism of action, imatinib offers an effective and well-tolerated therapeutic option for patients with BCR-ABL positive CML, GIST and other neoplastic diseases associated with activation of ABL, PDGFR alpha or beta, or c-kit. Participation in clinical trials is strongly suggested to further improve the use of imatinib. Open questions are the durability of responses on imatinib monotherapy, the optimum duration of therapy in good responders, the treatment of residual disease, and the cause and frequency of drug resistance. Phase III clinical trials addressing these questions by comparing regular dose with high dose therapy and introducing parallel or consecutive combination therapies are ongoing. In CML IV study of the German CML Study Group, which has been launched in July 2002, imatinib monotherapy is being compared with a combination therapy with interferon α or ara-C and with imatinib therapy after initial treatment with interferon α. Early transplantation is suggested in high risk patients and in patients with a low transplantation risk (EBMT score 0-1). As of October 2003, 300 patients have been recruited. CML study IV provides a comprehensive therapeutic approach for newly diagnosed CML patients consisting of imatinib, interferon α, ara-C, and allogeneic stem cell transplantation with step-wise recommendations according to the individual response and tolerability of the treatment[15].

References

1. Kantarjian HM, Sawyers CL, Hochhaus A, Guilhot F, Schiffer C et al. (2002) Imatinib mesylate (Gleevec™) induces hematologic and cytogenetic responses in the majority of patients with chronic myeloid leukemia in chronic phase: Results of a phase II study. N Engl J Med 346: 645-652
2. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F et al. (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348: 994-1004
3. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML et al. (2003) Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed patients with chronic myeloid leukemia. N Engl J Med. 349: 1421-1430
4. Müller MC, Gattermann N, Lahaye T, Deininger MWN, Berndt A, Fruehauf S et al. (2003) Dynamics of BCR-ABL mRNA expression in first line therapy of chronic myelogenous leukemia patients with imatinib or interferon α/ara-C. Leukemia 17: Oct 2 [Epub ahead of print]

5. Paschka P, Müller MC, Merx K, Kreil S, Schoch C, Lahaye T (2003) Molecular monitoring of response to imatinib (Glivec®) in CML patients pretreated with interferon alpha. Low levels of residual disease are associated with continuous remission. Leukemia 17: 1687-1694.
6. Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F et al. (2002) Glivec™ (imatinib mesylate) induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: Results of a phase II study. Blood 99: 1928-1937
7. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller C, Ottmann OG et al. (2002) Glivec™ (imatinib mesylate) induces hematologic and cytogenetic responses in patients with chronic myeloid leukemia in myeloid blast crisis: Results of a phase II study. Blood 99: 3530-3539
8. Mahon FX, Deininger MWN, Schultheis B, Chabrol J, Reiffers J, Goldman JM, Melo JV (2000) Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance Blood 96: 1070-1079
9. Hochhaus A, Kreil S, Corbin AS, La Rosée P, Müller MC, Lahaye T et al. (2002) Molecular and Chromosomal Mechanisms of Resistance to Imatinib (STI571) Therapy. Leukemia 16: 2190-2196
10. Verweij J, van Oosterom A, Blay JY, Judson I, Rodenhuis S, van der Graaf W et al. (2003) Imatinib mesylate (STI-571 Glivec®, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. Eur J Cancer 39: 2006-2011
11. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347: 472-480
12. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ et al. (2002) Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 347:481-487.
13. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J et al. (2003) A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 348:1201-1214
14. Baxter EJ, Hochhaus A, Bolufer P, Reiter A, Fernandez JM, Senent L et al. (2002) The t(4;22)(q12;q11) in atypical chronic myeloid leukaemia fuses BCR to PDGFRA. Hum Mol Genet 11: 1391-1397
15. Hochhaus A, Berger U, Reiter A, Engelich G, Lahaye T, Kreil S et al. (2002) Current therapy concepts in chronic myeloid leukemia. Study IV of the German CML Study Group Internist 43:1228-1244

Meet-the-Professor Session I

Role of Mabcampath in Allogeneic Transplantation

K.S PEGGS
Royal Free and University College London Medical School, London, UK.

Introduction

The most efficient method for prevention of graft-versus-host disease (GvHD) following allogeneic haematopoietic stem cell transplantation (SCT) is T-cell depletion of the graft. Concurrent T-cell depletion of the recipient facilitates engraftment and limits the requirement for escalation of the intensity of conditioning that is otherwise often required to prevent an increased incidence of graft rejection. However, donor lymphocytes contribute an anti-leukaemia effect and lymphocyte depletion may exacerbate problems with immune reconstitution. There is a fine balance between the risks of GvHD and host-versus-graft reactions, relapse and infection.

Pharmacology. Alemtuzumab (CAMPATH-1H) is a humanised IgG1 monoclonal antibody with specificity for the CD52 antigen, which is widely expressed on all human lymphoid cells as well as eosinophils, monocytes, dendritic cells and macrophages.[1] The half-life of alemtuzumab is dependent on the amount of target CD52 antigen in the patient. Following an in vivo dose of 20 mg/day for 5 days (-8 to –4) prior to allogeneic transplantation there is persistence of alemtuzumab in vivo past day 0 sufficient to cause T cell lysis by complement fixation and ADCC, and significant levels of antibody persist up to day +28 post-transplant.[2] The estimated terminal half-life of 15-21 days contrasts with the half-life of < 1 day previously estimated for the rat monoclonal antibody CAMPATH-1G. Delayed clearance of the antibody may impair immune reconstitution, affect rates of viral reactivation and limit efficacy of the donor T cell mediated GVL effect. Administration to the recipient as part of the conditioning regimen results in effective recipient T-cell and DC depletion in the peripheral blood[2] but it remains unknown if alemtuzumab leads to depletion of tissue DCs that might initiate GvHD. In addition, if sufficient antibody is circulating on the day of transplantation the graft will also be depleted of T-cells, which may contribute to a reduction in the incidence and severity of GvHD. The optimal dose of antibody to prevent GvHD and minimize post-transplant immune suppression is currently unknown. It can equally well be used by admixture with the infused stem cells, or by administration to the patient prior to the transplant.

Reduced Intensity Regimens incorporating Alemtuzumab. Many of the lessons learnt in the myeloablative setting have now been shown to be true in the reduced intensity setting, which forms the focus for the rest of this abstract. The most commonly used regimens combine alemtuzumab with fludarabine and an alkylating agent, usually melphalan or busulphan.[4,5] Alemtuzumab has also been added to the BEAM regimen and used in reduced intensity conditioning for lymphoma.[6] A partial list of reported regimens containing alemtuzumab or CAMPATH-1G is shown in Table 1. These regimens vary in their myeloablative and immunosuppressive properties and it is currently unclear which is optimal for any given clinical scenario.

Engraftment and Chimaerism. Engraftment is rapid with the regimen combining alemtuzumab with fludarabine and melphalan. The median time to neutrophil recovery (0.5×10^9/l) was 13 days (8–23), as was the median time to achieve platelets >20×10^9/l (range 3–96).[4] The incidence of graft rejection was <3% using peripheral blood stem cells from sibling donors and was 6% with bone marrow from unrelated donors.[4, 11] Lineage-specific chimaerism studies demonstrate most patients to be full donor chimeras as early as day 7 following the transplant, whilst some develop stable mixed chimaerism between 1 and 3 months post-transplant.[12] Since this approach to transplantation relies heavily on graft-versus-malignancy effects, the development of mixed T-cell

Table 1. Alemtuzumab containing reduced intensity regimens

Conditioning Regimen	Reference
Alemtuzumab 100 mg+Fludarabine 150 mg/m^2 +Melphalan 140 mg/m^2	4
Alemtuzumab 100 mg+Fludarabine 150 mg/m^2 +Busulphan 8 mg/kg	5
CAMPATH-1G 50 mg+BEAM	6
Alemtuzumab 100 mg+2 Gy TBI	7
Fludarabine 180 mg/m^2+ATG 40 mg/kg +Busulphan 6.4 mg/kg+*in vitro* T cell depletion with Alemtuzumab 20 mg	8
TBI 450 cGy+Fludarabine 120 mg/m^2+Alemtuzumab 40 mg	9
Alemtuzumab 100 mg+Fludarabine 120 mg/m^2 +Cyclophosphamide 2 g/m^2 + *in vitro* T cell depletion with Alemtuzumab	10

chimaerism following reduced intensity stem cell transplantation might be associated with a higher incidence of disease relapse and acts a trigger for using donor leucocyte infusions.

GvHD. Published results of sibling donor SCT using other reduced intensity conditioning regimens have shown a 38–60% incidence of grade II-IV acute GvHD, which is the primary cause of death in some patients. Incorporation of alemtuzumab in the HLA identical sibling setting reduces GvHD, with acute grade II–IV GvHD in only 5%.[4] For reduced intensity regimens without alemtuzumab, the experience with unrelated donor SCTs using a fludarabine+melphalan protocol is of high rates of severe GvHD, with 1 in 4 patients dying directly as a result of GvHD.[13] A similar regimen containing alemtuzumab was associated with a low incidence of GvHD despite a significant incidence of HLA disparity. Only 6% of patients had grade III-IV and 15% grade II acute GvHD.[11] Interestingly, registry data detailing myeloablative transplants revealed an unexpected and apparently paradoxical effect of post-transplant cyclosporin – it appeared to reduce the risk of dying from infection after 6 months. Although part of the benefit could be explained by a reduction in GvHD, the effect was still evident when patients with GvHD or graft rejection were excluded from analysis.

Disease-Specific Outcomes

Acute Myeloid Leukemia. We have reported 55 patients with acute leukemia/MDS who were considered too high risk for conventional myeloablative allogeneic transplantation.[14] Disease status at transplant was 'high risk' CR1 (n=12), CR2 (n=20) or relapsed/refractory disease (n=13). There were 10 patients with MDS. The median follow-up of patients who are alive is 25 months. Non-relapse mortality (NRM) at 90d was 13%. Six patients received donor lymphocyte infusions pre-emptively or at relapse. At the time of analysis, 20/55 patients were alive and in CR, with a 3 year DFS and OS of 34% and 39% respectively. Sub-group analysis indicated that the 3 year DFS (35%) and OS (48%) of patients with relapsed AML treated in CR2 appeared to be distinctly superior to that achieved with the use of salvage chemotherapy alone in these patients. Corresponding DFS for patients in 'high risk' CR1 and those with relapsed/refractory disease was 50% and 31% respectively. Similar results have been obtained in patients with myelodysplasia using a regimen containing alemtuzumab, fludarabine and busulphan.[5] Whether reduced intensity transplantation +/– alemtuzumab can achieve superior results to chemotherapy alone in relapsed AML remains to be determined in randomized comparative trials.

Hodgkin Lymphoma. The efficacy of allogeneic myeloablative transplantation in HL remains controversial, particularly because of very high TRM using TBI-based conditioning regimens.[15] We have experience in 41 patients with HL treated with our reduced intensity conditioning regimen.[16] Disease status at transplant was CR in 6, PR in 24, untested relapse in 1 and refractory in 10. Projected 4 year OS and PFS are 63% and 34% respectively (70% and 37% for related donors). Nineteen received donor lymphocytes from 3 months post transplantation for residual disease/progression (n=16) or mixed chimaerism (n=3). Three received specific anti-tumour chemotherapy prior to DLI. 9/16 demonstrated disease responses following DLI (7CR, 2PR), including 2 with prior anti-tumour chemotherapy. TRM was 9%, with an additional 11% mortality associated with DLI (overall procedural mortality 20%). Current PFS (incorporating DLI responses) is 42% at 4 years.

Non-Hodgkin Lymphoma/CLL. We reported results in 94 patients (median age 48 years) with NHL/CLL.[17] Sixty one had low-grade histology (LG-NHL) [CLL (n=12), Mantle Cell Lymphoma (n=10), Follicular Lymphoma (n=32), Waldenstrom's macroglobulinaemia (n=2), T-NHL (n=5)] and 33 had aggressive high-grade histology (HG-NHL) [de novo High Grade NHL (n=22), Transformed LG-NHL (n=11)]. A total of 38 patients (40%) had failed a previous autograft, 23 (70%) of the HG-NHL group and 15 (25%) of the LG-NHL group. The median number of prior treatment courses was 3 (range 1–6), with high-grade patients receiving more prior therapy than patients with low-grade disease. At transplant 23 patients were in CR, 61 in PR and 10 had refractory or progressive disease. With a median follow-up of 26 months (range 2–49), the actuarial 4 year overall survival (OS) for all patients is 61% (31% for HG-NHL and 77% for LG-NHL, respectively, p=<0.001). The 100 day and 1 year TRM for all patients were 14% and 15% respectively and were worse (p=<0.001) for patients with HG-NHL (30% vs 5% at 100 days and 33% vs 5% at 1 year post-transplant respectively). The current PFS is 52%. This was significantly superior for patients with LG-NHL (66%) than for HG-NHL (26%) (p=<0.001), and remained so even in the patients with HG-NHL who had chemosensitive disease who had a PFS of 34%. A total of 27 patients have relapsed (11 HG-NHL and 16 LG-NHL), with 15 dying of progressive disease (8 high-grade and 7 low-grade). Similar outcomes have been reported in a smaller number of patients using a regimen containing CAMPATH-1G + BEAM conditioning.[6]

Multiple Myeloma. In an effort to enhance graft-versus-myeloma activity a study of adjuvant dose-escalating donor lymphocyte infusions administered from 6 months post transplantation was planned in 20 patients with HLA-matched related (n=12) or unrelated (n=8) donors and chemotherapy-sensitive disease.[18] Acute GvHD following transplantation was minimal (3 grade II, no grade III/IV). Non-relapse mortality was relatively low (15%) and related mainly to infective causes. Disease responses by 6 months post transplantation were modest (2CR, 4PR, 2MR, 6 no change, 3 progressive, 3 not evaluable). Fourteen patients received escalating dose DLI for residual/progressive disease. Disease responses were commoner in those developing GvHD but response durations were disappointing (many than 12 months) and progression often occurred despite persisting full donor chimaerism. Two-year estimated overall and current progression-free survival were 71% and 30% respectively. Two-year estimated OS and current PFS were 71% and 30% respectively. In summary, whilst the conditioning regimen resulted in low TRM and GvHD, allowing subsequent DLI in the majority of cases, it had limited anti-tumour activity in myeloma and the graft-versus-myeloma effect of post-transplant DLI was disappointing.

Infectious Complication. The use of alemtuzumab has been associated with slower immune reconstitution and an increased incidence of viral infections. Pre-emptive anti-CMV therapy based on PCR-based assays in 101 patients effectively limited the mortality associated with CMV reactivation.[19] Fifty-one patients (50%) had a CMV infection at a median of 27 days post-transplant with a probability of 84.8% in patients at risk of CMV infection (donor or recipient CMV seropositive). The probability of recurrence of CMV infection was more common in unrelated donor transplant recipients. The median time to a CD4+ T cell >200/mm^3 was nine months in patients studied. In spite of the higher incidence of CMV infection there was no significant difference in overall survival and non-relapse mortality between CMV infected and uninfected patients. There also appears to be an increased incidence of adenovirus, RSV and parainfluenza virus in alemtuzumab-treated patients; however, many of these infections are not associated with serious clinical sequelae.[20] EBV PTLD occur with increased frequency following T cell depletion but appear less common following CAMPATH compared to ATG, perhaps because of coincident depletion of B cells, the latent reservoir of EBV.[21]

Donor Lymphocyte Infusions. DLI can promote full donor chimaerism and GVL but are associated with a high risk of GvHD early after transplant. We have experience of dose escalating DLI in 46 patients at our institution, who received a total of 109 DLIs (median 2, range 1-6) to treat mixed chimaerism, residual disease or disease progression.[22] Diseases treated included myeloma (n=19), Hodgkin lymphoma (n=13), non-Hodgkin lymphoma (n=10) and other (n=4). Thirty-two had an HLA-matched family donor and 14 an unrelated donor. Grade II-IV GvHD occurred in 5 sibling and 7 unrelated donor recipients. GvHD was more common (p=0.002), occurred at lower T-cell doses, and was more severe in the unrelated donor cohort. Conversion from mixed to multi-lineage full donor chimaerism occurred in 30 of 35 evaluable patients. Presence or absence of mixed chimaerism in the granulocyte lineage at the time of DLI did not predict for chimaerism response or development of GvHD. Disease responses were documented in 63% myeloma and 70% Hodgkin lymphoma patients, were limited in degree and durability in the former group, and were not predicted by changes in chimaerism status.

Summary

Alemtuzumab reduces the incidence of acute and chronic GvHD following stem cell transplantation and reduces GvHD-related mortality. There is a delay in immune reconstitution and an increased incidence of viral infections with the use of alemtuzumab, however many of these infections are asymptomatic, and at least in the case of CMV in the sibling setting, do not adversely effect transplant-related mortality. Disease relapse appears more common but approaches incorporating DLI may offset this tendency in immune responsive malignancies. Delivery of these therapies in a less toxic manner remains a priority for future research.

References

(1) Hale G, Swirsky DM, Hayhoe FG, Waldmann H. Effects of monoclonal anti-lymphocyte antibodies in vivo in monkeys and humans. Mol Biol Med. 1983;1:321-334.

(2) Morris E, Rebello P, Thomson K et al. Pharmacokinetics of alemtuzumab (CAMPATH-1H) used for in vivo and in vitro T-cell depletion in allogeneic transplants: relevance for early adoptive immunotherapy and infectious complications. Blood. 2003 Jul 1;102(1):404-406.

(3) Klangsinsirikul P, Carter GI, Byrne JL, Hale G, Russell NH. Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. Blood. 2002;99:2586-2591.

(4) Kottaridis P, Milligan DW, Chopra R et al. In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood. 2000;96:2419-2425.

(5) Kenyon MC, Mijovic A, Devereux S et al. Allogeneic stem cell transplantation for myelodysplastic syndromes: a single center experience [abstract]. Bone Marrow Transplant. 2000;25 (suppl. 1):235.

(6) Cull GM, Haynes AP, Byrne JL et al. Preliminary experience of allogeneic stem cell transplantation for lymphoproliferative disorders using BEAM-CAMPATH conditioning: an effective regimen with low procedure-related toxicity. Br J Haematol. 2000;108:754-760.

(7) Devereux S, Parker JE, Shafi T et al. Campath-1H reduces the risk of graft versus host disease following allogeneic hematopoietic stem cell transplantation and minimally myelosuppressive conditioning with low

dose total body irradiation and cyclosporine/mycophenolate mofetil immunosuppression [abstract]. Blood. 2001;98 (suppl. 1):355b.
(8) Barge RMY, Marijt WAF, Osanto S et al. Minimal GVHD following nonmyeloablative in vitro T cell depleted allogeneic stem cell transplantation which allows the infusion of graded increments of donor lymphocytes in patients with hematological malignancies and solid tumors [abstract]. Bone Marrow Transplant. 2002;29 (suppl. 2):147.
(9) Carrum G, Przepiorka D, Popat U et al. Submyeloablative conditioning regimens using anti-lymphocyte antibodies for allogeneic stem cell transplantation [abstract]. Blood. 2001;98 (suppl. 1):184a.
(10) Rizzieri DA, Long GD, Vredenburgh JJ et al. Non-myeloablative allogeneic transplantation using T depleted matched sibling peripheral blood stem cells [abstract]. Blood. 2001;98 (suppl. 1):420a.
(11) Chakraverty R, Peggs K, Chopra R et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood. 2002;99:1071-1078.
(12) Geary J, Verfuerth S, Bomford J, McKeag N, and Mackinnon S. Kinetics of donor engraftment following nonmyeloablative unrelated donor transplantation using lineage-specific PCR [abstract]. Bone Marrow Transplant. 2002;29 (suppl. 2):153.
(13) Khouri IF, Keating M, Korbling M et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine- based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J Clin Oncol. 1998;16:2817-2824.
(14) Tauro S, Craddock C, Peggs K et al. Allogeneic stem cell transplantation using a Campath-1H based reduced-intensity-conditioning regimen improves survival in adults with high risk acute myeloid leukaemia and myelodysplasia. [abstract]. ASH 2003.
(15) Milpied N, Fielding AK, Pearce RM, Ernst P, Goldstone AH. Allogeneic bone marrow transplant is not better than autologous transplant for patients with relapsed Hodgkin's disease. European Group for Blood and Bone Marrow Transplantation. J Clin Oncol. 1996;14:1291-1296.
(16) Peggs K, Thomson K, Chopra R et al. Long term results of reduced intensity transplantation in multiply relapsed and refractory Hodgkin Lymphoma: evidence of a therapeutically relevant graft-versus-lymphoma effect. ASH 2003.
(17) Morris E, Thomson K, Craddock C et al. Long-term follow-up of an Alemtuzumab (CAMPATH-1H) -containing reduced intensity allogeneic transplant regimen for Non-Hodgkin's Lymphoma (NHL). Blood 2002 Nov 15;100(11):139a
(18) Peggs KS, Mackinnon S, Williams CD et al. Reduced Intensity Transplantation with In Vivo T-cell Depletion and Adjuvant Dose-Escalating Donor Lymphocyte Infusions for Chemo-Sensitive Myeloma: Limited Efficacy of Graft-Versus-Tumor Activity. Biol Blood Marrow Transplant. 2003 Apr;9(4):257-265.
(19) Chakrabarti S, Mackinnon S, Chopra R et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: Potential role of Campath-1H in delaying immune reconstitution. Blood. 2002 Jun 15;99(12):4357-63.
(20) Chakrabarti S, Collingham KE, Marshall T et al. Respiratory virus infections in adult T cell-depleted transplant recipients: the role of cellular immunity. Transplantation. 2001;72:1460-1463.
(21) Peggs KS, Banerjee L, Thomson K, et al. Post-transplant lymphoproliferative disorders following reduced intensity conditioning with in-vivo T cell depletion. Bone Marrow Transplant. 2003 Apr;31(8):725-26.
(22) Peggs KS, Thomson K, Hart D, et al. Dose escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism and disease responses. Blood. In press.

TRANSPLANTATION STRATEGIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: CURRENT CONCEPTS OF THE GERMAN CLL STUDY GROUP AND THE EBMT

P. DREGER
Dept. Hematology, Allgemeines Krankenhaus St. Georg, Hamburg, Germany

As conventional cytotoxic treatment has only limited efficacy in chronic lymphocytic leukemia (CLL), recent efforts to develop curative treatment strategies for this disease have focussed on autologous and allogeneic stem cell transplantation (SCT).

Autologous Transplantation. Autologous SCT (auto-SCT) has been increasingly used in younger patients with CLL during the past decade. Although early studies indicated that this approach might be a potentially curative treatment in this otherwise incurable disease [1-4], more mature data do not support that auto-SCT can result in eradication of the leukemic clone in a relevant proportion of patients [5-8]. Especially in those individuals with high risk CLL as defined by an unmutated VH gene status relapse appears to be inevitable [9]. Not being curative, however, does not necessarily mean not being beneficial, but pursuing a toxic and expensive procedure like SCT requires evidence of its clinical value in terms of improved remission duration or survival.

Comparative studies between auto-SCT and standard palliative treatment are completely lacking to date. Since the results of prospective randomized studies will not be available in the near future, we performed a risk-matched comparison between 66 patients who had undergone a uniform SCT regimen and a database of 291 patients treated conventionally [10]. Matching variables were age, Binet stage, IgVH gene mutational status, and lymphocyte count. Forty-three pairs fully matched for all 4 variables were identified. In both cohorts, 70% of the patients were in advanced stage, 67% showed an unmutated VH status, and 58% had a high lymphocyte count, indicating predominance of poor-risk cases. With the exception of time to study entry, patient groups were well balanced for a broad variety of additional risk factors including adverse genetic abnormalities and CD38 expression. With an overall median follow-up of 70 and 86 months, respectively, survival was significantly longer for the SCT patients than for the conventionally treated patients when calculated from diagnosis (hazard ratio (HR) 0.39 (95% confidence interval (95%CI) 0.16–0.92), p=0.03 (log rank)), or from study entry (HR 0.32 (95%CI 0.14–0.76), p=0.006). The benefit for the auto-SCT group remained significant when the analyses were restricted to those 58 patients who had an unmutated VH status. Cox regression analysis confirmed auto-SCT as independent favorable prognostic factor for survival from diagnosis as well as from study entry (HR 0.38, 95%CI 0.15-0.97, p=0.04; and HR 0.37, 95%CI 0.15-0.93, p=0.03, respectively).

In conclusion, this study provides evidence that auto-SCT might prolong survival of younger patients with high-risk CLL. Although patients with unmutated VH status have a significantly poorer outcome after auto-SCT than those with mutated VH, the present analysis indicates that unmutated patients will, nevertheless, particularly benefit from high-dose therapy due to the extremely poor results of conventional treatment in this subset. Because of the limitations inherent to this kind of study, our data do not definitely prove a survival advantage but provide for the first time a solid rationale for prospective randomized trials on this issue.

Based on these and other promising results, auto-SCT in CLL is currently being investigated in prospective randomized trial by the EBMT in cooperation with the GCLLSG (**GCLLSG CLL3R trial**). The aim of this study is to assess the efficacy of a consolidating autotransplant in comparison to no further treatment after having achieved remission upon first- or second-line therapy for symptomatic CLL (**Fig. 1**). The endpoints of the CLL3R trial are event-free survival, overall survival and quality of life. To date, almost one third of the 300 patients needed has been accrued, mainly from France, the UK, and Germany.

A novel approach aiming at complete elimination of residual CLL cells from the patient by auto-SCT is followed by the **GCLLSG CLL3C protocol**, which incorporates a limited amount of the CD52 antibody alemtuzumab into the high-dose regimen (**Fig. 1**). Preliminary results from this study suggest that complete molecular remissions can be achieved, but attention has to be given to the risk of infections associated with this regimen.

Allogeneic Transplantation. Allogeneic SCT (allo-SCT) has been shown to result in long-term disease control in a proportion of patients with resistant CLL [1;11-14], and there is some evidence that graft-versus-leukemia activity (GVL) is of crucial importance for the efficacy of allo-SCT [15-17]. To date, however, it is unknown whether GVL can be effective in patients with unmutated V_H status and unfavorable caryotype, respectively. Information on this issue is particularly important as allo-SCT in CLL is increasingly performed using nonmyeloablative or reduced-intensity conditioning [18-21], implying that the contribution of GVL for disease control becomes even more essential.

To this end, the GCLLSG in cooperation with the EBMT has set up the CLL3X study aiming at assessing the feasibility and efficacy of allo-SCT after reduced-intensity conditioning with fludarabine and cyclophosphamide in younger patients with aggressive CLL (**Fig 1**). As of November 2003, 33 patients from 9 centres have been included in this protocol. With a median follow-up of 22 months, the 2-year event-free survival is currently 80%. Of note, 8 of 10 patients available for sensitive ASO primer RQ-PCR monitoring show

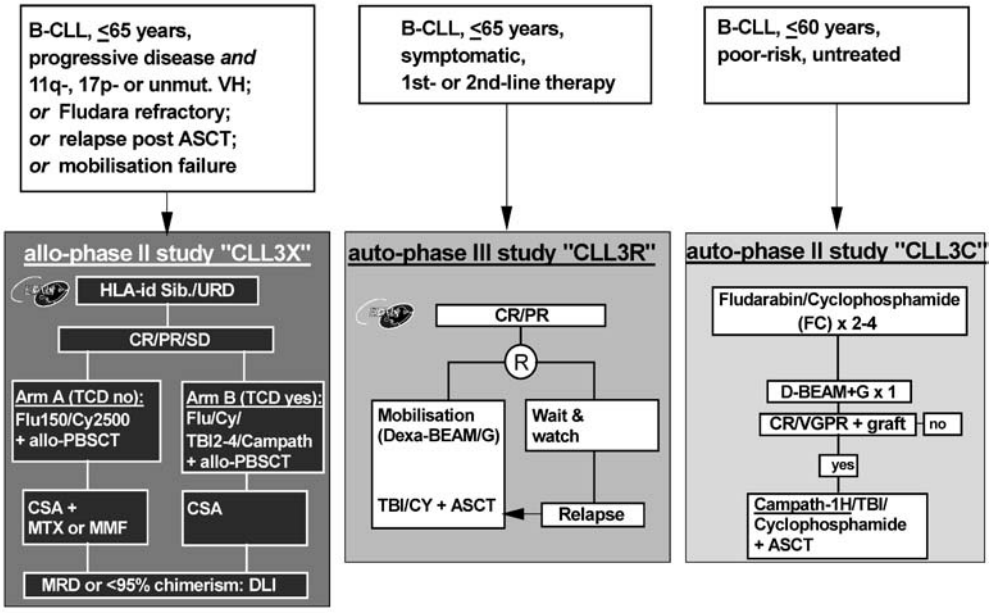

Figure 1: Current transplant concepts of the GCLLSG

ongoing complete molecular remissions occurring with the onset of chronic graft-versus-host-disease or immune interventions such as cyclosporin withdrawal or donor lymphocyte infusions.

In order to investigate the influence of in-vivo T cell depletion, recently a second arm was added to the study which allows the use of alemtuzumab and low-dose total body irradiation in the context of the conditioning regimen.

Conclusions. Taken together, a consolidating auto-SCT might be a promising approach for younger symptomatic patients with an unfavorable genetic risk profile (i.e. unmutated VH and/or adverse FISH karyotype) but still responsive disease. Selected patients with advanced or progressive CLL and unfavorable genetics should be considered for allografting. However, it must be kept in mind that both autologous and allogeneic stem cell transplantation are still experimental procedures which should not be performed outside of approved clinical trials.

References

1. Rabinowe SN, Soiffer RJ, Gribben JG, Daley H, Freedman AS, Daley J, Pesek K, Neuberg D, Pinkus G, Leavitt PR, Spector NA, Grossbard ML, Anderson K, Robertson MJ, Mauch P, Chayt-Marcus K, Ritz J, Nadler LM (1993): Autologous and allogeneic bone marrow transplantation for poor prognosis patients with B-cell chronic lymphocytic leukemia. Blood 82:1366-1376.
2. Khouri IF, Keating MJ, Vriesendorp HM, Reading CL, Przepiorka D, Huh YO, Andersson BS, van Besien K, Mehra RC, Giralt SA, Ippoliti C, Marshall M, Thomas MW, O'Brien S, Robertson LE, Deisseroth AB, Champlin RE (1994): Autologous and allogeneic bone marrow transplantation for chronic lymphocytic leukemia: Preliminary results. J Clin Oncol 12:748-758.
3. Dreger P, von Neuhoff N, Kuse R, Sonnen R, Glass B, Uharek L, Schoch R, Löffler H, Schmitz N (1998): Early stem cell transplantation for chronic lymphocytic leukaemia: A chance for cure? Brit J Cancer 77:2291-2297.
4. Sutton L, Maloum K, Gonzalez H, Zouabi H, Azar N, Boccaccio C, Charlotte F, Cosset JM, Gabarre J, Leblond V, Merle-Beral H, Binet JL (1998): Autologous hematopoietic stem cell transplantation as salvage treatment for advanced B cell chronic lymphocytic leukemia. Leukemia 12:1699-1707.
5. Pavletic ZS, Biermann PJ, Vose JM, Bishop MR, Wu D-D, Pierson J, Kollath J, Weisenburger D, Kessinger A, Armitage JO (1999): High incidence of relapse after autologous stem-cell transplantation for B-cell chronic lymphocytic leukemia or small lymphocytic lymphoma. Ann Oncol 10:1023-1026.
6. Khouri IF, Keating MJ (1998): High-dose chemotherapy for chronic lymphocytic leukemia: eligibility, timing, and benefit? Ann Oncol. 9:131-132.
7. Esteve, J., Montserrat, E., Dreger, P., Meloni, G., Pavletic, S., Catovsky, D., Dearden, C., Scime, R., Sutton, L., Desablens, B., Kimby, E., Coiffier, B., Brunet, S., Sanz, M. A., Besalduch, J., Caballero, D., Juliusson, G., Conde, E., Del Potro, E., and Schmitz, N. Stem Cell Transplantation (SCT) for Chronic Lymphocytic Leukemia (CLL): Outcome and Prognostic Factors after Autologous and Allogeneic Transplants. Blood 98 Suppl.1, 482a. 2001.
8. Dreger, P., van Biezen, A., Brand, R., Esteve, J., Gratwohl, A., Kimby, E., Michallet, M., Milligan, D. W., and Niederwieser, D. Prognostic factors for survival after autologous stem cell transplantation for chronic lymphocytic leukemia (CLL): the EBMT experience. Blood 96, 482a. 2000.
9. Ritgen M, Lange A, Stilgenbauer S, Dohner H, Bretscher C, Bosse H, Stuhr A, Kneba M, Dreger P (2003): Unmutated immunoglobulin variable heavy chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukemia. Blood 101:2049-2053.
10. Dreger, P., Stilgenbauer, S., Benner, A., Ritgen, M., Schmitz, N., and Döhner, H. The prognostic impact of autologous stem cell transplantation in patients with chronic lymphocytic leukemia: a risk-matched analysis based on the VH gene mutational status. Blood (in press).
11. Michallet M, Archimbaud E, Rowlings PA, Bandini G, Horowitz MM, Bortin MM, Atkinson K, Deeg J, Gahrton G, Goldman JM, Jouet J-P, Montserrat E, Rai KR, Rozman C, Speck B, Gratwohl A, Gale RP (1996): HLA-identical sibling bone marrow transplants for chronic lymphocytic leukemia. Ann Intern Med 124:311-315.
12. Khouri I, Przepiorka D, van BK, O'Brien S, Palmer JL, Lerner S, Mehra RC, Vriesendorp HM, Andersson BS, Giralt S, Korbling M, Keating MJ, Champlin RE (1997): Allogeneic blood or marrow transplantation for chronic lymphocytic leukaemia: timing of transplantation and potential effect of fludarabine on acute graft-versus-host disease. Br J Haematol 97:466-473.
13. Pavletic ZS, Arrowsmith ER, Bierman PJ, Goodman SA, Vose JM, Tarantolo SR, Stein RS, Bociek G, Greer JP, Wu CD, Kollath JP, Weisenburger DD, Kessinger A, Wolff SN, Armitage JO, Bishop MR (2000): Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia. Bone Marrow Transplant 25:717-722.
14. Doney KC, Chauncey T, Appelbaum FR (2002): Allogeneic related donor hematopoietic stem cell transplantation for treatment of chronic lymphocytic leukemia. Bone Marrow Transplant 29:817-823.
15. Rondon G, Giralt S, Huh Y, Khouri I, Andersson B, Andreeff M, Champlin R (1996): Graft-versus-leukemia effect after allogeneic bone marrow transplantation for chronic lymphocytic leukemia. Bone Marrow Transplant 18:669-672.
16. Mattsson J, Uzunel M, Remberger M, Ljungman P, Kimby E, Ringden O, Zetterquist H (2000): Minimal residual disease is common after allogeneic stem cell transplantation in patients with B cell chronic lymphocytic leukemia and may be controlled by graft-versus-host disease. Leukemia 14:247-254.
17. Esteve J, Villamor N, Colomer D, Cervantes F, Campo E, Carreras E, Montserrat E (2001): Stem cell transplantation for chronic lymphocytic leukemia: different outcome after autologous and allogeneic transplantation and correlation with minimal residual disease status. Leukemia 15:445-451.
18. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, O'Brien S, Giralt S, Ippoliti C, von WB, Gajewski J, Donato M, Claxton D, Ueno N, Andersson B, Gee A, Champlin R (1998): Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J.Clin.Oncol. 16:2817-2824.

19. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, Chauncey TR, Gooley TA, Hegenbart U, Nash RA, Radich J, Wagner JL, Minor S, Appelbaum FR, Bensinger WI, Bryant E, Flowers ME, Georges GE, Grumet FC, Kiem HP, Torok-Storb B, Yu C, Blume KG, Storb RF (2001): Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood 97:3390-3400.
20. Dreger P, Brand R, Hansz J, Milligan D, Corradini P, Finke J, Lambert-enghi Deliliers G, Martino R, Russel NH, van Biezen A, Michallet M, Niederwieser D (2003): Low treatment-related mortality but retained graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using reduced-intensity conditioning. Leukemia 17:841-848.
21. Schetelig J, Thiede C, Bornhauser M, Schwerdtfeger R, Kiehl M, Beyer J, Sayer HG, Kroger N, Hensel M, Scheffold C, Held TK, Hoffken K, Ho AD, Kienast J, Neubauer A, Zander AR, Fauser AA, Ehninger G, Siegert W (2003): Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group. J Clin.Oncol. 21:2747-2753.

Treosulfan/Fludarabin: A New Conditioning Regimen in Allogeneic Transplantation

J. CASPER[1], W. KNAUF, I.- BLAU[2], T. RUUTU, L. VOLIN[3], H. WANDT, K. SCHAFER-ECKART[4], J. HOLOWIECKI, S. GIEBEL[5], J. ASCHAN[6], A. ZANDER, N. KROGER[7], G. DOELKEN[8], and M. FREUND[1]
[1]University of Rostock, Germany; [2]University of Berlin, Germany; [3]University of Helsinki, Norway; [4]5th Med. Hospital Nuremberg, Germany; [5]Medical Academy Katowice, Poland; [6]University of Huddinge, Sweden; [7]University of Hamburg, Germany; [8]Ernst-Moritz-Arndt University Greifswald, Germany

Keywords: Marrow and stem cell transplantation; Allogeneic transplantation; Conditioning regimen; Acute myeloid leukemia

Abstract

Recently, the water-soluble bifunctional alkylating agent treosulfan demonstrated broad stem cell toxicity, immunosuppressive as well as antileukemic activity. Due to its well known low non-hematologic toxicity profile, treosulfan was considered an alternative agent for conditioning prior to allogeneic transplantation. A first clinical study, combining 3×10 g/m^2 of treosulfan with 5×30 mg/m^2 of fludarabine, demonstrated the feasibility of this conditioning. A fast, reliable and complete development of the donor hematopoiesis was evident as well as a low non-hematologic toxicity, transplantation-related mortality and relapse rate. In a second study treosulfan was escalated from 3×10 to 3×12 and 3×14 g/m^2. In this protocol, 55 pts (patients) not amenable to standard conditioning suffering from various hematological malignancies were included. Complete donor chimerism was reached by day 28 in 80% of the pts. So far, 8 pts (11%) died without disease progression and 11 pts (20%) relapsed. Treosulfan was very well tolerated. Especially no hepatic VOD, severe cardiac or pulmonary toxicity was noted. Acute GvHD (°II–IV) occurred in 44% and chronic GvHD in 45% of pts. Considering the poor prognosis of these study populations, treosulfan-based conditioning is considered to be safe and efficient. New phase II clinical protocols in AML and MDS will be initiated.

Introduction

For many years, conditioning therapy prior to allogeneic transplantation was restricted to a very limited number of drugs and total body irradiation irrespectively of the indication for transplantation. This has changed in the last few years with introduction of new conditioning approaches, such as non-myeloablative[1] or mini-transplantation[2]. Side-effects like severe mucositis and leukopenia are no longer unavoidable in the early posttransplantation period, when doses of conditioning therapy are significantly reduced. Other side-effects such as pulmonary toxicity[3] or veno-occlusive disease induced by busulfan[1, 4–6] may not be excluded even after reduced-intensity conditioning. Therefore, the introduction of alternative conditioning agents such as treosulfan may on one side help to further reduce side-effects and on the other side allow the development of highly effective conditioning regimens. Treosulfan (L-treitol-1,4-bis-methanesulfonate) is a prodrug of a bifunctional alkylating cytotoxic agent that is approved for the treatment of ovarian carcinomas in a number of European countries.[7, 8] Hematotoxicity is dose-limiting in conventional therapy.[9] When treosulfan was first used for conditioning prior to allogeneic transplantation in patients not amenable to standard conditioning therapy, a low extramedullary toxicity profile and low non-relapse mortality was evident as well as a low relapse rate resulting in a comparably high overall and event-free survival rates.[10] Similar results are reported from a combination of treosulfan (3×12 or 3×14 g/m^2) and cyclophosphamide (2×60 mg/kg) (18 pts).[11]

In the most recent study, 5×30 mg of fludarabine were combined with treosulfan 3×10, 3×12 and 3×14 g/m^2 to define the optimal dose of treosulfan and to develop refined patient and disease specific conditioning regimens. Results from this study as well as results of the combined data of AML and MDS patients from the two treoulfan/fludarabine protocols will be presented.

Patients, Material and Methods

Treosulfan combined with fludarabine was evaluated in 3 cohorts of ≥15 pts with hematological malignancies at high-risk of organ toxicity and otherwise not amenable to standard conditioning. Patient accrual started in January 2002, and stopped in June 2003. A total number of 55 pts was included (19 AML, 10 MM, 8 NHL, 7 MDS, 6 CML, 3 CLL, 1 ALL, 1 MH). A median follow-up of 8.9 months (range of those surviving 2.5–19.9) has been reached at the time of analysis.

Preparative regimen consisted of treosulfan (10 g/m^2: 20 pts; 12 g/m^2: 18 pts; 14 g/m^2: 17 pts) given on day -6, -5, -4 and fludarabine (30 mg/m^2 i.v.) day -6 to -2. No prophylactic anti-convulsive treatment was given. Pts with MUD/1misMRD (28/1) received rabbit-ATG (2 mg/kg) on day -3, -2, -1. Unmanipulated bone marrow or peripheral blood stem cells were transplanted on day 0. GvHD prophylaxis consisted of short course MTX and standard dose CsA.

For the analysis of AML and MDS patients from the two treosulfan/fludarabine studies, a total of 36 pts (study I 10 pts.; study II 26 pts. in the three dose levels) were available. These pts have been transplanted with de novo AML (7 pts in 1.CR, 7 pts in ≥2. CR, 5 pts in ≥1. PR, 1 pat in relapse and 1 pat with progressive disease), sAML (4 pts), MDS (7 pts), and sMDS (4 pts) with a median age of 50.5 years (range 20 to 66,6 pts older than 60 years).

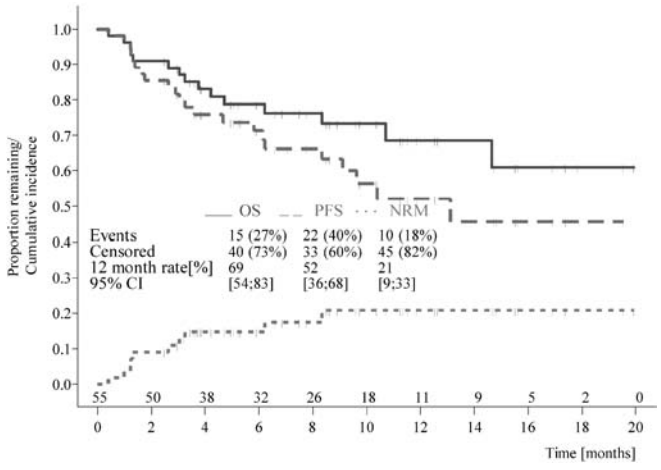

Figure 1: Overall and Progression-Free Survival, Non-Relapse Mortality of all 55 pts

Transplants originated from a matched related donor (16), mismatched related donor (1) or matched unrelated donor (19) were used.

Results

Leukocyte engraftment was reached at a median of 13 d (range 10–24), platelet engraftment at a median of 13 d (range 9–36). No primary, but 1 secondary graft failure was reported (CML, 2. CP with rapid disease progression shortly after transplantation).

Complete donor chimerism was reached by day 28 in 80% of 55 pts. So far, 8 pts died (disease progression: 2 AML, sepsis: 2, aGvHD: 2, myocardial infarction: 1, EBV infection: 1, (NRM rate: 11%). 11 pts (20%) relapsed (5 AML, 2 MM, 2 CML, 1 NHL, 1 MDS).

Reported CTC °III/IV adverse events (preliminary data) included increased liver transaminases, but no VOD, fever/infection, mucositis (only 3 pts), diarrhea, hyper-/hypotension, renal failure (3 reversible, 3 in context with multiorgan failure). Acute GvHD (°II–IV) occurred in 44% and chronic GvHD in 45% of pts so far. An overall and event-free survival at 12 months of 69% and 52% respectively has been reached (Figure 1).

AML and MDS patients were the indication of the largest group of patients in this as well as the first study with treosulfan and fludarabine. Analyzing the results of these patients after a median follow-up of 166.5 days (range 1–1414) resp. of 213.5 days for surviving pts, an overall survival of 65% and an event-free survival of 44% has been reached. 8 of 36 pts suffered from relapse, 4 of them had either a sMDS, an AML in PR, relapsed, or progressive disease prior to transplantation. The non-relapse mortality amounts to 24% in the Kaplan Meier estimates after one year or later and was due to infections (5 pts), GvHD (1 pat), and a myocardial infarction most likely not related to the conditioning regimen.

Discussion

Treosulfan-based conditioning lead to toxicity reduced, well tolerable and efficient regimens in patients otherwise not eligible for allogeneic transplantation. Certain toxic side-effects reported after reduced-intensity conditioning are less prevalent or did not occur in these protocols. While reduced-dose combinations with busulfan may still lead to severe hepatic toxicity (Bearman Grade II)[12, 13] or to VOD,[1, 6] the liver toxicity (ALT/AST elevation or bilirubinemia) observed was transient and without clinical relevance. No VOD occurred in the patients treated with treosulfan. Pneumonitis and pulmonary toxicity[12–15] or severe left ventricular failure/cardiac toxicity[13, 14] associated with reduced-dose busulfan and fludarabine or melphalan combinations were likewise not observed. The resulting low treatment-related mortality in high risk patient populations as well as the comparably high overall and event-free survival rates are promising. Looking at single indications in the two treosulfan and fludarabine studies, the largest indications AML and MDS as equally promising. Further treosulfan-based studies may, therefore, lead to the development of highly effective and toxicity-reduced conditioning regimens specific for individual indications.

References

1) Slavin S, Nagler A, Naparstek E, et al. Nonmyeloblative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91:756–763.
2) Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood. 1997;89:3048–3054.
3) Akasheh MS, Freytes CO, Vesole DH. Melphalan-associated pulmonary toxicity following high-dose therapy with autologous hematopoietic stem cell transplantation. Bone Marrow Transplant. 2000;26:1107–1109.
4) Ringden O, Remberger M, Ruutu T, et al. Increased risk of chronic graft-versus-host disease, obstructive bronchiolitis, and alopecia with busulfan versus total body irradiation: long-term results of a randomized trial in allogeneic marrow recipients with leukemia. Nordic Bone Marrow Transplantation Group. Blood. 1999;93:2196–2201.
5) Schetelig J, Kroger N, Held TK, et al. Allogeneic transplantation after reduced conditioning in high risk patients is complicated by a high incidence of acute and chronic graft-versus-host disease. Haematologica. 2002;87:299–305.
6) Kroger N, Schetelig J, Zabelina T, et al. A fludarabine-based dose-reduced conditioning regimen followed by allogeneic stem cell transplantation from related or unrelated donors in patients with myelodysplastic syndrome. Bone Marrow Transplant. 2001;28:643–647
7) Masding J, Sarkar TK, White WF, et al. Intravenous treosulfan versus intravenous treosulfan plus cisplatinum in advanced ovarian carcinoma. Br J Obstet Gynaecol. 1990;97:342–351.
8) Gropp M, Meier W, Hepp H. Treosulfan as an effective second-line therapy in ovarian cancer. Gynecol Oncol. 1998;71:94–98.
9) Harstrick A, Wilke H, Eberhardt W, et al. A Phase I dose escalation trial of intravenous treosulfan in refractory cancer. Onkologie. 1996; 19:153–156.
10) Casper J, Knauf W, Kiefer T, Wolff D, Steiner B, Hammer U, Wegener R, Kleine HD, Wilhelm S, Knopp A, Hartung G, Dolken G, Freund, Related Articles, Links Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. Blood. 2003 Aug 28 [Epub ahead of print]
11) Holler E, Trenschel R, Casper J, et al. Treosulfan/Cyclophosphamide conditioning in high-risk leukemia patients. A clinical phase I and pharmacokinetic study. Bone Marrow Transplant. 2003; 31 (Suppl. 1): Abstr. No. P642
12) Nagler A, Slavin S, Varadi G, Naparstek E, Samuel S, Or R. Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for malignant lymphoma. Bone Marrow Transplant. 2000;25:1021–1028.
13) Bornhauser M, Kiehl M, Siegert W, et al. Dose-reduced conditioning for allografting in 44 patients with chronic myeloid leukaemia: a retrospective analysis. Br J Haematol. 2001;115:119–124.
14) Ritchie DS, Seymour JF, Roberts AW, Szer J, Grigg AP. Acute left ventricular failure following melphalan and fludarabine conditioning. Bone Marrow Transplant. 2001;28:101–103.
15) Badros A, Barlogie B, Siegel E, et al. Improved outcome of allogeneic transplantation in high-risk multiple myeloma patients after nonmyeloablative conditioning. J Clin Oncol. 2002;20:1295–1303.

Meet-the-Professor Session II

Novel Treatment Strategies in Follicular Lymphoma

C. BUSKE,.H. DREYLING, M. UNTERHALT, and W. HIDDEMANN
Dept. of Internal Medicine III, University of Munich, Klinikum Großhadern, Munich, Germany

Abstract

Malignant lymphomas are a heterogenous group of malignancies, belonging to the 10 most frequent types of cancers worldwide. In indolent lymphoma only patients with limited stage I/II (Ann Arbor) can be potentially cured by local irradiation. However, about 85% of cases present with advanced stage; for these patients no established therapeutic strategy with curative potential exist. The development of the anti-CD20 monoclonal antibody rituximab has been one of the major contributions in the field of anti-lymphoma treatment in the recent years. Various clinical trials have suggested the combination of chemotherapy and rituximab to be highly effective in *de novo* as well as relapsed indolent lymphomas.

Introduction

85% of cases with indolent lymphoma present with advanced stage III/IV disease and currently no established therapeutic strategy with curative potential exists for these patients. Thus, palliative chemotherapy is being initiated only in symptomatic patients. In recent years, novel treatment options have been developed. One of the promising new concepts is the introduction of myeloablative chemo- or radiochemotherapy supported by bone marrow or peripheral blood stem cell transplantation. The approach of dose intensification, however, is limited to the minority of patients due to the increased treatment related short-term and long-term toxicity. Therefore, there is an urgent need for the development of innovative therapeutic strategies with increased lymphoma specificity and reduced treatment – related toxicity. One of the most attractive novel therapeutic strategies in patients with malignant lymphomas is the antibody-based therapy. Based on its immunobiological mechanism this therapeutic approach promises high lymphoma specificity, reduced toxicity and – because of its different mechanism of action – a synergistic effect with conventional chemotherapy. This therapeutic rationale has gained increasing clinical relevance through the development of the chimeric human-mouse anti-CD20 mAb Rituximab. Several phase II trials have shown that Rituximab has a high to moderate single agent activity in pretreated patients with indolent lymphomas and high activity in first line therapy with up to 90% remission rates. In follicular lymphoma (FL), the most frequent subtype of indolent lymphoma, prospective randomized trials have so far not been available. Thus, the 'German Low Grade Lymphoma Study Group' (*GLSG*) initiated two prospective randomized trials of Rituximab in combination with chemotherapy versus chemotherapy alone in patients in first-line or relapsed/refractory FL.

Results

First line therapy. In 2000, the GLSG initiated a prospective randomized phase III study to evaluate the efficacy of a combined immuno-chemotherapy in first line treatment of lymphoma. Patients were randomly assigned to receive either up to 6 courses of a standard CHOP chemotherapy (cyclophosphamide 750 mg/m^2 d1; doxorubicine 50 mg/m^2 d1; vincristine 1.4 mg/m^2 d1; prednisone 100 mg/m^2 d1-5) or a combined immuno-chemotherapy (CHOP+Rituximab 375 mg/m^2 d1). Responders subsequently underwent an interferon-alpha maintenance therapy or a myeloablative consolidation followed by autologous stem cell transplantation. In 373 currently evaluable patients with FL, the addition of rituximab led only to a moderate improvement of CR rate (21% vs. 17%) and overall response (97% vs. 93%) in comparison to chemotherapy alone (table 1). However, follow up analyses indicated a substantial increase of the time to treatment failure (TTF) after combined immuno-chemotherapy. Toxicity in both treatment arms was comparable with only a slight increase of grade III/IV neutropenia (42% vs. 37%) and allergy-like symptoms (4% vs. 0%) in the R-CHOP arm. In summary, rituximab plus CHOP chemotherapy is a highly efficient first line treatment for patients with FL with a comparable toxicity compared to CHOP alone.

Table 1:

	CHOP	CHOP-Rituximab
patients evaluable	187	201
completed induction	178	195
CR	17%	21%
PR	75%	76%
MR/SD	4%	2%
PD	3%	1%
ED	1%	1%
CR+PR	93%	97%

Table 2:

	FCM	FCM+Rituximab
patients evaluable	30	35
completed induction	28	32
CR	25%	44%*1
PR	50%	50%
MR/SD	7%	–
PD	18%	3%
ED	–	3%
CR+PR	75%	94%*2

*1 p=0.106; *2 p=0.047

Salvage therapy. For salvage therapy the *GLSG* started a multicenter national trial in patients with relapsed or refractory FL. Patients received a fludarabine-containing regimen (FCM) with fludarabine 25 mg/m^2 d 1-3, cyclophosphamide 200 mg/m^2 d 1-3, mitoxantrone 8 mg/m^2 d 1 every 28 days. A total of 4 courses were given. Patients were prospectively randomized for FCM alone or FCM plus Rituximab (375 mg/m^2 on day 1, R-FCM). Subsequently patients were assigned to a rituximab maintenance therapy (4×375 mg/m^2 after 12 and 36 weeks) or a watch & wait strategy. No significant differences of toxicity were observed in the two study arms. 60 patients are currently evaluable for initial response (table 2): addition of rituximab induced a significant increase in the rate of complete remissions with 44% in the immunochemotherapy arm versus 25% in the FCM arm (p=0.106) (table 2). The progression free survival (PFS) was significantly prolonged in the R-FCM arm compared to FCM with the PFS not reached versus 21 months, respectively, after a median follow-up of three years. Furthermore, addition of rituximab to FCM resulted in a clear trend towards a prolonged overall survival compared to chemotherapy alone after a median follow-up of 23 months.

Conclusion. Taken together, these two prospective randomized clinical trials confirm that the therapeutic concept of a combined immunochemotherapy consisting of rituximab and an established chemotherapeutic regimen is highly effective in the first line and salvage treatment of patients with follicular lymphoma. Particularly encouraging are the results in relapsed or refractory patients, whereas longer follow-up analyses have yet to define the role of rituximab in newly diagnosed patients. Of note, this increase in efficacy is not accompanied by a significantly higher treatment related toxicity. Accordingly, combining an immunobiological antibody-based approach with its different mode of action with conventional chemotherapy is a very powerful tool in the development of future multimodal and thus highly efficient anti-lymphoma strategies. Further follow-up of the presented studies will indicate whether rituximab plus chemotherapy will finally lead to an improved overall survival of patients with follicular lymphoma.

References:

1. Buske C, Feuring-Buske M, Unterhalt M, Hiddemann W. Monoclonal antibody therapy for B cell non-Hodgkin's lymphomas: emerging concepts of a tumour-targeted strategy (1999). Eur J Cancer 35:549-557
2. Hiddemann W, Dreyling M, Unterhalt M. Rituximab plus chemotherapy in follicular and mantle cell lymphomas (2003). Semin Oncol 30:16-20
4. Czuczman MS, Fallon A, Mohr A, Stewart C, Bernstein ZP, McCarthy P, Skipper M, Brown K, Miller K, Wentling D, Klippenstein D, Loud P, Rock MK, Benyunes M, Grillo-Lopez AJ, Bernstein SH. Rituximab in combination with CHOP or fludarabine in low-grade lymphoma (2002). Semin Oncol 29:36-40
5. Hiddemann W, Dreyling MH, Forstpointner R, Kneba M, Woermann B, Lengfelder E, Schmits R, Reiser M, Metzner B, Schmitz N, Truemper L, Eimermacher H, Parwaresch R.Combined Immuno-Chemotherapy (R-CHOP) Significantly Improves Time To Treatment Failure in First Line Therapy of Follicular Lymphoma – Results of a Prospective Randomized Trial of the *German Low Grade Lymphoma Study Group* (*GLSG*). (2003). Blood 104a
6. Dreyling MH, Forstpointner R, Repp R, Hermann S, Haenel A, Metzner B, Pott C, Hartmann *F,* Rothmann F, Parwaresch R, Unterhalt M, Hiddemann W.*5.*Combined Immuno-Chemotherapy (R-FCM) Results in Superior Remission and Survival Rates in Recurrent Follicular and Mantle Cell Lymphoma – Final Results of a Prospective Randomized Trial of the *German Low Grade Lymphoma Study Group* (*GLSG*). (2003). Blood 103a

New Treatment Strategies in Lymphomas: Aggressive Lymphomas

B. COIFFIER
Hospices Civils de Lyon & Université Claude Bernard, Lyon, France

Abstract

This review will cover the use of these monoclonal antibodies alone or in combination with chemotherapy for the treatment of aggressive lymphomas. Rituximab, an unconjugated anti-CD20 chimeric antibody, is certainly the most widely used but other unconjugated or radiolabeled monoclonal antibodies may catch up quickly. Rituximab combined with chemotherapy allows increasing the complete response rate, to decrease progression during treatment or relapse, to increase duration of response, event-free survival and overall survival. This benefit is now demonstrated in several randomized studies in different settings. Less data are available for the use of Rituximab in maintenance after chemotherapy or autologous transplant.

The use of monoclonal antibodies (MAb) for the treatment of lymphoma patients appeared some 7 years ago and, firstly, they have been developed for so-called 'low-grade' or indolent lymphomas (1). Murine antibodies have been used with toxin or isotopes attached to them (2-4) and, in this case, the antibody is used to specifically transport the active agent, often a radionucleide, to lymphoma cells. In the case of unmodified, naked, monoclonal antibodies, such as rituximab, the chimeric human-mouse antibody fixes the antigen on the membrane of lymphoma cells with the murine antibody part and stimulates the immune host mechanisms through the human Fc part. The fact of fixing the antigen on the cell surface may also trigger a cascade of biologic events leading to the cell death through the apoptotic process.

Rituximab alone for the treatment of lymphomas. First studies with MAbs were done on patients with relapsing or refractory indolent lymphomas, mostly follicular (5). The first study evaluating the response rate in patients with aggressive lymphoma (DLCL or MCL) patients was conducted in Europe four years ago (6). This study included patients in first or second relapses with 'intermediate or high-grade lymphoma' according to the Working Formulation. The response rate was higher in patients in first or second relapse than in primary refractory patients and in those with smaller tumors. This study showed that aggressive lymphomas may respond to rituximab therapy and it opened the development of rituximab in all types of B cell lymphomas.

Phase II studies combining rituximab and chemotherapy. One important phase II study has been presented with the combination of CHOP and rituximab in aggressive B-cell lymphomas (7). In this study, 33 patients with previously untreated advanced aggressive B-cell NHL received an infusion of rituximab (375 mg/m^2) on day -2 of each cycle of CHOP chemotherapy for 6 cycles. The overall response rate was 94% (31 out of 33 patients). Twenty patients reached a CR (61%), 11 patients a PR (33%), and 2 patients were classified as having progressive disease. The median duration of response and time to progression had not been reached after a median observation time of 26 months, 29 of the responding patients remaining in remission during this period, including 15 of 16 patients with an IPI score >2. This combination of CHOP and rituximab did not increase the toxicity of both type of chemotherapy.

Several phase II studies with a combination of rituximab and different chemotherapy regimens such as DHAP, EPOCH, VNCOP-B, fludarabine-based regimens, and ICE have been presented (8-10). The constant findings of these studies was the fact that the combination of chemotherapy plus rituximab did not seem to increase the toxicity of the chemotherapy regimen and seemed to allow a higher response rate and a longer duration of response than in historical controls. In this regard, the 55% response rate obtained with R-ICE in patients with DLCL and refractory to the first line therapy is demonstrative of the improvement of response (11). R-EPOCH was also associated with a 71% response rate in these refractory patients (10).

Randomized trials combining chemotherapy and rituximab for diffuse large cell lymphomas. The GELA (Groupe d'Etude des Lymphomes de l'Adulte) has presented the first randomized study demonstrating the benefit of adding rituximab to chemotherapy for the treatment of patients with diffuse large B-cell lymphoma (12). In the recent American Society of Hematology meeting, several other studies have demonstrated the benefit of this combination in DLCL patients and in patients with other lymphomas (13-18).

The GELA study compared 8 cycles of CHOP to 8 cycles of CHOP plus rituximab (R-CHOP) in elderly patients with DLCL. The classical doses of CHOP were given every 3 weeks and rituximab was given at the dose of 375 mg/m^2 the same day of the CHOP. Three hundred and ninety-nine newly diagnosed elderly patients were included in this trial, 197 in CHOP arm and 202 in R-CHOP arm. At the end of treatment, 75% of the patients had reached a CR or an undocumented CR (CRu) in the R-CHOP arm compared to 63% in the CHOP arm (p=0.005). Twenty-two percent of the patients treated with CHOP had a progression during the treatment compared to 9% in the R-CHOP arm. With a median follow-up of 4 years, 138 events (70%) were observed in the CHOP arm and 99 (49%) in the R-CHOP arm, most of them being a progression during or after treatment (p=0.002). This higher response rate and lower progression rate observed with the combination of CHOP and rituximab translated into statistically longer event-free survival, disease-free survival, and overall survival (Figure 1).

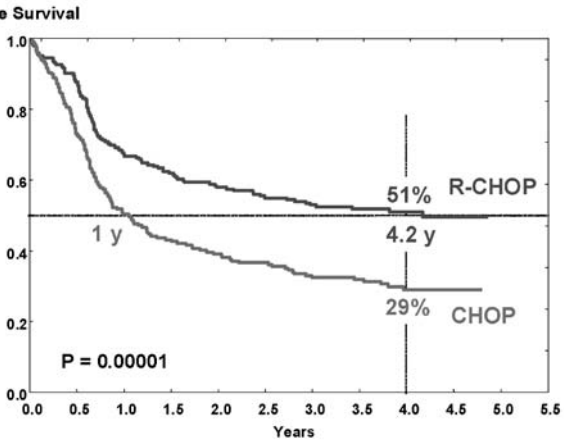

Figure 1. Event-free survival of the 399 patients of the GELA randomized study comparing CHOP and R-CHOP in elderly patients with diffuse large B-cell lymphoma.(12)

Table 1. Comparison of survivals in patients with diffuse large B-cell lymphoma treated in British Columbia before and after the registration of rituximab for this indication (March 1st, 2001).

	Before registration	After registration	P value
R-CHOP	15%	91%	
2-y PFS	52%	71%	0.0009
2-y OS	53%	77%	0.0001

The benefit of the addition of rituximab to the CHOP chemotherapy was even more important in patients with low-risk disease according to the age-adjusted International Prognostic Index: the improvement over CHOP alone at 4 years was over 50% (67% of the patients event-free compared to 36%). This study demonstrated that the addition of rituximab to CHOP chemotherapy led to significant prolongation of event-free and overall survivals in elderly patients with DLCL, without significant additional toxicity. The benefit of the combination was extremely important for patients expressing bcl-2 protein, an abnormality usually associated with chemoresistance, poor response to treatment, and shorter survival: patients treated with CHOP had a poorer outcome if they expressed bcl-2 protein (19). This observation translates the fact that rituximab induces a sensitization to the activity of chemotherapy drugs by acting on the apoptosis mechanisms (20).

In the intergroup study led by ECOG (E4494 (18)), elderly patients were randomized between CHOP and R-CHOP with a second randomization for responders between a consolidation with rituximab (4 injections every 6 months for 2 years) and nothing. The main differences with the GELA study were the number of cycles of CHOP that may be reduced to 6 for patients responding quickly to the chemotherapy and the number of rituximab injections. Rituximab was given once every two cycles of CHOP, so patients received half of the dose of the GELA study. This study showed a benefit for R-CHOP over CHOP alone in term of progression-free survival but not for overall survival. There is a trend for a longer disease-free survival in patients receiving Rituximab maintenance, particularly for the group of patients treated with CHOP alone. Because of the length of maintenance duration and the 4-arm design, these results must be considered as preliminary but they confirm the GELA results. The less important benefit added by Rituximab might be interpreted as the results of an suboptimal number of cycles of CHOP and infusions of rituximab.

Another very important observation was presented by physicians from the British Columbia Cancer Agency (16): they have compared the survival of patients with DLCL treated from the date of rituximab registration in Canada (March 1st, 2001) to that of patients treated during the same period of time but before this date. Even if some patients in the pre-rituximab period did receive rituximab in part of trials and some patients were treated with CHOP only in the rituximab period, they observed a significant increase in progression-free and overall survivals (Table 1).

Rituximab as maintenance therapy after chemotherapy. Because the efficacy of rituximab seems more important when the tumor mass is smaller, its efficacy may theoretically be higher in patients responding to chemotherapy either in first line or in relapse, thus as a maintenance therapy to prevent recurrences. Currently, only preliminary data have been presented. In the Intergroup US study, responders were randomized to observation or rituximab 4 in-

fusions every 6 months for 2 years (13). With a median follow-up of 2.7 years and half of the patients analyzed, patients treated with maintenance seem to have a longer progression-free survival, particularly for those treated without rituximab in induction. Currently, there is not sufficient data to use rituximab as maintenance therapy in patients with aggressive lymphoma. Moreover, because of the possible synergism between chemotherapy and rituximab, if both had to be given to a patient, the combination of both during induction therapy would be the best choice.

Monoclonal antibodies in the setting of stem cell transplantation. Rituximab has been used either for improving the salvage chemotherapy or for purging the transplant, even if few data exist for DLCL. It has also been used after autologous or allogeneic transplant to decrease the relapse rate (21). In this setting, it seems that rituximab may complete the response for patients with persisting abnormalities. However, the benefit may only be demonstrated by randomized studies.

In conclusion, these different studies all showed that rituximab has some activity by itself in DLCL and, more importantly, that this activity is increased when it is combined with chemotherapy. More studies will come and will allow the definition of the right use of rituximab in combination with chemotherapy: which regimen? How many infusions of rituximab? Maintenance therapy? Because of the long half-life of rituximab, the day of the infusion in combination with chemotherapy does not matter and doing both the same day is probably the easiest treatment for the patient.

References

1. Grillo-Lopez AJ, White CA, Varns C, et al. Overview of the clinical development of rituximab: First monoclonal antibody approved for the treatment of lymphoma. Sem Oncol 1999;26(5 Suppl 14):66-73.
2. Grossbard ML, Fidias P, Kinsella J, et al. Anti-B4-blocked ricin: A phase II trial of 7 day continuous infusion in patients with multiple myeloma. British Journal of Haematology 1998;102(2):509-15.
3. Kaminski MS, Zasadny KR, Francis IR, et al. Iodine-131 - Anti-B1 radioimmunotherapy for B-cell lymphoma. Journal of Clinical Oncology 1996;14(7):1974-81.
4. Witzig TE, White CA, Wiseman GA, et al. Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. Journal of Clinical Oncology 1999;17(12): 3793-803.
5. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. Journal of Clinical Oncology 1998;16(8):2825-33.
6. Coiffier B, Haioun C, Ketterer N, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma. A multicenter phase II study. Blood 1998;92(6): 1927-32.
7. Vose JM, Link BK, Grossbard ML, et al. Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol 2001;19(2): 389-97.
8. Hainsworth JD, Litchy S, Burris HA, et al. Rituximab as first-line and maintenance therapy for patients with indolent non-Hodgkin's lymphoma. J CLin Oncol 2002;20(20):4261-7.
9. Joyce RM, Kraser CN, Tetrealt JC, et al. Rituximab and ifosfamide, mitoxantrone, etoposide (RIME) with Neupogen (R) support for B-cell non-Hodgkin's lymphoma prior to high-dose chemotherapy with autologous haematopoietic transplant. Eur J Hematol 2001;66(Suppl 64):56-62.
10. Wilson WH, Gutierrez M, O'Connor P, et al. The role of rituximab and chemotherapy in aggressive B-cell lymphoma: A preliminary report of dose-adjusted EPOCH-R. Sem Oncol 2002;29(1 Suppl 2):41-7.
11. Kewalramani T, Zelenetz A, Bertino J, et al. Rituximab significantly increases the complete response rate in patients with relapsed or primary refractory DLBCL receiving ICE as second-line therapy. In: American Society of Hematology; 2001: Blood; 2001.
12. Coiffier B, Lepage E, Brière J, et al. CHOP Chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B-cell lymphoma. N Engl J Med 2002;346:235-42.
13. Habermann TM, Weller EA, Morrison VA, et al. Phase III Trial of Rituximab-CHOP (R-CHOP) vs. CHOP with a Second Randomization to Maintenance Rituximab (MR) or Observation in Patients 60 Years of Age and Older with Diffuse Large B-Cell Lymphoma (DLBCL). In; 2003; San Diego, CA: Blood; 2003.
14. Jiang Y, McLaughlin P, Thomaides A, et al. Quantification and Monitoring of the t(14;18) Translocation Copy Number by Real-Time PCR in Patients with Follicular Lymphoma Treated with FND Plus Concurrent or Delayed Rituximab. In; 2003; San Diego, CA: Blood; 2003.
15. Marcus R, Imrie K, Belch A, et al. An International Multi-Centre, Randomized, Open-Label, Phase III Trial Comparing Rituximab Added to CVP Chemotherapy to CVP Chemotherapy Alone in Untreated Stage III/IV Follicular Non-Hodgkins Lymphoma. In; 2003; San Diego, CA: Blood; 2003.
16. Sehn LH, Donaldson J, Chhanabhai M, et al. Introduction of Combined CHOP-Rituximab Therapy Dramatically Improved Outcome of Diffuse Large B-Cell Lymphoma (DLBC) in British Columbia (BC). In; 2003; San Diego, CA: Blood; 2003.
17. Dreyling MH, Forstpointner R, Repp R, et al. Combined Immuno-Chemotherapy (R-FCM) Results in Superior Remission and Survival Rates in Recurrent Follicular and Mantle Cell Lymphoma - Final Results of a Prospective Randomized Trial of the German Low Grade Lymphoma Study Group (GLSG). In; 2003; San Diego, CA: Blood; 2003.
18. Hiddemann W, Dreyling MH, Forstpointner R, et al. Combined Immuno-Chemotherapy (R-CHOP) Significantly Improves Time To Treatment Failure in First Line Therapy of Follicular Lymphoma - Results of a Prospective Randomized Trial of the German Low Grade Lymphoma Study Group (GLSG). In; 2003; San Diego, CA: Blood; 2003.
19. Mounier N, Briere J, Gisselbrecht C, et al. Rituximab plus CHOP (R-CHOP) overcomes bcl-2-associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). Blood 2003;101(11):4279-84.
20. Emmanouilides C, Jazirehi AR, Bonavida B. Rituximab-mediated Sensitization of B-non-Hodgkin's lymphoma (NHL) to cytotoxicity induced by paclitaxel, gemcitabine, and vinorelbine. Cancer Biother Radiopharm 2002;17(6):621-30.
21. Horwitz SM, Negrin RS, Blume KG, et al. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin's lymphoma. Blood 2003:2003-04-1257.
22. Coiffier B. Diffuse large cell lymphoma. Current Opinion in Oncology 2001;13(5):325-34.

MAIN SESSIONS

Special Lecture

Targeted Therapies in Myeloid Leukemias

D.G. GILLILAND
Harvard Medical School, Brigham & Women's Hospital and Harvard Institute of Medicine, Boston, USA

Introduction

Significant progress has been made over the past two decades in treatment of acute myeloid leukemia. However, the majority of adults who develop AML still die from complications of disease or therapy. There is thus a need to improve the current therapeutic approach to leukemia. Recent advances in our understanding of the molecular pathogenesis of leukemia have led to the preclinical and clinical development of molecularly targeted drugs. These may be useful additions to the armamentarium of drugs currently used to treat AML.

Genetics of AML

We have a detailed, though still incomplete, understanding of many of the molecular events associated with the development of AML. Many of these insights have been derived from cloning and characterization of recurring chromosomal translocations in AML, but point mutations in genes are increasingly being identified that contribute to pathogenesis of disease. A simplistic model for pathogenesis of AML can be generated based on classes of mutations that fall into two broad complementation groups. One of these groups contains mutations that are known to confer proliferative and/or survival advantage with minimal impact on differentiation in primary hematopoietic progenitors. These include activating mutations in RAS, FLT3, KIT and other receptor tyrosine kinases. Epidemiologic evidence that supports this class of mutation as a complementation group includes the observation that these mutations are only very rarely identified in the same patient. Experimental evidence to support complementation includes a limited data set showing that each of these engenders a similar myeloproliferative phenotype *in vivo*, and a limited data set indicates that one of these can substitute for the other in murine models of AML, as described in more detail below. For example, RAS or FLT3 can cooperate to generate nearly identical murine models of acute promyelocytic leukemia in cooperation with the PML-RARα transgene.

A second complementation group is comprised of mutations that often involve hematopoietic transcription factors, or transcriptional co-activators. These include rearrangements involving core binding factor, such as AML1-ETO or CBFß-SMMHC, those involving retinoic acid receptor alpha, such as PML-RARa, and those involving members of the HOX family of transcription factors. These fusion genes are also mutually exclusive in AML - that is, AML1-ETO is never observed in leukemias with the PML-RARa fusion and so on. There are several shared functional characteristics of this class of gene rearrangements. These include activity as dominant negative inhibitors of the wildtype alleles through recruitment of the nuclear co-repressor complex, association with impaired hematopoietic differentiation, and association with immortalization or "self-renewal" phenotypes. For example, the core binding factor fusions such as AML1-ETO and CBFß-SMMHC are dominant negative inhibitors of native core binding factor, and impair normal hematopoietic development during embryogenesis. Dominant negative inhibition is thought to be due to aberrant recruitment of the nuclear co-repressor complex by AML1-ETO. Expression of AML1-ETO in adult hematopoietic cells using conditional alleles confers properties of immortalization in serial replating assays in vivo. These properties are similar to those of PML-RARa, which is associated with impaired differentiation at the promyelocyte stage of hematopoietic development, and aberrantly recruits the nuclear co-repressor complex. Evidence that PML-RARa is important in engendering the block in differentiation includes the observation that all-trans retinoic acid (ATRA) targets the PML-RARa fusion and results in normal maturation and differentiation of myeloid lineage cells.

Epidemiologic data thus supports a model for genesis of AML that requires at least two broad classes of mutations - one that confers proliferative and survival advantage to hematopoietic progenitors with minimal effects on differentiation, and those that impair hematopoietic differentiation, and perhaps in doing so confer properties of immortalization by disrupting normal pathways for apoptosis of terminally differentiated cells. There is also convincing experimental evidence to support this model in cell culture and murine models of leukemia.

Phenotypic consequences of expressing activating mutations in signal transduction pathways: lessons from analysis of myeloproliferative syndromes

Myeloproliferative syndromes, such as chronic myeloid leukemias, chronic myelomonocytic leukemia, systemic mastocytosis, hypereosinophilic syndrome and juvenile myelomonocytic leukemia are thus far universally associated with activating mutations in signal transduction pathways, including BCR-ABL, TEL-PDGFßR, TEL-JAK2, FIP1L1-PDGFRA, oncogenic RAS alleles, activating mutations in PTPN11 (SHP2) and NF1 loss of function, respectively.

Table 1. Mutations that do not co-segregate in AML (or CML blast crisis)

BCR-ABL	AML1-ETO
TEL-PDGFßR	CBFß-SMMHC
KIT	PML-RARa
N-RAS	NUP98-HOXA9
K-RAS	C/EBPA loss of function
FLT3-ITD	AML1 loss of function
	MLL rearrangements
	CBP, p300, TIF2 fusions
Signal transduction mutations	Transcription factors

Table 2. Examples of mutations that co-segregate in AML

FLT3-ITD	and	AML1-ETO or PML-RARa or MOZ-TIF2 or C/EBPa or AML1
N-RAS	and	AML1-ETO or PML-RARa
KIT	and	CBFß-SMMHC
TEL-PDGFßR	and	AML1-ETO
BCR-ABL	and	NUP98-HOXA9

Table 3. Murine models of cooperativity in AML

Signaling		Transcription	Phenotype
BCR-ABL	+	NUP98-HOXA9	CML blast crisis
TEL-PDGFßR	+	AML1-ETO	CMMoL blast crisis
FLT3-ITD	+	PML-RARα	APL
FLT3-ITD	+	MOZ-TIF2	AML (M5)
K-RAS	+	PML-RARα	APL

Murine models of these diseases indicate that each of these activating mutations expressed alone in primary hematopoietic progenitors causes a myeloproliferative syndrome with many of the features of the human phenotype, including normal maturation and differentiation of myeloid lineage cells. Furthermore, mutations that result in loss of kinase activity in the tyrosine kinases abrogate the disease phenotype, indicating that the kinases are validated targets for therapeutic intervention in this context.

Chromosomal translocations in AML frequently target hematopoietic transcription factors

Chromosomal translocations in AML most often target transcription factors that play a role in normal hematopoietic development. These include numerous distinct translocations involving core binding factor, retinoic acid receptor alpha, members of the HOX family of transcription factors, and even transcriptional modulators such as MLL and the co-activators CBP, p300 and TIF2. The available evidence indicates that these gene rearrangements aberrantly recruit co-activator and co-repressor complexes, resulting in dysregulated transcription during hematopoietic development. For example, the core binding factor and retinoic acid receptor rearrangements (such as AML1-ETO and PML-RARa) aberrantly recruit the nuclear co-repressor complex, and act as dominant negative inhibitors of the native proteins. Phenotypically this may result in impaired hematopoietic differentiation, and in immortalization of hematopoietic progenitors, as observed in the case of the AML1-ETO fusion. The causality of PML-RARa in the block in maturation at the promyelocyte stage is apparent from the response of promyelocytic leukemia cells to all-trans-retinoic acid (ATRA). ATRA targets the PML-RARa protein, and results in release of co-repressors, with subsequent transcriptional transactivation. Phenotypically, leukemic cells undergo terminal differentiation and apoptosis, indicating that the PML-RARa fusion protein is a critical determinant of the block in differentiation. The success of ATRA in PML has fueled efforts to target the co-repressor complex in other leukemias using HDAC inhibitors.

Experimental evidence for cooperating mutations in AML

Epidemiologic data indicates that mutations in signal transduction pathways that confer proliferative and survival advantage to cells, and mutations affecting hematopoietic transcription factors, fall into at least two complementation groups. For example, FLT3-ITD or activation loop mutations, oncogenic N-RAS and K-RAS mutations, and activating mutations in KIT are presently collectively in as many as 50% of all AML, but these mutations only very rarely co-segregate in the same leukemia. Similarly, gene rearrangements involving core binding factor, retinoic acid receptor alpha, MLL, HOX family members, or loss of function mutations in C/EBPalpha or AML1, collectively occur frequently in AML, but two of these together are never identified in the same leukemia.

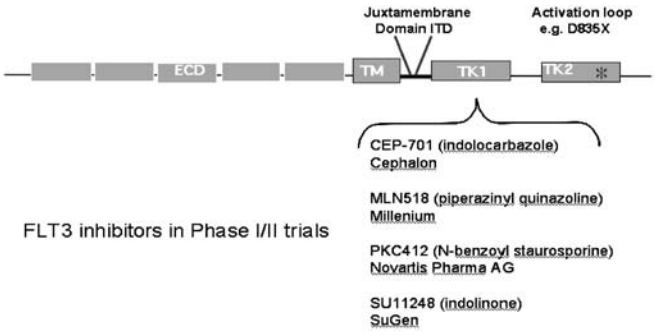

Figure 1. Small molecule inhibitors of FLT3 in clinical trials

In contrast, components from each of these groups are often identified in the same leukemia. For example, FLT3-ITD or activation loop mutations have been identified in core binding factor leukemias, MLL leukemias and are quite frequent in PML-RARa, as discussed in this meeting by Dr. Grimwade. Oncogenic RAS mutations have also been identified in core binding factor, MLL, and PML-RARa associated leukemias.

These data suggest cooperativity between at least these two broad classes of mutations. Experimental data derived from analysis of primary hematopoietic progenitors provides further support. First, expression of any of these mutant genes is not sufficient to cause AML. Expression of any of the signal transduction mutations results in a myeloproliferative phenotype, but not AML. Similarly, expression of AML1-ETO in a conditional model confers certain properties of immortalization in vitro, but does not result in leukemia in murine models unless chemical mutagens are utilized. Similarly, expression of PML-RARa in the promyelocyte compartment under the control of the MRP8 or Cathepsin G promoters results in AML with a long latency and incomplete penetrance, indicating a requirement for a second mutation.

Finally murine models directly demonstrate cooperativity between these two classes of mutations. Murine models of cooperativity in AML we and others have developed that recapitulate the human genotype include BCR-ABL and NUP98-HOXA9; TEL-PDGFβR and AML1-ETO; FLT3-ITD and PML-RARa; FLT3-ITD and MOZ-TIF2; and K-RAS and PML-RARa. Collectively these data indicate that each of these gene rearrangements contributes to the leukemia phenotype, and that strategies that target each of these components, respectively, may enhance therapeutic efficacy in AML.

Targeted therapies based on genetic insights in AML

Therapies that target signal transduction mutations. Components of the signal transduction apparatus are attractive molecular therapeutic targets. The paradigm is imatinib for therapy of BCR-ABL positive CML. Imatinib is a potent inhibitor not only of ABL, but also of PDGFRB, PDGFRA, KIT and ARG. There are now compelling clinical examples of imatinib efficacy in a spectrum of malignancies associated with activating mutations in each of these kinases, including TEL-PDGFRB positive CMML (and other PDGFRB rearrangements in CMML); hypereosinophilic leukemia associated with the FIP1L1-PDGFRA fusion, GIST tumors associated with activating mutations in KIT; and although rare, preclinical data indicates that the TEL-ARG fusion associated with AML is also imatinib sensitive.

Considerable effort has been dedicated to extrapolating the imatinib paradigm to treatment of AML. FLT3 is the single most commonly mutated gene in AML, and may also be a target for therapy even when the wildtype allele is overexpressed. A number of FLT3 selective inhibitors are currently in clinical trials in AML, including CEP-701, SU11248, MLN518 and PKC412. Some early data on response the Phase I/II trials will be presented in this meeting by Dr. Stone and others. Overall, it appears that these potent FLT3 inhibitors have acceptable toxicity profiles at doses required for pharmacologic inhibition of receptor in vivo, and initial data indicates that each of these has activity in reduction of AML blasts, in particular in the peripheral blood. However, initial data also clearly indicates that FLT3 inhibitors as single agents will not be sufficient to induce CR in the majority of AML, and will likely need to be used in combination with other agents.

Additional efforts have focused on targeting oncogenic RAS using farnesyl-transferase inhibitors (FTIs). As will be discussed in this meeting by Dr. Karp, these agents have activity in AML, but we do not yet have a complete understanding of the molecular basis for response. KIT, although relatively rarely mutated in AML are also attractive targets for therapeutic intervention. The most common KIT alleles in AML, D816V and D816Y, are not inhibited by imatinib, but are inhibited by several of the FLT3 inhibitors described above. Efforts are also underway to target the downstream effectors of kinases and RAS, including BCL-2 antisense strategies as will be discussed by Dr. Marcucci, as well as targeted cell surface receptors and leukemia epitopes using antibody or ligand that delivers cytotoxic agents such as calicheamicin or protein toxins to leukemic cells.

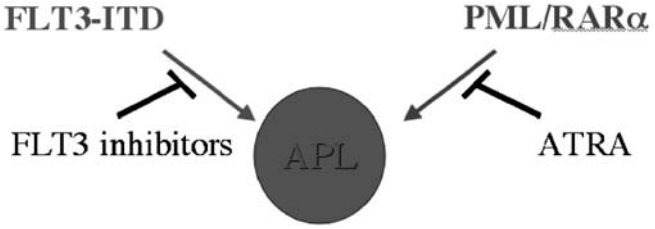

Figure 2. Molecular targeting of multiple mutations in AML

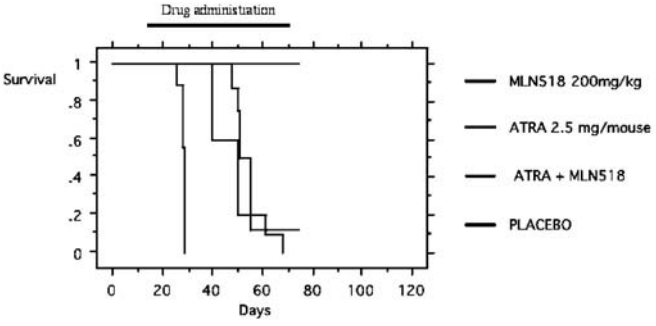

Figure 3. Prolonged survival in FLT3-ITD + PML-RARα leukemia treated with MLN518 and ATRA

Finally, whole genome screens for novel mutations that activate signal transduction pathways in leukemia are underway, including high-throughput sequencing of all known tyrosine kinases in the human genome.

Therapies that target aberrant transcription in AML

Based on the extraordinary success of ATRA in treating APL, as will be discussed in a session at this meeting, considerable effort has focused on development of histone deacetylase inhibitors in AML. This strategy is fueled in part by the observation that several gene rearrangements in AML, such as the core binding factor mutations, also aberrantly recruit the nuclear co-repressor complex. Dr. Gottlicher will discuss the re-invention of valproic acid as a histone deacetylase inhibitor, and other strategies to identify specific inhibitors are underway in academic centers and industry. It is not yet clear how efficacious these agents will be, or what spectrum of activity against the large number of HDAC family members will provide a suitable therapeutic window.

Combinations of molecularly targeted therapy: FLT3 inhibitors + ATRA in APL as a proof-of-principle

The use of ATRA has dramatically improved survival in APL patients with t(15;17). However, long term disease free survival still requires use of intensive induction chemotherapy with ATRA, and 20-30% of patients still die from complications of disease of therapy. A significant subset of APL patients harbor activating mutations in FLT3. Dr. Grimwade has demonstrated that 46% of APL patients in the UK MRC AML 1- and 12 trials had activating mutations in FLT3, and that these were associated with a poor prognosis. FLT3 mutation did not segregate independently from high WBC as a poor prognostic indicator, suggesting that FLT3 mutations may contribute to leukocytosis in APL.

To test the possibility that this high risk group of APL might benefit from combination of molecularly targeted therapy, we developed a murine model of APL due to cooperativity between FLT3-ITD and PML-RARa. We tested the effect of ATRA alone, the FLT3 inhibitor MLN518 alone, or the combination in treatment of leukemia induced by FLT3 -ITD and PML-RARa. There was a statistically significant prolongation of survival for either FLT3 inhibitors or ATRA alone compared with placebo, but the combination was statistically significantly superior to single agents in prolongation of survival. These data suggest that incorporation of FLT3 inhibitors in this poor prognostic subset of APL patients may have therapeutic efficacy.

Summary

There is still a compelling need to improve therapeutic outcome in AML. However, during the past several years our understanding of the genetic basis of AML, and the nature of the mutations that contribute to the phenotype, have been elucidated in cell culture and murine models of leukemia. The validation of various mutant leukemogenic gene products has in turn led to the development of an expanding group of molecular targeted therapies that have potential to improve the therapeutic window for treatment of AML.

Main Session I

The Recent JALSG Study for Newly Diagnosed Patients with Acute Promyelocytic Leukemia (APL)

R. OHNO and N. ASOU for the Japan Adult Leukemia Study Group
Aichi Cancer Center, Nagoya, and Kumamoto University Hospital, Kumamoto, Japan

Abstract

Based on the prognostic factors obtained from our previous APL92 study, in the JALSG APL97 study, we intensified chemotherapy for patients with leukocyte counts ≥3,000/μL and ≥10,000/μL, also intensified consolidation chemotherapy, and then tested whether further chemotherapy is required in patients with negative RT-PCR for PML/RARα after the completion of consolidation therapy. Of 256 presently evaluable patients, 244 (95%) achieved CR. Predicted 5-year EFS is 67% and predicted 5-year overall survival 84%.

Introduction

The introduction of all-*trans* retinoic acid (ATRA) to clinics in late '80s has marked a major advance in the treatment of acute promyelocytic leukemia (APL) [1]. With ATRA-based induction therapy, around 90% of newly diagnosed patients with APL now achieve CR, and over 70% of patients are curable with subsequent post-remission therapy with or without ATRA [2, 3]. The ATRA therapy was called a differentiation therapy at the beginning, which is still true as an observed phenomenon. Subsequent analysis of the differentiation, however, has elucidated its mechanism, and now the ATRA treatment is regarded as a molecular targeted therapy aimed at the pathogenetic molecule of this leukemia, i.e. PML/RARα. Thus, ATRA has turned to be the first successful molecular targeted drug in the history of cancer therapy.

Several groups, including ourselves [4-6], have reported improved treatment outcomes with ATRA alone or in combination with chemotherapy during remission induction therapy followed by intensive post-remission chemotherapy in patients with newly diagnosed APL, as compared with chemotherapy alone. However, a considerable number of patients who had received ATRA followed by chemotherapy actually relapsed [2-3]. To develop a better treatment regimen to minimize the relapse, we firstly analyzed prognostic factors in newly diagnosed patients treated in the APL92 study of the Japan Adult Leukemia Study Group (JALSG). Then based on the analysis, we started the APL97 study in 1977.

The JALSG APL92 Study and Prognostic Factors. The JALSG APL92 study from 1992 to 1997 was the first study of our group which employed ATRA for newly diagnosed adult patients with APL. Patients received daily ATRA (45 mg/m^2) alone until complete remission (CR) if their initial leukocyte counts were <3,000/μL, and daily ATRA+daunorubicin (DNR) 40 mg/m^2× 3 days+enocitabine (BHAC) 200 mg/m^2×5 days if leukocyte counts were ≥3,000/μL. During therapy, if peripheral blasts exceeded 1,000/μL, DNR × 3 days+BHAC ×5 days were added. After CR, 3 courses of consolidation and 6 courses of maintenance/intensification chemotherapy were given. Of 376 registered, 369 were evaluable (median age, 46, ranging from 15 to 86 years, and median leukocyte counts, 2,000/μL), and 333 (90%) achieved CR: 99% of patients of age <30, 89% of age 30 to 49, 90% of age 50 to 69 and 68% of age ≥70; 93% of leukocyte counts <3,000/μL, 91% of 3,000 to 10,000/μL, and 83% of ≥ 10,000/μL; and 94% of patients treated by ATRA alone, 89% by ATRA+initial chemotherapy, 88% by ATRA+later chemotherapy, and 86% by ATRA+initial and later chemotherapy. Among 223 patients who were initially treated with ATRA alone, 126 (57%) continued ATRA alone and 119 (94%) of them obtained CR, and 97 patients (43%) received additional chemotherapy and 85 (88%) of them achieved CR. Thus, 126 (34%) of 369 patients achieved CR with ATRA alone. Twelve patients (3%) died within 7 days after the start of therapy, and 28 (8%) died within 28 days mainly due to visceral bleeding. Of note, 121 (98%) of 123, who constituted the last one-third of the registered patients, achieved CR probably owing to the accumulated clinical experience of attending physicians in the use of ATRA. At the median follow-up of 45 months, the predicted 6-year overall survival and event-free survival (EFS) of all cases were 65% and 52%, respectively. EFS of patients with initial leukocytes <10,000/μL and ≥10,000/μL was 57% and 38%, respectively.

Favorable prognostic factors for the achievement of CR were younger age (<30 years), no or mild purpura, high serum total protein, and low LDH at presentation as well as no or mild DIC during induction therapy. Favorable prognostic factors for EFS were initial leukocyte counts <10,000/μL, no or mild DIC during induction therapy, and no sepsis during induction therapy [5-6].

From this study, we learned that more effective chemotherapy was needed for patients with leukocyte counts ≥10,000/μL both in induction and consolidation therapy in order to increase the CR and cure rates.

The JALSG APL97 Study. Based on the lesson learned from the previous study, in the JALSG APL97 study, we intensified chemotherapy for patients with leukocyte counts ≥3,000/μL and ≥10,000/μL, also intensified consolidation chemotherapy, and then tested whether further chemotherapy is required in patients with negative RT-PCR for PML/RARα after the completion of all consolidation therapy. For remission-induction therapy, all patients received 45 mg/m^2 of ATRA orally daily until achieving CR. Patients with leukocyte counts <3,000/μL at the start of therapy received ATRA alone (Group A). Patients with leukocyte counts between 3,000 and 10,000/μL or with peripheral blast and promyelocyte counts more than 1,000/μL received ATRA combined with idarubicin (IDR) 12 mg/m^2/day for 2 days by 30-minute infusion and cytosine arabinoside (Ara-C) 80 mg/m^2/day for 5 days by continuous infusion (Group B). Patients with leukocyte counts ≥10,000/μL received ATRA combined with IDR 12 mg/m^2/day for 3 days and Ara-C 100 mg/m^2/day for 5 days (Group C). When the blast and promyelocyte counts in the peripheral blood increased higher than 1,000/μL during the treatment, patients received IDR 12 mg/m^2 for 2 days and Ara-C 100 mg/m^2 for 5 days in addition to ATRA (Group D). Heparin and/or other anti-fibrinolysis agents (gabexate mesilate or nafamostat mesilate) and platelet transfusion were given if needed.

After achieving CR, patients received 3 courses of consolidation chemotherapy. The first consolidation consisted of mitoxantrone (MIT, 7 mg/m^2, 30-minute infusion) for 3 days and Ara-C (200 mg/m^2, continuous infusion) for 5 days. The second consolidation consisted of daunorubicin (50 mg/m^2, 30-minute infusion) for 3 days, etoposide (ETP, 100 mg/m^2, 1-hour infusion) for 5 days, DNR (50 mg/m^2) for 3 days, and Ara-C (140 mg/m^2, continuous infusion) for 5 days. The third consolidation consisted of IDR 12 mg/m^2 for 3 days and Ara-C 140 mg/m^2 for 5 days.

Patients were tested for PML/RARα fusion gene by RT-PCR before the start of therapy and after the completion of 3 courses of consolidation therapy. Those who were negative for the fusion gene after the consolidation therapy were randomized to receive either 6 courses of maintenance/intensification therapy every 6 weeks or no further therapy. The first maintenance/ intensification therapy consisted of BHAC (170 mg/m^2, 2-hour infusion, day 1 through 5), DNR (30 mg/m^2, 30-minute infusion, days 1 and 4) and 6MP (70 mg/m^2, orally, days 1 through 7). The second consisted of BHAC and MIT (5 mg/m^2, 30-minute infusion, days 1 and 2). The third consisted of BHAC, ETP (80 mg/m^2, 1-hour infusion, days 1, 3, and 5) and vindesine (VDS, 2 mg/m^2, bolus infusion, days 1 and 8). The fourth consisted of BHAC, ACR (14 mg/m^2, 30-minute infusion, days 1 through 4) and 6-MP. The fifth and sixth courses were the same as the first and third, respectively. Patients who were positive for the fusion gene were scheduled to receive ATRA 45 mg/day daily for 4 weeks, and then the above 6 courses of mainte-nance/intensification therapy.

From May 1997 to June 2002, 304 patients with newly diagnosed adult patients were registered. Presently, data of 256 patients were available. Of these, 244 (95%) achieved CR: 80 (98%) of 82 patients in Group A, 62 (98%) of 63 in Group B, 45 (88%) of 51 in Group C and 57 (95%) of 60 in Group D. Of 136 patients with leukocytes <3,000/μL who were initially treated with ATRA alone, 80 (58%) stayed on ATRA alone and 56 (41%) required additional chemotherapy due to the increase of blasts and promyelocytes. Thus, 82 (32%) of 256 patients were treated with ATRA alone and 98% of them achieved CR. No significant factor at presentation was identified to predict the increase of blasts and promyelocytes among these patients receiving ATRA alone. Fourteen (5%) patients died during the induction therapy and 7 (3%) during the consolidation therapy. Predicted 5-year EFS is 67%: 81% for Group A, 73% for Group B, 53% for Group C and 56% for Group D (p=0.006). Predicted 5-year overall survival is 84%, since relapsed patients were salvaged by stem cell transplantation, arsenic trioxide, a new retinoid, Am80 and/or readministration of ATRA.

At diagnosis 240 (98%) of 243 patients tested were positive for PML/RARα. At the time of achievement of CR, 61 (50%) of 121 patients tested were positive for PML/RARα. After the completion of 3 courses of consolidation therapy, none of 220 patients tested were positive for this fusion gene. So far, there seems to be no significant difference in EFS between two groups who received maintenance/intensification chemotherapy or no further therapy.

Next Strategy for the Increase of Cure Rate. The recent JALSG studies show that the intensification of chemotherapy during the ATRA-based induction therapy as well as during consolidation therapy produces better EFS and overall survival in newly diagnosed adult patients with APL. However, the CR rate and EFS of patients with higher initial leukocytes (≥10,000/μL) are still unsatisfactory, even though the EFS has improved compared with that in the JALSG APL92 study (53% versus 38%). The next step for the increase of CR rate in this cohort would be further intensification of chemotherapy, although intensified chemotherapy may bring therapy-related mortality. The 3 courses of consolidation therapy was just intensive enough to induce genetic CR in all patients tested for PML/RARα by RT-PCR, and since there was 7 (3%) death during consolidation phase, further intensification of chemotherapy in this phase would not be justified. Further chemotherapy does not seem to improve EFS in patients who were PCR negative after the 3 courses of consolidation therapy. Therefore, ATRA, arsenic trioxide or Am-80 would be the choice of drug as maintenance therapy if applied, although it should prospectively be tested whether further molecular targeting therapy would prolong EFS of APL, especially of patients with high initial leukocyte counts.

References

1) Huang M, Ye Y, Chen S, Chai J, Lu J, Zhoa L, Gu L, Wang Z (1988) Use of all-trans-retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567-572.
2) Tallman MS, Nabhan C, Feusner JH, Rowe JM (2002) Acute promyelocytic leukemia: evolving therapeutic strategies. Blood 99:759-767.
3) Ohno R, Asou N, Ohnishi K (2003) Treatment of acute promyelocytic leukemia: strategy toward further increase of cure rate. Leukemia 17:1454-1463.
4) Kanamaru A, Takemoto N, Tanimoto M, Murakami H, Asou N, Kobayashi T, Kuriyama K, Ohmoto E, Sakamaki H, Tsubaki K, Hiraoka A, Yamada O, Oh H, Furusawa S, Matsuda S, Minato K, Ueda T, Ohno R and the Japan Adult Leukemia Study Group (1995) All-trans retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. Blood 85:1202-1206.
5) Asou N, Adachi K, Tamura J, Kanamaru A, Kageyama S, Hiraoka A, Omoto E, Akiyama H, Tsubaki K, Saito K, Kuriyama K, Oh H, Kitano K, Miyawaki S, Takeyama K, Yamada O, Nishikawa K, Takahashi M, Matsuda S, Ohtake S, Suzushima H, Emi N, Ohno R (1998) Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Japan Adult Leukemia Study Group. J Clin Oncol 16:78-85.
6) Asou N, Adachi K, Tamura J, Kanamaru A, Kageyama S, Hiraoka A, Omoto E, Akiyama H, Tsubaki K, Saito K, Kuriyama K, Oh H, Kitano K, Miyawaki S, Takeyama K, Yamada O, Nishikawa K, Takahashi M, Matsuda S, Ohtake S, Ohno R (2001) Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia: the APL92 study Japan Adult Leukemia Study Group (JALSG). Cancer Chemother Pharmacol 48 (Suppl 1) S65-S71.

Risk-Adapted Therapy for Acute Promyelocytic Leukemia: The PETHEMA Approach

M.A. SANZ
Hematology Service. University Hospital La Fe. Valencia. Spain

Through the combination of ATRA and conventional chemotherapy, cure is now a reality for most patients with acute promyelocytic leukemia (APL). In fact, several modern approaches based on this combination have led to prolonged disease-free survival and potential cure for more than 80% of patients achieving complete remission. The current consensus on the most appropriate induction therapy consists of the simultaneous administration of ATRA and anthracycline-based chemotherapy [8]. It is likely that the standard form of chemotherapy will rely on the use of anthracycline alone, because no apparent advantage has been observed by adding other cytotoxic agents, such as cytarabine. Nevertheless, this issue remains a matter of investigation. On the other hand, there is not the same degree of consensus on the most appropriate consolidation therapy, except for giving at least two cycles of anthracycline-based chemotherapy. Apparently, therapeutic efficacy did not differ according to the number of cycles and type of drugs combined with anthracyclines. As a consequence, the role of non-anthracycline drugs has been seriously brought into question also for consolidation therapy. This is particularly true after the encouraging results recently reported by the Spanish PETHEMA group [4]. Using less intensive monochemotherapy with anthracyclines for both induction and consolidation therapy, which led to a significant reduction in treatment-related toxicity during the consolidation phase and a high degree of compliance, the outcome results of the LPA96 study of the PETHEMA group were similar to those obtained in other major studies using anthracycline-based chemotherapy combina-tions. Given the common therapy backbone and the similar outcome results of their respective trials, both the GIMEMA and PETHEMA groups carried out a joint study [5] that defined a simple model for relapse prediction based on the initial white blood cell and platelet counts. Based on this study, the GIMEMA and PETHEMA groups decided to apply this predictive model to design their next studies, thereby adopting a risk-adapted strategy. In November 1999, aiming to improve the antileukemic efficacy in patients with increased relapse risk, the PETHEMA started the new trial LPA99 based on a risk-adapted strategy. The results obtained in 426 consecutive patients with newly diagnosed PML/RARα positive APL who were enrolled in these two consecutive studies (LPA96 and LPA99) have been recently reported in Blood [6]. This study shows that combining ATRA with anthracycline monochemotherapy for induction and consolidation, followed by ATRA and low dose methotrexate and mercaptopurine for maintenance therapy, results in extremely high antileukemic efficacy, moderate toxicity and a high degree of compliance in patients with APL. The novel addition of ATRA to consolidation therapy, combined with a moderate increase in the dose of anthracycline for intermediate- and high-risk patients, resulted in higher antileukemic activity with no additional severe toxicity. The three-year cumulative incidence of relapse for patients in the LPA96 and LPA99 studies was 17.2 and 7.5 percent, respectively (P=0.008). Patients treated with ATRA in consolidation therapy showed an overall reduction in the relapse rate from 20.1 to 8.7 percent (P=0.004). In intermediate-risk patients the rate decreased from 14.0 to 2.5 percent (P= 0.006). This relapse rate of 7.5 percent at three-years in the overall LPA99 series, with 2.6 percent in the low and intermediate group (together accounting for 75 percent of total patients), compares favorably with all other studies published so far. This improved antileukemic efficacy, also translated into significantly better disease-free and overall survival, was certainly caused by the modified consolidation therapy. Although it is unclear which part of the reinforced consolidation therapy (ATRA or chemotherapy or both) may have led to the impact observed in the outcome, it is likely that the addition of ATRA has had a significant role, as demonstrated in several studies for induction and maintenance therapy [1, 2, 3, 4, 7]. Based on these results and those still unpublished by the GIMEMA (personal communication), we believe that the current consensus on the simultaneous administration of ATRA and chemotherapy for induction and maintenance therapy of APL could be extended to the consolidation phase. Based on risk-adapted strategies, future clinical investigations should focus on developing new therapeutic approaches to decrease the relapse rate in high-risk patients with hyperleukocytosis at presentation and progressively decreasing treatment intensity for the remaining patients.

References

1. Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH, on behalf of the MRC Adult Leukemia Working Party (1999). Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the randomized MRC trial. Blood 93:4131-4143.
2. Fenaux P, Chastang C, Sanz MA, et al (1999). A randomized comparison of ATRA followed by chemotherapy and ATRA plus chemotherapy, and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. Blood 94:1192-1200.
3. Mandelli F, Diverio D, Avvisati G, et al (1997). Molecular remission in PML/RARα-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Blood 90:1014-1021.
4. Sanz MA, Martín G, Rayón C, et al (1999). A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and

reduced toxicity in newly diagnosed PML/RARα-positive acute promyelocytic leukemia. Blood 94:3015-3021.
5. Sanz MA, Lo Coco F, Martín G, et al (2000). Definition of relapse risk and role of non-anthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood 96:1247-1253.
6. Sanz MA, Martin G, Gonzalez M, et al (2003). Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA Group Blood. Prepublished October 23, 2003; DOI 10.1182/blood-2003-07-2462
7. Tallman MS, Andersen JW, Schiffer CA, et al (1997). All-trans retinoic acid in acute promyelocytic leukemia. N Engl J Med 337:1021-1028.
8. Tallman MS, Nabhan Ch Feusner JH, Rowe JM (2002). Acute promyelocytic leukaemia: evolving therapeutic strategies. Blood 99:759-767.

Monitoring of Minimal Residual Disease in Acute Promyelocytic Leukemia

E. LENGFELDER, A. REITER, S. SAUSSELE, A. WEISSER, and R. HEHLMANN
III. Medizinische Universitätsklinik, Klinikum Mannheim,
Fakultät für Klinische Medizin Mannheim, University of Heidelberg, Germany

Key words: Acute promyelocytic leukemia, Minimal residual disease, Qualitative and quantitative RT-PCR

About 90% of patients with newly diagnosed acute promyelocytic leukemia (APL) are in complete hematologic remission after the induction therapy with all-trans retinoic acid (ATRA) and intensive chemotherapy and more than 70% of these patients are expected to be cured by subsequent consolidation and maintenance therapy. About 10 to 30% of patients will relapse in dependence on individual risk factors and modifications of first line therapy [8]. Although second remission rates up to 90% may be reached using variable salvage regimens, the probability of survival is reduced by about half in comparison to first line therapy. The identification of different risk-groups and the development of potent salvage strategies with acceptable toxicity are therefore challenging tasks in the treatment of APL. Some pretreatment patient characteristics have been associated with a higher risk of relapse, e.g. presenting leukocyte and platelet counts [1,14], CD56 status [12], presence of *FLT3* mutations [6] and *PML* breakpoint pattern [5], but all lack the precision to predict the relapse risk of the individual case. In addition, the prognostic importance of several factors may be influenced by the modification of the therapy, which limits their value [8].

Qualitative nested RT-PCR. It has become evident that testing *PML-RARA*-positive by qualitative nested RT-PCR after the end of consolidation or reappearance of a positive PCR during follow up is associated with a subsequent overt hematologic relapse [1, 7, 13, 14].

This has provided the rationale for the monitoring of minimal residual disease (MRD) by detection of *PML-RARA* fusion transcripts with conventional qualitative RT-PCR assays during and after first-line or relapse treatment schedules including autologous and allogeneic transplant procedures. According to the results of the largest series reported by the GIMEMA group, 70% relapses could be successfully predicted on the basis of prior molecular relapse. Median time from molecular conversion to frank relapse was 3 months (range, 1 to 14 months). However, achievement and persistence of PCR negativity (sensitivity threshold – 1 in 10^4) cannot be equated with cure, since approximately 30% patients testing PCR negative in the marrow at the end of consolidation or during follow up relapse despite of the favorable PCR results [3]. These data demonstrate that *PML-RARA* positivity is providing important prognostic information for directing necessary additional treatment intensification for the patients with impending relapse, whereas PCR negativity does not reliably predict a favorable outcome. These results further indicate that attenuation of the treatment intensity on the basis of negative PCR results may lead to a deterioration of the outcome.

Several strategies have been developed to improve the predictive value of MRD monitoring in APL: Firstly, by correlating the MRD results with the scheduled chemotherapy, it was suggested that the kinetics of clearance of residual leukemic cells might be a prognostic marker, but clinical studies provided conflicting data. Whereas the GIMEMA and PETHEMA groups found no correlation between PCR status after induction and subsequent risk of relapse, the MRC study found that detection of transcripts at any stage following induction or during consolidation was significantly (p=0.006) associated with an increased risk of relapse, with PCR status following the third course of chemotherapy being most predictive [1, 11, 13, 14].

A second strategy is the use of modified or alternative assays with increased sensitivity for the detection of the *PML-RARA* fusion gene or the reciprocal *RARA-PML* fusion gene which is an additional target for monitoring residual disease because it can be detected with a higher sensitivity of about 1 to 2 logs. However, this increase in sensitivity did not improve the predictive value of MRD assessment at the end of consolidation in one study because *RARA-PML* negative patients relapsed and *RARA-PML* positive did not [1]. However, for patients that express *RARA-PML*, serial monitoring of this transcript in parallel with the more conventional *PML-RARA* assay could be of value, providing earlier warning of impending relapse and hence permitting more rapid initiation of pre-emptive therapy.

Thirdly, modified assays with improved sensitivity of up to 10^{-6} detected residual *PML-RARA* fusion transcripts in the majority of patients in long-term remission making it difficult to distinguish between patients prone to relapse from those who are still likely to be cured [15].

Quantitative RT-PCR. Because conventional qualitative RT-PCR fails to detect significant residual disease in a proportion of patients who ultimately relapse, sensitive quantitative approaches have been developed for the evaluation of the kinetics of residual disease [2].

In several relatively small studies with real-time quantification using hybridization or hydrolysis technology, it was demonstrated that a sensitive and accurate quantification of *PML-RARA* fusion transcripts may provide a superior approach for monitoring MRD compared to qualitative RT-PCR, with the

majority of investigated patients showing significantly higher transcript levels of *PML-RARA* before onset of clinical relapse [4].

The largest series so far evaluating quantitative PCR for MRD monitoring in APL patients was retrospectively evaluated by the US Intergroup considering 123 patients treated on protocol 0129, which included randomization for ATRA as a component of induction and/or maintenance therapy [4]. This study showed that patients with relatively high *PML-RARA* transcript levels at the end of consolidation (*PML-RARA*/*GAPDH* copy ratio $>10^{-5}$) had a significantly increased risk of relapse in comparison to patients with lower or undetectable *PML-RARA* transcripts (disease free survival 33% vs 65% at 3 years). Interestingly, half of the patients who ultimately relapsed had levels of MRD that were below the detection threshold of the quantitative *PML-RARA* assay. This underlines that treatment decisions cannot be solely based on a negative PCR status at the end of consolidation therapy and that a molecular surveillance is needed during further follow up. Furthermore, the Intergroup study revealed intermittent PCR positivity in remission in patients who appear to be cured of their disease.

These observations demonstrate that further correlation of clinical data with the results of the quantitative PCR are needed to define the predictive value of this method exactly. Nevertheless, it can be expected that the advent of quantitative RT-PCR assays will contribute to improvements in patient care. This is supported by preliminary data which suggest that initiation of second-line therapy may be more effective and less toxic at the time of molecular relapse than at the point of hematologic relapse resulting in improved long-term survival [9,10].

A quantification of the leukemic burden by a quantitative RT-PCR of *PML-RARA* at diagnosis followed by a close monitoring after each treatment course and during follow up might contribute to the comparability of the different treatment approaches in APL. It might further be helpful to identify patients who will profit from an early intensification of treatment as presently followed by the German AML Cooperative Group (AMLCG) incorporating high dose ara-C into the induction therapy leading to a rapid reduction of the malignant clone on the clinical and molecular levels [7] and those patients who are sufficiently treated with less intensive approaches. Further investigations will show whether parallel monitoring of the MRD with a quantitative RT-PCR of the reverse transcript *RARA-PML* can give additional information.

References

1. Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH (1999) Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the randomized MRC Trial. Blood 93:4131-4143.
2. Cassinat B, Zassadowski F, Balitrand N, Barbey C, Rain JD, Fenaux P, Degos L, Vidaud M, Chomienne C (2000) Quantitation of minimal residual disease in acute promyelocytic leukemia patients with t(15;17) translocation using real-time RT-PCR. Leukemia 14:324-328.
3. Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pane F, Saglio G, Martinelli G, Petti MC, Santoro A, Pelicci PG, Mandelli F, Biondi A, Lo Coco F (1992) Early detection of relapse by prospective reverse transcriptase- polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. Blood 92:784-789.
4. Gallagher RE, Yeap BY, Bi W, Livak KJ, Beaubier N, Rao S, Bloomfield CD, Appelbaum FR, Tallman MS, Slack JL, Willman CL (2003) Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: Assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood 101:2521-2528.
5. Grimwade D. The pathogenesis of acute promyelocytic leukaemia: evaluation of the role of molecular diagnosis and monitoring in the management of the disease (1999) Br J Haematol 106:591-613.
6. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH, Linch DC (2001) The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 98:1752-1759.
7. Lengfelder E, Reichert A, Schoch C, Haferlach T, Löffler H, Staib P, Heyll A, Seifarth W, Saussele S, Fonatsch C, Gassmann W, Ludwig WD, Hochhaus A, Beelen D, Aul C, Sauerland MC, Heinecke A, Hehlmann R, Wörmann B, Hiddemann W, Büchner T (2000) Double induction strategy including high dose cytarabine in combination with all-trans retinoic acid: effects in patients with newly diagnosed acute promyelocytic leukemia. German AML Cooperative Group. Leukemia 14:1362-1370.
8. Lengfelder E, Gnad U, Büchner T, Hehlmann R (2003) Treatment of relapsed acute promyelocytic leukemia. Onkologie 26:373-379.
9. Lo Coco F, Diverio D, Avvisati G, Petti MC, Meloni G, Pogliani EM, Biondi A, Rossi G, Carlo-Stella C, Selleri C, Martino B, Specchia G, Mandelli F (1999) Therapy of molecular relapse in acute promyelocytic leukemia. Blood 94:2225-2229.
10. Lo Coco F, De Santis S, Esposito A, Divona M, Diverio D (2002) Molecular monitoring of hematologic malignancies current and future issues. Semin Hematol 39(2 Suppl 1):14-17.
11. Mandelli F, Diviero D, Avvisati G, Luciano A, Barbui T, Bernasconi B, Broccia G, Cerri R, Falda M, Fioritoni G, Leoni F, Liso V, Petti MC, Rodeghiero F, Saglio G, Vegna ML, Visani G, Jehn U, Willemze R, Muus P, Pelicci PG, Biondi A, Lo Coco F, for the Gruppo Italiano Mallatie Ematologiche Maligne dell'Adulto and Associazione Italina di Ematologia ed Oncologia Pediatrica Cooperative Groups (1997) Molecular remission in PML/RARα-positive acute promyelocytic leukemia by combined all-trans retinoic acid and Idarubicin (AIDA) therapy. Blood 90:1014-1021.
12. Murray CK, Estey E, Paietta E, Howard RS, Edenfield WJ, Pierce S, Mann KP, Bolan C, Byrd JC (1999) CD56 expression in acute promyelocytic leukemia: a possible indicator of poor treatment outcome? J Clin Oncol 17:293-297.
13. Sanz MA, Martin G, Rayon C, Esteve J, Gonzalez M, Diaz-Mediavilla J, Bolufer P, Barragan E, Terol MJ, Gonzalez JD, Colomer D, Chillon C, Rivas C, Gomez T, Ribera JM, Bornstein R, Roman J, Calasanz MJ, Arias J, Alvarez C, Ramos F, Deben G (1999) A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARalpha-positive acute promyelocytic leukemia. PETHEMA group. Blood 94:3015-3021.
14. Sanz MA, Lo Coco F, Martin G, Avvisati G, Rayon C, Barbui T, Diaz-Mediavilla J, Fioritoni G, Gonzalez JD, Liso V, Esteve J, Ferrara F, Bolufer P, Bernasconi C, Gonzalez M, Rodeghiero F, Colomer D, Petti MC, Ribera JM, Mandelli F (2000) Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood 96:1247-1253.
15. Tobal K, Liu Yin JA (1998) RT-PCR method with increased sensitivity shows persistence of PML-RARA fusion transcripts in patients in long-term remission of APL. Leukemia 12:1349-1354.

Curative Therapeutic Approaches to APL

M. S. TALLMANN
Northwestern University Feinberg School of Medicine,
Robert H. Lurie Comprehensive Cancer Center, Chicago, USA

Abstract

Acute promyelocytic leukemia (APL) has become the most curable subtype of acute myeloid leukemia in adults. It represents the only established example of successful differentiation therapy. With current therapy which includes all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy for induction, anthracycline-based consolidation and maintenance with ATRA and/or low-dose chemotherapy, approximately 75–85% of patients with acute promyelocytic leukemia (APL) remain alive and disease-free at 5 years, and most patients are likely to be cured, an unprecedented achievement in the field of hematologic malignancies. However, several causes for failure to be cured need to be addressed. The first is early death which occurs in approximately 10% and is frequently attributable to hemorrhage due to the characteristic coagulopathy. The second is relapse, particularly in intermediate- and high-risk patients. Analyses of new prognostic factors may permit refinement of current risk classification and identify patients warranting alternative therapy. Finally, long-term consequences of current treatment will be important to recognize, including delayed cardiomyopathy, extramedullary relapse related to sanctuary sites, and the potential for second malignancies. For patients who do relapse, arsenic trioxide appears to be the treatment of choice since the majority of patients achieve a second complete morphologic, cytogenetic, and even molecular remission. While some patients achieving a second complete remission have prolonged disease-free survival with consolidation and maintenance arsenic, high-dose chemotherapy with autologous hematopoietic stem cell transplantation appears to offer the highest likelihood of cure. Such a strategy or anti-CD33 antibodies, recently shown to be active in APL, might be considered for high-risk patients in first remission.

Induction and Postremission Therapy in APL. In less than two decades, the prognosis for patients with acute promyelocytic leukemia (APL) has changed from highly fatal to highly curable. The major advance has been the addition of all-trans retinoic acid (ATRA) to anthracycline-based chemotherapy. Although ATRA as a single agent induces complete remission (CR) in the majority of patients through differentiation of the leukemic promyelocytes, the majority of patients will relapse unless additional therapy is given.[1] The best results appear to be those achieved when ATRA is combined with cytotoxic chemotherapy for induction. Large cooperative group studies show that when ATRA is combined with anthracycline-based chemotherapy for induction, several cycles of anthracycline-based consolidation are administered and maintenance with either ATRA, low-dose chemotherapy or both is given, approximately 75–85% of patients can be expected to be disease-free at 5 years and are presumed to be cured.[2–7] This excellent outcome has been achieved through a series of studies which demonstrate first, that ATRA significantly reduces the complete remission (CR) rate when compared to chemotherapy alone; second, that ATRA given concurrently with induction chemotherapy leads to a significantly lower relapse rate compared to when ATRA and chemotherapy were given sequentially; third, some form of maintenance therapy with either ATRA, low-dose chemotherapy or both, appears to further decrease the relapse rate, and fourth, increased doses of anthracycline in induction and ATRA during consolidation may improve results in patients with intermediate- and high-risk disease. (Table 1) Finally, several studies have suggested that APL may be one subtype of acute myeloid leukemia (AML) where the inclusion of cytarabine in induction or consolidation adds little, if any, benefit. Data to support this hypothesis come from both retrospective[6] as well as prospective studies.[7, 8]

Causes of Treatment Failure

Early Death from Hemorrhage. There are several causes for treatment failure in patients with APL. First, the early death rate remains approximately 10%. Hemorrhage, attributable to the complex coagulopathy characteristic of this disease, remains an important cause of early death.[9-12] (Table 2) Overall, the impact of ATRA on the early death rate has been modest. The cause of bleeding is complex and attributable to disseminated intravascular coagulation, fibrinolysis and proteolysis.[13–15] Tissue factor, the major initiator of blood coagulation, is downregulated by ATRA. Similarly, markers of fibrinolysis indicate that ATRA also inhibits this process. Annexin II, a calcium-dependent glycoprotein which serves as the receptor for plasminogen and tissue type-plasminogen activator, is highly expressed on leukemic promyelocytes and is downregulated by ATRA. The downregulation of procoagulant factors appears more rapid and more complete after ATRA than chemotherapy.[16, 17] However, markers of fibrinolysis appear to be essentially completely downregulated to normal, leaving mild persistent procoagulant activity unopposed. It may be useful to consider studying the potential role of early administration of an anticoagulant such as activated protein C. The routine use of antifibrinolytics at this time cannot be recommended.

Relapse. A second cause for treatment failure is relapse, particularly in intermediate-and high-risk patients. Identification of prognostic factors has permitted the classification of patients into low-, intermediate-, and high-risk groups.[5]

Table 1: Long-Term Outcome with ATRA-Based Regimens

Study	N	Regimen	DFS (%)
APL91[2]	54	ATRA+DNR+Ara-C	63
No. Am. Intergroup[3, 5]	49	ATRA+DNR+Ara-C+maint.	74
GIMEMA[9]	108	ATRA+IDA+maint.	89
PETHEMA[6]	109	ATRA+IDA+maint (no Ara-C)	85
PETHEMA[7]	225	ATRA+IDA+ maint (no Ara-C) with intensified ADA for intermediate and high-risk ATRA in consolidation	90

Table 2: Prospective Trials of ATRA in APL

Trial	N	Induction	% CR	% ED	% ED due to Hemorrhage	% DFS/ EFS
Randomized						
APL 91[2]	54	ATRA	97	9	60	79
	47	(+Chemo) Chemo	81	8	75	50
APL 93[4]	109	ATRA→	95	8	32	75
	99	Chemo ATRA+Chemo	94	7		86
No Am. Intergroup[3, 5]	172	ATRA	72	11	56	67
	99	Chemo	69	14	50	32
Nonrandomized						
GIMEMA[9]	240	ATRA+Chemo	95	5	73	79
GIMEMA (older adults)[10]	134	ATRA+Chemo	86	12	25	81
JALSG[11]	196	ATRA+/–Chemo	88	9	94	62
PETHEMA[7]	123	ATRA+Chemo	89	10	67	92

The Spanish cooperative group PETHEMA has intensified anthracycline exposure in induction and added ATRA during consolidation and now reports excellent outcome for even those patients with intermediate- and high-risk disease.[7] The relapse rate in most studies now ranges between 5 and 20%. Maintenance therapy appears to have significantly decreased the relapse rate. The APL 93 study by the European APL group has suggested that combined therapy with ATRA and low-dose chemotherapy, which includes 6-mercaptopurine and methotrexate provides the lowest rate of relapse.[4] New strategies to further prevent relapse for patients at high-risk could include early exposure to arsenic trioxide[18] or anti-CD33 antibodies such as gemtuzumab ozogamicin[19,20] or M195.[21] The mechanism by which maintenance therapy is effective is not clear. New evidence suggests that periodic low-dose chemotherapy (so-called “metronomic” chemotherapy dosing) may preferentially provide an anti-vascular effect to newly formed endothelial cells such as those associated with new tumor formation.[22]

Prognostic Factors

Risk classification has been provided by the PETHEMA group. This classification is based strictly on presenting white blood cell count and platelet count. Additional prognostic factors of potential importance include expression of CD56[23, 24] and FLT3 internal tandem gene duplication mutations which have been shown to have a deleterious effect on outcome in some,[25, 26] but not other studies.[27] The influence of cytogenetic abnormalities in addition to the t(15;17) is not clear.[28–31]

Treatment of Patients with Relapsed APL

Arsenic trioxide has emerged as the treatment of choice for patients with relapsed APL.[18, 32, 33] Although some patients can be reinduced into a second remission with ATRA, either with or without chemotherapy, arsenic trioxide offers the advantage of inducing molecular remission in the majority of patients after two cycles of therapy.[33] In addition, this strategy permits the avoidance of further anthracycline exposure which may be potentially important in patients who may proceed to stem cell transplantation. Patients who relapse may do so in extramedullary sites. The Italian cooperative group GIMEMA has suggested that relapse in the central nervous system may be a particularly prominent site.[34] These data together with anecdotal reports suggest that it may be worthwhile to consider prophylactic intrathecal therapy and/or high-dose cytarabine therapy as consolidation and to mobilized peripheral blood stem cell prior to autologous transplantation in second CR. The outcome for patients who achieve a second CR with arsenic trioxide appears best if an autologous stem cell transplant is carried out with molecularly negative previously harvested stem cells.[35]

Long Term Complications

Because leukemic promyelocytes appear to be quite sensitive to anthracyclines, the doses of anthracyclines in induction and consolidation have been increased. Therefore, the risk of cardiomyopathy is present. A preliminary report suggests that this may become an important issue.[36–38] In addition, increased exposure to anthracyclines may potentially increase the risk of secondary malignancies, particularly in myelodysplastic syndrome and AML. Whether this in fact will occur requires further study of long-term survivors.

References

1. Huang M, Ye Y, Chen S, et al: Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567-572, 1988
2. Fenaux P, Le Deley M, Castainge S, et al: Effect of all trans-retinoic acid in newly diagnosed acute promyelocytic leukemia: results of a multicenter randomized trial. European APL91 Group. Blood 82:3241-3249, 1993
3. Tallman M, Andersen J, Schiffer C, et al: All-trans retinoic acid in acute promyelocytic leukemia. New England Journal of Medicine 337:1021-28, 1997
4. Fenaux P, Chastang C, Chevret S, et al: A randomized comparison of all-trans retinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. Blood 94:1192-1200, 1999
5. Tallman M, Andersen J, Schiffer C, et al: All-trans retinoic acid in acute promyelocytic leukemia: long-term outcome and prognostic factor analysis from the North American Intergroup Protocol. Blood 100:4298-4302, 2002
6. Sanz M, LoCoco F, Martin G, et al: Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood 96:1247-1253, 2000
7. Sanz M: Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline mnochemotherapy: a multicenter study of the PETHEMA group. Blood, in press
8. Estey E, Thall P, Pierce S, et al: Treatment of newly diagnosed acute promyelocytic leukemia without cytarabine. Journal of Clinical Oncology 15:483-490, 1997
9. Mandelli F, Diverio D, Avvisati G, et al: Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood 90:1014-21, 1997
10. Mandelli F, Latagliata R, Avvisati G, et al: Treatement of elderly patients (> or + 60 years) with newly diagnosed acute promyelocytic leukemia. Results of the Italian multicenter group GIMEMA with ATRA and idarubicin (AIDA) protocols. Leukemia 17:1085-1090, 2003
11. Asou N, Adachi K, Tamura J, et al: Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Japan Adult Leukemia Study Group. J Clin Oncol 16:78-85, 1998
12. Sanz M, Martin G, Rayon C, et al: A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARa-positive acute promyelocytic leukemia. Blood 94:3015-21, 1999
13. Barbui T, Finazzi G, Falanga A: The impact of all trans-retinoic acid on the coagulopathy of acute promyelocytic leukemia. Blood 91:3093-3102, 1998
14. Tallman M, Hakimian D, Kwaan H, et al: New insights into the pathogenesis of coagulation dysfunction in acute promyelocytic leukemia. Leukemia and Lymphoma 11:27-36, 1993
15. Di Bona E, Avvisati G, Castaman G, et al: Early haemorrhagic morbidity and mortality during remission induction with or without all -trans retinoic acid in acute promyelocytic leukemia. British Journal of Haematology 108:689-95, 2000
16. Dombret H, Scrobohaci M, Ghorra P, et al: Coagulation disorders associated with acute promyelocytic leukemia: Corrective effect of all-trans retinoic acid treatment. Leukemia 7:2-9, 1993
17. Tallman M, Lefebvre P, Cohen I, et al: Procoagulant, profibrinolytic and proinflammatory mediators in patients with previously untreated aucte promyelocytic leukemia (APL). Blood 86:172a, 1995
18. Niu C, Yan H, Yu T, et al: Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 94:3315-24, 1999
19. Estey E, Giles F, Beran M, et al: Experience with gemtuzumab ozogamicin („Mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia. Blood 99:4222-24, 2002
20. Petti M, Pinazzi M, Diverio D, et al: Prolonged molecular remission in advanced acute promyelocytic leukemia after treatment with gemtuzumab ozogamicin (Mylotarg CMA-676). Br J Haematol 115:63-65, 2001
21. Jurcic J, Deblasio T, Dumont L, et al: Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HUM195 in acute promyelocytic leukemia. Clinical Cancer Research 6:672-80, 2000
22. Bocci G, Nicolou K, Kerbel R: Protracted low dose effects on endothelial cells proliferation and survival in vitro reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res 62:6938-6943, 2002
23. Murray C, Estey E, Paietta E, et al: CD56 expression in acute promyelocytic leukemia: A possible indicator of poor treatment outcome. J Clin Oncol 17:293-297, 1999
24. Ferrara F, Morabito F, Martino B, et al: CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukemia treated with simultaneous ATRA and chemotherapy. J Clin Oncol 18:1295-1300, 2000
25. Gallagher R, Sandra J, Binu-John S, et al: Independent prognostic significance of FLT3 internal tandem duplication (ITD) mutations in acute promyelocytic leukemia (APL) patients treated on ECOG protocol E2491. Blood 100:326a, 2002
26. Noguera N, Breccia M, Divona M, et al: Alterations of the FLT3 gene in acute promyelocytic leukemia: association with diagnostic characteristics and analysis of clinical outcome in patients treated with the Italian AIDA protocol. Leukemia 16:2185-2189, 2002
27. Shih L-Y, Kuo M-C, Liang D-C, et al: Internal tandem duplication and Asp835 mutations of the FMS-like tyrosine kinase 3 (FLT3) gene in acute promyelocytic leukemia. Cancer 98:1206-1216, 2003
28. Slack J, Arthur D, Lawrence D, et al: Secondary cytogenetic changes in acute promyelocytic leukemia - prognostic importance in patients treated with chemotherapy alone and in association with the intron 3 breakpoint of the PML gene: a Cancer and Leukemia Group B study. J Clin Oncol 15:1786-1795, 1997
29. Hiorns L, Swansbury G, Mehta J, et al: Additional chromosome abnormalities confer worse prognosis in acute promyelocytic leukaemia. Br J Haematol 96:314-321, 1997
30. Xu L, Xhao W-L, Xiong S-M, et al: Molecular cytogenetic characterization and clinical relevance of additional, complex and/or variant chromosome abnormalities in acute promyelocytic leukemia. Leukemia 15:1359-1368, 2000
31. De Botton S, Chevret S, Sanz M, et al: Additional chromosomal abnormalities in acute promyelocytic leukemia (APL) do not confer poor prognosis: Results of APL 93 trial. Br J Haematol 111:801-806, 2000
32. Zhang P, Wang S, Hu X: Arsenic trioxide treated 72 cases of acute promyelocytic leukemia. Clin J Hematol 17:58-62, 1996
33. Soignet S, Frankel S, Douer D, et al: United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. Journal of Clinical Oncology 19:3852-60, 2001
34. Specchia G, Lo Coco F, Vignetti G, et al: Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with all-trans retinoic acid: a report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. Journal of Clinical Oncology 19:4023-28, 2001
35. Meloni G, Diverio D, Vignetti G, et al: Autologous bone marrow transplantation for acute promyelocytic leukemia in second remission: prognostic relevance of pretransplant minimal residual disease assessment by reverse-transcription polymerase chain reaction of the PML/RAR alpha fusion gene. Blood 90:1321-1325, 1997
36. Thomas X, Le Q, Fiere D: Anthracycline-related toxicity requiring cardiac transplantation in long-term disease-free survivors with acute promyelocytic leukemia. Ann Hematol 81:504-507, 2002
37. Lobe I, Rigal-Huguet F, Vekhoff A, et al: Myelodysplastic syndrome after acute promyelocytic leukemia: the European APL group experience. Leukemia 17:1600-1604, 2003
38. Latagliata R, Petti M, Fenu S, et al: Therapy-related myelodysplastic syndrome-acute myelogenous leukemia in patients treated for acute promyelocytic leukemia: an emerging problem. Blood 99:822-24, 2002

Main Session II

Epigenetic Regulation of Tumor Suppressors in t(8:21)-containing AML

G. YANG[1], W. KHALAF[2], L. VAN DE LOCHT[3], J. H. JANSEN[3], B. A. VAN DER REIJDEN[3], C. MÜLLER-TIDOW[4], H. RUUD DELWEL[5], H. SERVE[4], D. W. CLAPP[2], and S. W. HIEBERT[1,6*]
[1] Department of Biochemistry Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA; [2] Departments of Microbiology, Immunology and Pediatrics, Herman B Wells Center for Pediatric Research, Indianapolis, IN, USA; [3] Department of Hematology, University Medical Center St. Radboud, Nijmegen, The Netherlands; [4] Department of Medicine, Hematology and Oncology, University of Münster, Münster Germany; [5] Department of Hematology, Erasmus University, Rotterdam, The Netherlands; [6] Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA.

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Abstract

The t(8;21) is perhaps the most frequent chromosomal translocation associated with acute myeloid leukemia. The translocation creates a fusion protein that consists of the DNA binding domain of the RUNX1 transcription factor fused to the MTG8 transcriptional co-repressor to create a potent transcriptional repressor. Here, we discuss the possibility that the t(8;21) fusion protein represses tumor suppressors that regulate the RAS signaling pathway and the p53 oncogenic checkpoint.

Results and Discussion

The t(8;21) is one of the most frequent chromosomal translocations associated with acute myeloid leukemia (AML), accounting for 10–15% of these cases [9]. The translocation fuses the DNA binding domain of RUNX1 to nearly all of MTG8 (myeloid translocation gene on chromosome 8; also known as ETO), which appears to function as a transcriptional co-repressor. MTG8 associates with histone deacetylases 1, 2, and 3 and the mSin3 and N-CoR co-repressors (Amann et al., 2001), which themselves recruit histone deacetylases [1, 3, 4, 5]. Thus, the fusion protein is a potent transcriptional repressor, but the direct targets of transcriptional regulation that mediate cellular transformation are unknown.

In order to identify genes that are regulated by RUNX1, and therefore, targets of the chromosomal translocation fusion proteins that contain the RUNX1 DNA binding domain, we adopted a candidate approach. Based on the lack of p53 mutations in these tumors, we scanned the promoters of genes that regulate the levels of p53. We found that RUNX1-MTG8 repressed the promoter of the *p14ARF* tumor suppressor in reporter assays and reduced the endogenous levels of *p14ARF* expression in multiple cell types [6]. Chromatin immunoprecipitation assays demonstrated that RUNX1 and RUNX1-MTG8 bound to the *p14ARF* promoter *in vivo*. In acute myeloid leukemia samples containing the t(8;21), the levels of *p14ARF* mRNA were markedly lower when compared to other acute myeloid leukemia samples. Therefore, *p14ARF* is epigenetically regulated by RUNX1-MTG8 [6]. Using *p14ARF* as a transcriptional target gene, we have been able to define the domains of MTG8 that are required for repression of an endogenous tumor suppressor (Linggi and Hiebert, unpublished data).

Taking a similar approach, we scanned the promoters of genes that function as tumor suppressors in myeloid cells. Mutation of *NF1* is a common genetic alteration and patients with *NF1* mutations are predisposed to neurological malignancies and juvenile monomyelocytic leukemia. However, *NF1* mutation is not observed in other forms of leukemia. To investigate whether *NF1* is a target for transcriptional repression in acute myeloid leukemia (AML), we used quantitative RT-PCR analysis of *NF1* mRNA expression in AML patient samples (Yang et al., submitted). While there was variation among the different FAB subtypes, the AML M2 group had lower levels of *NF1* mRNA. This subgroup is characterized by mutations in C/EBPα as well as the t(8;21) [7,8]. Therefore, we used visual scanning and computer based algorithms to identify binding sites for C/EBPα and RUNX1. We identified 10 consensus-binding sites for RUNX1 as well as multiple C/EBPα binding sites. In transient transfection assays RUNX1 and C/EBPα modestly activated a reporter gene driven by the *NF1* promoter. However, when these factors were co-expressed, we observed a dramatic, cooperative transactivation of the *NF1* promoter (up to 90-fold). By contrast, the t(8;21) fusion protein, RUNX1-MTG8, repressed the *NF1* promoter in reporter assays (Yang et al., submitted). In addition, we were able to trap RUNX1-MTG8 physically associated with endogenous *NF1* using chromatin immunoprecipitation assays. RUNX1-MTG8 also repressed the expression of the endogenous *NF1* gene (Yang et al., submitted). Finally, when RUNX1-MTG8 was expressed in primary myeloid progenitor cells, the fusion protein repressed *Nf1* and sensitized these cells to GM-CSF, but not to IL-3, thus phenocopying mutation of *NF1* (Yang et al., submitted). Thus, *NF1* may be an important tumor suppressor in AML.

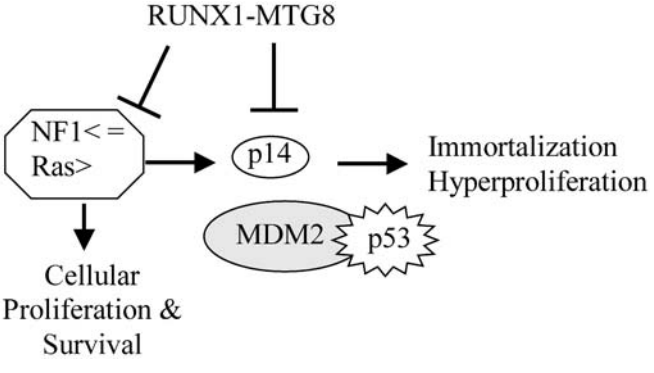

Figure 1. Molecular model of t(8;21)-induced leukemogenesis.RUNX1-MTG8 repression *NF1* stimulates RAS-dependent signaling that stimulates cellular proliferation And survival and that would cooperate with mutantions in Flt-3 or RAS. In addition, repression of p14ARF inactivates the “oncogene” checkpoint and allows MDM2-mediated degradation of p53.

References

1. Alland, L., Muhle, R., Hou, H., Jr., Potes, J. Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997). Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature *387*, 49-55.
2. Amann, J. M., Nip, J., Strom, D. K., Lutterbach, B., Harada, H., Lenny, N., Downing, R., Meyers, S., and Hiebert, S. W. (2001). ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. Mol Cell Biol *21*, 6470-6483.
3. Heinzel, T., Lavinsky, R. M., Mullen, T. M., Soderstrom, M., Laherty, C. D., Torchia, ., Yang, W. M., Brard, G., Ngo, S. D., Davie, J. R*., et al.* (1997). A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional epression. Nature *387*, 43-48.
4. Kao, H. Y., Downes, M., Ordentlich, P., and Evans, R. M. (2000). Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. Genes Dev *14*, 55-66.
5. Li, J., Wang, J., Nawaz, Z., Liu, J. M., Qin, J., and Wong, J. (2000). Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. Embo J *19*, 4342-4350.
6. Linggi, B., Muller-Tidow, C., van de Locht, L., Hu, M., Nip, J., Serve, H., Berdel, W. E., van der Reijden, B., Quelle, D. E., Rowley, J. D., *et al.* (2002). The t(8;21) fusion protein, AML1 ETO, specifically represses the transcription of the p14(ARF) tumor suppressor in acute myeloid leukemia. Nat Med *8*, 743-750.
7. Pabst, T., Mueller, B. U., Harakawa, N., Schoch, C., Haferlach, T., Behre, G., Hiddemann, W., Zhang, D. E., and Tenen, D. G. (2001a). AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in t(8;21) myeloid leukemia. Nat Med *7*, 444-451.
8. Pabst, T., Mueller, B. U., Zhang, P., Radomska, H. S., Narravula, S., Schnittger, S., Behre, G., Hiddemann, W., and Tenen, D. G. (2001b). Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet *27*, 263-270.
9. Rowley, J.D.(1999). The role of chromo-some translocations in leukemogenesis. Semin Hematol *36*, 59-72.

Core Binding Factor Acute Myeloid Leukemia. Cancer and Leukemia Group B (CALGB) Study 8461

C.D. BLOOMFIELD,[1] A.S. RUPPERT,[1,2] K. MRÓZEK,[1] J.E. KOLITZ,[3] J.O. MOORE,[4] R.J. MAYER,[5] C.G. EDWARDS,[1] L..J. STERLING,[1] J.W. VARDIMAN,[6] A.J. CARROLL,[7] M.J. PETTENATI,[8] J. STAMBERG,[9] J.C. BYRD,[1] G. MARCUCCI[1], and R.A. LARSON[6]

[1)] The Ohio State University, Columbus, OH, USA
[2)] CALGB Statistical Center, Durham, NC, USA
[3)] North Shore University Hospital, Manhasset, NY, USA
[4)] Duke University Medical Center, Durham, NC, USA
[5)] Dana-Farber Cancer Institute, Boston, MA, USA
[6)] University of Chicago, Chicago, IL, USA
[7)] University of Alabama at Birmingham, Birmingham, AL, USA
[8)] Wake Forest University School of Medicine, Winston-Salem, NC, USA
[9)] University of Maryland Cancer Center, Baltimore, MD, USA

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The most common core binding factor (CBF) acute myeloid leukemias (AML) involve two cytogenetic groups: t(8;21)(q22;q22) and its variants [hereafter abbreviated t(8;21)] and inv(16)(p13q22) or t(16;16)(p13;q22) and their variants [abbreviated inv(16)]. Among adult *de novo* AML, these groups comprise approximately 7% and 8% of patients, respectively [4]. Since both t(8;21) and inv(16) result in disruption of the CBF transcription factor and activation of leukemogenic pathways involving histone deacetylation and inhibition of gene transcription, and confer a relatively favorable prognosis with current therapeutic strategies, many clinical trials and reports have considered these two cytogenetic subgroups of AML together. In this presentation, we have compared the presenting features and outcome of a large number of adult patients with t(8;21) or inv(16). We have then updated a number of CALGB publications with a focus on the results for t(8;21) and inv(16) separately.

Between August of 1984 and april of 2003, 305 cases of CBF AML were enrolled on CALGB 8461, a prospective study of cytogenetics in adult AML. Of these patients, 142 had t(8;21) and 163 inv(16). The two groups were similar in presenting age (median 37 and 40 years, respectively), with 21 patients in each group being older than 60. For most other presenting findings the groups differed significantly. Clinically, patients with t(8;21) were less frequently Caucasian, and less often presented with splenomegaly, lymphadenopathy and gingival hypertrophy. Patients with t(8;21) more frequently had secondary cytogenetic abnormalities and FAB M2, whereas FAB M4Eo/M4 was much more common in patients with inv(16). Patients with t(8;21) had lower median hemoglobin levels, white blood cell counts, and percent circulating and marrow blasts at diagnosis.

Of the 305 cases, 137 with t(8;21) and 157 with inv(16) started treatment on a CALGB therapeutic trial for newly diagnosed adults with AML [4, 7, 8]. There were no significant differences in therapeutic outcomes measured, with 89% of t(8;21) and 86% of inv(16) patients achieving a complete remission (CR). Other endpoints measured were overall survival (OS) and cumulative incidence of relapse (CIR). The CIR was used to evaluate the time until relapse in patients achieving a CR, where death without relapse was acknowledged to exist as a competing risk, and patients alive without relapse were censored. The CIR does not view relapses and deaths in CR with equal importance, but rather treats relapse as the type of treatment failure of primary importance [6]. With a median follow-up of 7.4 years, both the CIR and OS for t(8;21) patients were similar to those for inv(16) patients (P=0.50 and P=0.34, respectively). Specifically, the CIR for t(8;21) patients was 48% (standard error, SE=0.05) at 5 years and 49% (SE=0.05) at 10 years; for patients with inv(16), CIR was 58% (SE=0.05) at both time points. For t(8;21) patients, the percentages alive at 5 and 10 years were 48% (95% CI=39%, 57%) and 45% (95% CI=36%, 54%), and for inv(16) 53% (95% CI=43%, 61%) and 48% (95% CI=39%, 57%), respectively.

In 1994 at the Plenary Session of the 36th Annual Meeting of the American Society of Hematology, we were the first to show that higher doses of post-induction cytarabine improved outcome for patients with CBF AML [1]. When these data were published in manuscript form in 1998, the median follow-up of living patients was 7.7 years [2]. With an updated median follow-up of CBF patients of 13.1 years there remains a significant benefit of higher doses of post-induction cytarabine regarding CIR (P=0.004). The 10-year CIR for patients receiving low-dose cytarabine (100 mg/m^2 by continuous infusion (ci) qd×5 days) is 79% (SE=0.10), as opposed to 42% (SE=0.12) for patients receiving intermediate-dose cytarabine (400 mg/m^2 by ci qd×5 days) and 32% (SE=0.11) for patients receiving high-dose cytarabine (HDAC, 3g/m^2 iv q12 hours on days 1, 3 and 5). The proportion of patients relapsing in each cytarabine group is also 79%, 42% and 32%, respectively (P=0.01). However, the OS among groups is not significantly different (P=0.60), with the 10-year survival probabilities of 58% (95% CI=36%, 80%), 52% (95% CI=29%, 75%), and 73% (95% CI=52%, 93%), respectively. The numbers of patients with t(8;21) or inv(16) in each post-induction therapy arm are very small. Still, the results are similar in both cytogenetic groups, with significant differences in CIR (P=0.03) among the three arms for patients with t(8;21), where CIR improved as the dose of cytarabine increased. A similar trend for improved CIR (P=0.09) was observed in patients with inv(16). There were no significant differences in OS among the low-dose, intermediate-dose, and high-dose cytarabine groups for either patients with t(8;21) or patients with inv(16).

Since our initial study showing that higher doses of cytarabine post-induction improved outcome in CBF AML [1], CALGB has employed at least 1 cycle of HDAC in all subsequent trials for newly diagnosed patients with CBF AML under the age of 60 years. In 1999, we published the impact of 3 or 4 cycles of HDAC post induction compared to 1 cycle in patients with t(8;21) [3]. With a median follow-up of approximately 5 years, patients receiving 3 or 4 cycles had a significantly improved disease-free survival (P=0.03) and OS (P=0.04) compared with patients receiving 1 cycle. We have now updated these results. With a median follow-up for surviving patients of 8.7 years, the patients receiving 3 or 4 cycles of HDAC continue to have improved CIR and OS (P=0.001 and P=0.07, respectively). The 8-year CIR for patients receiving 3 or 4 cycles of HDAC is 21% (SE=0.10) compared to 64% (SE=0.09) for those receiving 1 cycle, and the 8-year OS is 76% (95% CI=58%, 94%) compared to 43% (95% CI = 25%, 61%). With the exception of 1 recurrent leukemia at 7 years, all relapses occurred within the first 2 years.

We have recently performed the same analysis for patients with inv(16) [5]. The benefit of 3 or 4 cycles compared to 1 cycle of HDAC, although evident, is less striking for patients with inv(16) (CIR, P=0.03; OS, P=0.89). With a median follow-up for living patients of 8.9 years, the 8-year CIR for patients receiving 3 or 4 cycles is 43% (SE=0.10) compared to 70% (SE=0.11) for those receiving 1 cycle, and the 8-year OS is 67% (95% CI=49%, 85%) compared to 70% (95% CI=50%, 90%). In contrast with the t(8;21) patients, relapses still occurred at 2, 3, and 4 years after diagnosis in patients with inv(16). There was no relapse beyond 5 years.

We have also compared outcome of inv(16) patients with that of t(8;21) patients who received the same number of HDAC cycles. Among those receiving 1 cycle of HDAC, inv(16) patients have better OS than t(8;21) patients (P=0.05), with 70% (95% CI=50%, 90%) versus 43% (95% CI=25%, 61%) of patients alive at 8 years. The CIR is similar for both cytogenetic groups (P=0.67) receiving 1 cycle of HDAC, with a relatively high 8-year CIR of 70% (SE=0.11) for inv(16) and 64% (SE=0.09) for t(8;21) patients. Both groups show improved CIR when receiving 3 or 4 cycles of HDAC, but the t(8;21) patients appear to benefit more from repeated HDAC treatment than the inv(16) patients (P=0.11). The respective 8-year CIR probabilities are 21% (SE=0.10) and 43% (SE=0.10). The OS is similar (P=0.77) among patients receiving 3-4 cycles of HDAC, with 76% (95% CI=58%, 94%) of t(8;21) patients and 67% (95% CI=49%, 85%) of inv(16) patients alive at 8 years. These data suggest significant room for improvement in reducing the relapse rate for patients with inv(16) who are treated with 3 or 4 cycles of HDAC, although the salvage rate for such patients is substantial.

As a result of the improved outcome for patients with CBF AML receiving 3 or 4 cycles of HDAC, all patients with CBF AML under the age of 60 have received 3 cycles of post-induction HDAC in our last 2 CALGB trials, CALGB 9621 and CALGB 19808 [7]. CALGB 9621 is now closed to patient accrual; with a median follow-up for living patients of 4.0 years, the 3-year CIR and OS for all patients with CBF achieving CR is 48% (SE=0.08) and 66% (95% CI=51%, 80%), respectively. There is no significant difference in CIR nor in OS between the cytogenetic groups (P=0.78 and P=0.54). For t(8;21) patients, the 3-year CIR and OS are 50% (SE=0.12) and 65% (95% CI=43%, 86%), and for inv(16) patients, they are 48% (SE=0.11) and 67% (95% CI=47%, 87%), respectively.

Conclusions

Overall, these data confirm the improved outcome of AML patients with t(8;21) or inv(16) treated with multiple cycles of HDAC as consolidation therapy. At the present, therefore, this approach should be considered as the therapeutic standard for primary adult CBF AML and the benchmark to which future treatments should be compared. Nevertheless, there appear to be differences in the outcome between the two CBF groups of patients. Administration of repetitive courses of HDAC seems to convey more therapeutic advantage in patients with t(8;21) compared to those with inv(16). Patients with inv(16) appear to have a relatively higher risk of relapse despite 3 or 4 cycles of HDAC. By contrast, the OS rates are very similar in both groups suggesting that, once relapse has occurred, patients with inv(16) respond more favorably to salvage treatments. These data, albeit encouraging, underscore the need for improvement and call for novel therapeutic strategies that capitalize on the improved results obtained with repetitive HDAC administration. In this regard, novel compounds such as those targeting specific molecular defects, like the abnormal recruitment of histone deacetylase activity that contributes to leukemogenesis in these cytogenetic subgroups of patients, are particularly appealing.

References

1. Bloomfield CD, Lawrence D, Arthur DC, Berg DT, Schiffer CA, Mayer RJ (1994) Curative impact of intensification with high-dose cytarabine (HiDAC) in acute myeloid leukemia (AML) varies by cytogenetic group. Blood 84:111a
2. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R, Patil SR, Davey FR, Berg DT, Schiffer CA, Arthur DC, Mayer RJ (1998) Frequency of prolonged remission duration after high-dose cytarabine in-

tensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res 58:4173-4179
3. Byrd JC, Dodge R, Carroll A, Baer MR, Edwards C, Stamberg J, Qumsiyeh M, Moore JO, Mayer RJ, Davey F, Schiffer CA, Bloomfield CD (1999) Patients with t(8;21)(q22;q22) and acute myeloid leukemia have a superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. J Clin Oncol 17:3767-3775
4. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PRK, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD (2002) Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 100:4325-4336
5. Byrd JC, Ruppert AS, Mrózek K, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Stamberg J, Koduru PRK, Moore JO, Mayer RJ, Davey FR, Larson RA, Bloomfield CD (2003) Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): Results from Cancer and Leukemia Group B (CALGB 8461). J Clin Oncol, in press
6. Gooley TA, Leisenring W, Crowley J, Storer BE (1999) Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 18:695-706
7. Kolitz JE, George SL, Barrier R, Hoke E, Hurd DD, Velez-Garcia E, Powell BL, Marcucci G, Baer MR, Hohl RJ, Mrózek K, Carroll AJ, Edwards CG, Caligiuri MA, Vardiman JW, Bloomfield C, Larson RA (2003) Treatment of core binding factor (CBF) acute myeloid leukemia (AML) with post-remission high-dose cytarabine (HiDAC): results from CALGB 9621. Blood, in press
8. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, Omura GA, Moore JO, McIntyre OR, Frei E (1994) Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med 331:896-903

Double Induction Therapy in Core Binding Factor Acute Myeloid Leukemias: First Results of the AMLCG

W. HIDDEMANN[1], C. SCHOCH[1], T. HAFERLACH[1], W. KERN[1], A. HEINECKE[2], C. SAUERLAND[2], and Th. BÜCHNER[3] for the German AML Cooperative Group (AMLCG)
[1] Department of Internal Medicine III, University of Munich, Germany; [2] Department of Medical Informatics and Biomathematics, University of Münster, Germany; [3] Department of Internal Medicine A, University of Münster, Germany

The Core Binding Factor (CBF) acute myeloid leukemias (AML) represent a distinct subgroup of AML that is characterized by specific cytogenetic and biologic features as well as by a favourable clinical outcome. Cytogenetically CBF-AML comprise predominantly AML with t(8;21)(q22;q22) and inv(16)(p13;q22) or t(16;16)(p13;q22). Biologically they involve a family of heterodimeric transcription factors containing a common beta subunit (CBFbeta) associated with one of three alpha subunits (CBFalpha). Clinically CBF-AML have a favourable outcome with a high initial response rate and a high proportion of long term survivors (1–4). Still, their optimal therapy is under debate, particularly the role and dose of Cytosine Arabinoside (AraC) during induction or postremission therapy.

In 1999 the German AML Cooperative Group (AMLCG) embarked on a prospective randomised comparison of different strategies for induction and postremission therapy. For induction two forms of double induction therapy with TAD-HAM versus HAM-HAM were compared. Postremission therapy comprised a randomised comparison of 3 years monthly maintenance therapy versus myeloablative radiochemotherapy followed by autologous stem cell transplantation. In addition, priming with G-CSF during induction and postremission courses was evaluated. Up front randomisation between the different treatment arms was stratified for pretherapeutic rsik factors comprising karyotype, age, LDH serum level and de novo AML versus AML arising from a preceding MDS or after prior chemotherapy for another malignant disorder (Fig. 1).

As of November 2003 1321 patients were entered into the trial. An overall remission rate of 61% was achieved for all patients, the median relapse free interval was 19 months, the median overall survival was 27 months. While no significant differences in CR rates were found among patients receiving TAD-HAM versus HAM-HAM, a tendency towards a longer relapse free interval and a longer overall survival was observed for the HAM-HAM treated cases.

Cytogenetic analysis was successful in 97% of patients. 131 patients (8%) had CBF leukemias, 1077 patients revealed an intermediate karyotype (66%) and 417 cases had unfavourable cytogenetics (26%). For CBF leukemias the CR rate was 68% as compared to 59% for other AML subgroups. Patients with alterations of chromosome 16 had a higher CR rate of 72% versus 64% for cases with t(8;21). This difference was mainly due to a higher mortality during induction for patients with t(8;21) (28% versus 16%). Further analysis of the two induction arms revealed different effects of TAD-HAM versus HAM-HAM. In patients with t(8;21) TAD-HAM resulted in a higher CR rate than HAM-HAM (68% versus 59%). In contrast, cases with inv(16) or t(16;16) had a higher CR rate after HAM-HAM (86% versus 58%). Similar differences emerged for the relapse free survival which was higher after TAD-HAM in patients with alterations of chromosome 16 while it was higher after HAM-HAM in cases with t(8;21).

These data suggest a different sensitivity towards high dose AraC containing regimens during remission induction for patients with inv(16)/t(16;16) and t(8;21) respectively.

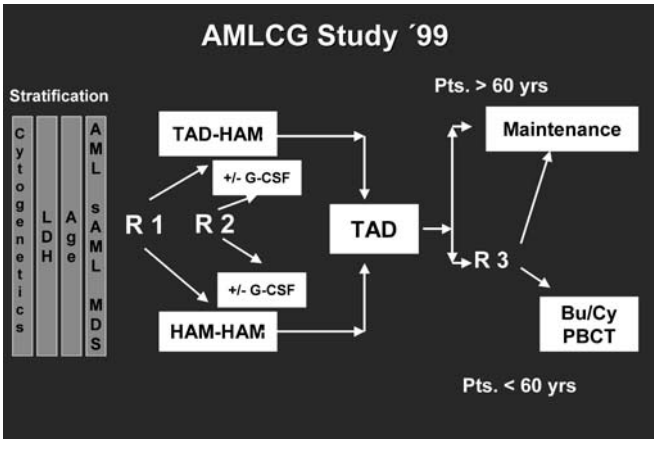

Figure 1

Literatur

1. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R, Patil SR, Davey FR, Berg DT, Schiffer CA, Arthur DC, Mayer RJ (1998) Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res 58:4173-4179
2. Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD; Cancer and Leukemia Group B (CALGB 8461) (2002) Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B. Blood 100:4325-4336
3. Delaunay J, Vey N, Leblanc T, Fenaux P, Rigal-Huguet F, Witz F, Lamy T, Auvrignon A, Blaise D, Pigneux A, Mugneret F, Bastard C, Dastugue N, Van den Akker J, Fiere D, Reiffers J, Castaigne S, Leverger G, Harousseau JL, Dombret H (2003) French Acute Myeloid Leukemia Intergroup; Groupe Ouest-Est des Leucemies Aigues Myeoblastiques; Leucemies Aigues Myeoblastiques de l'Enfant; Acute Leukemia French Association; Bordeaux-Grenoble-Marseille-Toulouse cooperative groups. Blood 102:462-469
4. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MCR AML 10 trial. The Medical Research Council adult and Children's Leukaemia Working Parties. Blood 92:2322-2333

Prognosis of Core Binding Factor (CBF) AML – Overviews of the French AML Intergroup

J. DELAUNAY, S. NGUYEN, H. DOMBRET
for the French AML Intergroup, Hôpital St. Louis, Paris, France

Aim

As patients with t(15;17) AML are now considered and treated separately with differentiating agents, t(8;21) and inv(16)/t(16;16) AML remain the two good-risk cytogenetic subtypes frequently collectively considered in a single CBF-AML group. We performed two parallel overviews in children and adults with CBF-AML prospectively enrolled in 6 different trials conducted in France between 1987 and 1998 (LAME-91, BGMT-87 and BGMT-91, GOELAM-1 and GOELAM-2, ALFA-9000; median follow-up, 5.2 years). A total of 271 patients, including 161 t(8;21) and 110 inv(16)/t(16;16) cases, were retrospectively selected on standard cytogenetics and analyzed with the aim to investigate respective prognostic factors for CR achievement, relapse incidence, and DFS in these two AML subtypes in large number of patients and to evaluate allogeneic stem cell transplantation (SCT) *versus* chemotherapy as post-CR treatment according to the intent-to-treat principle.

Results in t(8;21) AML

Median age was 29 years. Median WBC was 12.5 G/L (75% percentile, 25 G/L). CR rate was 96%. At 3 years, cumulative incidence of relapse and DFS were 34% and 56%, respectively. WBC was the only identified prognostic factor for outcome of CR patients. To further take into account the spontaneous differentiation potential of the leukemic clone, a WBC index was derived as the product of WBC by the marrow blast percentage. This WBC index was a more powerful factor than the original WBC, allowing to distinguish three subgroups of patients with different outcomes (low index, less than 2.5; intermediate index, between 2.5 and 20; high index, 20 or more). In multivariate analysis, the WBC index was the only prognostic factor for relapse incidence and DFS. Outcome of CR patients: 1) was not influenced by age and cytogenetic findings; 2) was similar among patients allocated to receive SCT or chemotherapy.

Results in inv(16)/t(16;16) AML

Median age was 34 years. Median WBC was 44 G/L (75% percentile, 89 G/L). CR rate was 93%. Bad-prognosis factors for CR achievement were higher WBC and lower platelet count (optimal cutpoints at 120 and 30 G/L, respectively). At 3 years, cumulative incidence of relapse and DFS were 42% and 48%, respectively. In multivariate analysis, advanced age (optimal cutpoint, 35 years) was the only factor for shorter DFS, because of a higher incidence of relapse rather than death in CR. Outcome of CR patients: 1) was not influenced by WBC and cytogenetic findings; 2) was similar among patients allocated to receive SCT or chemotherapy. Interestingly, advanced age was associated with a trend for more frequent additional chromosome abnormalities.

Conclusions

Prognostic factor identification markedly contrasts within each of the two CBF-AML subtypes. In t(8;21) AML, both c-Kit mutations (exon 17) (*Cairoli et al. Leukemia 2003*) and AML1-ETO fusion transcript level (*Schnittger et al. Blood 2003*) might represent molecular explanations to the prognostic impact of the WBC index. In inv(16)/t(16:16) AML, the impact of advanced age on relapse incidence needs to be related to molecular features, including c-Kit mutations (exon 8) (*Care et al. BJH 2002*) and *maybe CBFβ*-MYH11 transcript level.

Individual Patient Data Based Meta-Analysis on 410 Patients 16 to 60 Years of Age with Core Binding Factor Acute Myeloid Leukemia: a Survey of the German AML Intergroup

R.F. SCHLENK[1], A. BENNER[2], T. BÜCHNER[3], C. SAUERLAND[3], C. SCHOCH[4], G. EHNINGER[5], M. SCHAICH[5], B. MOHR[5], D. NIEDERWIESER[6], R. KRAHL[6], R. PASOLD[7], A. GANSER[8], J. KRAUTER[8], K. DÖHNER[1], H.DÖHNER[1], and G. HEIL[8]
for the German AML-Intergroup
[1] Dept. of Internal Medicine III, University of Ulm; [2] Central Unit of Biostatistics, German Cancer Research Center Heidelberg; [3] Dept. of Internal Medicine A, University of Münster; Germany; [4] Dept. of Internal Medicine III, Ludwig-Maximillians University Munich; Germany; [5] Dept. of Internal Medicine I, University of Dresden; Germany; [6] Dept. of Hematology/Oncology, University of Leipzig, Germany; [7] Ernst von Bergmann Klinik, Potsdam, Germany; [8] Dept. of Hematology/ Oncology, University of Hannover, Germany

Acute myeloid leukemias exhibiting t(8;21) or inv(16)/t(16;16) karyotypes are referred to as core binding factor (CBF) acute myeloid leukemia (AML), which is characterized by high first complete remission (CR) rates and by improved disease free survival (DFS) following dose-intensified cytarabine for consolidation therapy. We performed a meta-analysis in 410 young adults (median age: 42 years, range 16-60) intensively treated between 1993 and 2002 in 8 prospective, multicenter German AML trials.

The response after double induction therapy was as follows: CR 87% and 89%, early or hypoplastic death (ED/HD) 10% and 8.5%, resistant disease (RD) 3% and 2.5%. In patients with t(8;21), there was a significantly higher HD-rate after second induction therapy with high dose cytarabine compared to intermediate and standard dose cytarabine. DFS was 60% and 58% and overall survival (OS) 65% and 74% in the t(8;21)- and abn(16q22)-group, respectively.

For postremission therapy, *intention-to-treat* analysis revealed no difference for both groups between chemotherapy, autologous and allogeneic transplantation, whereas *as-treated* analysis showed a significant benefit in DFS for patients with abn(16q22) after allogeneic transplantation. In the t(8;21)-group, significant prognostic variables for longer DFS and OS were lower WBC and higher platelet count, for shorter OS loss of a sexual chromosome (LOS); in the abn(16q22)-group trisomy 22 was the only significant prognostic variable for longer DFS. After relapse, the CR rate was significantly better for patients with abn(16q22) resulting in a significantly better survival after relapse compared to patients with t(8;21).

In conclusion, we identified WBC, platelet count and LOS in the t(8;21)-group and trisomy 22 in the abn(16q22)-group as prognostic variables. *As-treated* analysis revealed better DFS for patients with abn(16q22) after allogeneic transplantation. In relapsed patients prognosis was significantly better in patients with abn(16q22) compared to those with t(8;21).

Main Session III

Farnesyltransferase Inhibitors (FtIs) in Myeloid Malignancies

J.E. KARP[1] and J. E. LANCET[2]
[1] Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, Room 289, Baltimore, Maryland, USA; [2] University of Rochester Cancer Center, 601 Elmwood Avenue, Box 610, Rochester, New York, USA, 1

ABSTRACT

Farnesyltransferase inhibitors (FTIs) are small-molecule inhibitors that selectively inhibit farnesylation of a number of intracellular substrate proteins such as Ras. Preclinical work has revealed their ability to effectively inhibit tumor growth *in vitro* and *in vivo* in animal models across a wide range of malignant phenotypes. Myeloid malignancies are appropriate disease targets, in that they express relevant biologic targets, such as Ras, Mitogen-Activated Protein Kinase (MAPK), AKT, and others that may depend upon farnesyl protein transferase (FTase) activity to promote proliferation and survival. Phase I trials in acute leukemias and myelodysplasia have demonstrated biologic and clinical activities as determined by target enzyme inhibition, low toxicity, and both complete and partial responses. As a result, phase II trials have been initiated in a variety of hematologic malignancies and disease settings, in order to further validate clinical activity and to identify downstream signal transduction targets that may be modified by these agents. It is anticipated that these studies will serve to define the optimal roles of FTIs in patients with hematologic malignancies and provide insight into effective methods by which to combine FTIs with other agents.

Keywords: Myeloid leukemias, Myelodysplasia, Myeloproliferative disorders, Signal transduction, RAS, Farnesyltransferase inhibitors

Farnesyl transferase inhibitors (FTIs) represent a new class of small molecule signal transduction inhibitors that impede critical cell growth and survival signals. These agents are potent and selective competitive inhibitors of intracellular farnesyl protein transferase (FTase), an enzyme that catalyzes the transfer of a farnesyl moiety to the cysteine terminal residue of a substrate protein [1]. A host of intracellular proteins are substrates for prenylation via FTase, including Ras, Rho-B, Rac, membrane lamins, and centromeric proteins that interact with microtubules to promote the completion of mitosis [2]. Interruption of prenylation may prevent substrates from undergoing maturation which, in turn, may result in the inhibition of cellular events that depend on the function of those substrates. Myeloid malignancies exhibit activity of diverse pathways involving Ras early in transduction and progressing through downstream intermediaries such as RhoB, Mitogen-Activated Protein Kinase, and phosphatidylinositol-3 kinase (PI_3K)/AKT that depend on FTase activity to promote proliferation and survival and, as such, are ripe disease for study of FTIs [reviewed in 3].

Clinical Trials in Acute Myelogenous Leukemia (AML)

A dose-escalation Phase I trial of the non-peptidomimetic methylquinolone FTI R115777 (Tipifarnib, ZARNESTRA™, Johnson and Johnson Pharmaceuticals Research and Development, LLC)) was conducted in adults with relapsed or refractory acute leukemias at doses ranging from 100 mg BID to 1200 mg BID for 21 out of 28 days [4]. Reliable inhibition of FTase occurred at or above the 300 mg bid dose level, inhibition of protein processing of farnesylated proteins (the nuclear protein lamin A and the chaperone protein HDJ-2) occurred at 600 mg BID, and dose-limiting toxicity consisting of readily reversible central neurotoxicity was observed at 1200 mg BID. Drug accumulated in bone marrow in a dose-dependent manner and in concentrations 2-3-fold higher than serum throughout the duration of administration. An overall response rate of 29% was achieved, including 2 complete remissions (CRs) in patients with relapsed AML (2/25, 8%). Responses occurred across all dosing cohorts without strict relationship to degree of FTase inhibition in sampled leukemic cells and independently of *ras* mutations, since *ras* mutations were not detected in any patient's leukemia. A multi-center Phase II trial of R115777 600 mg BID for 21 out of 28 days in adults with relapsed or refractory AML [5] confirmed: 1) biologic activity, with 20% of patients having a >50% reduction in marrow blasts and 12% having a reduction in the bone marrow blasts to less than 5%; and 2) clinical activity, with an overall response rate of roughly 14% (complete or partial remission, or stable disease for >8 weeks). Importantly, clinical response conferred a survival benefit (median approximately 280–300 days for responders vs. 50–60 days for non-responders).

Based on the results of these initial trials, we are conducting a Phase II trial of oral R115777 at 600 mg BID for 21 days every 28–42 days in previously untreated, poor-risk AML, focusing on patients over age 65 and also adults ≥18 yrs with MDS/AML or treatment-related AML [6]. The median age for 100 patients entered on trial is 74 yrs (range 34-86) and adverse cytogenetics are documented in 60%. Median number of cycles received was 2 (range 1-6). Of 92 evaluable patients, 19 (20%) achieved complete remission (CR), with an overall response rate (CR+PR) of 33%. Of note is that CR and overall re-

sponse rates for patients ≥age 75 are the same as for the younger cohorts. Median CR duration is 5.8 months (range 1.5–12+) and the median survival for all responders (CR+PR) has not yet been reached, with >60% alive at 15+ months. Median survival for the entire group of patients is 8 months, with non-responders surviving a median 5 months. Nine patients (9%) died during therapy, mainly from infection. Non-hematologic grade 3–4 toxicities consisting of transient neurotoxicity, renal impairment and rash occurred in 6 (7%). Laboratory correlates demonstrated inhibition of FT activity as measured by the appearance of unfarnesylated HDJ-2 in marrow blasts after 8 days of treatment from 17/'23 (74%) patients tested to date, with clinical response (CR or PR) in roughly half. In contrast, in 6 patients where unfarnesylated HDJ-2 did not emerge, only 1 achieved clinical response, suggesting the possibility that failure to inhibit HDJ-2 farneyslation may have negative predictive value. Early data also suggest a possible relationship between clinical response and the presence of trisomy 8 in AML blasts. Additional correlates under investigation include measurement of changes in phosphorylation in key signaling intermediates (ERK, AKT) and examination of DNA microarray-based gene expression patterns prior to and during therapy.

Myelodysplasia (MDS)

Poor-risk MDS presents another germane disease target for FTI therapy. In a Phase I study by Kurzrock, et al [7], dose limiting toxicity consisting of fatigue occurred at 900 mg BID. Objective responses (hematologic improvement or PR) were observed in 33% of all patients and 50% of those with *N-ras* mutations. A Phase II trial at 600 mg BID confirmed clinical activity, including the achievement of CRs in 2/16 (12.5%) [8]. However, toxicity (including myelosuppression) necessitated removal from study in 4 (25%), raising the possibility that lower doses may be more appropriate for MDS patients in order to prevent discontinuation of therapy. Encouraging results in MDS are being obtained with other FTIs, as well. A Phase II study of SCH66336 demonstrated erythroid or platelet responses in 3 of 15 patients without severe toxicities [9]. In a Phase I trial of BMS-214662, 2 of 6 patients with high-grade MDS had >50% reduction in marrow blasts, while a third patient achieved hematologic improvement as evidenced by platelet count normalization [10].

Chronic Myelogenous Leukemia (CML) and Other Myeloproliferative Disorders (MPDs)

The use of FTIs in CML is supported by the fact that the BCR-ABL fusion protein utilizes signaling cascades including Ras and PI_3K/AKT that likely depend on intact FTase activity. In Cortes' study of R115777 600 mg BID [11], complete or partial hematologic (but not cytogenetic) responses occurred in 7/22 (33%) patients with previously treated CML, including STI-resistant CML, with responses seen in 60% of chronic phase CML and 20% of patients with accelerated CML. Also included in this trial were 8 patients with MPD and associated myelofibrosis, of whom 25% achieved a >50% reduction in spleen size and achievement of CR or PR. Interestingly, Cortes uncovered a relationship between elevated plasma VEGF levels prior to therapy and response to R115777. Similar clinical results have been obtained by Gotlib [12] in STI-refractory-CML and in undifferentiated MPDs, with hematologic responses seen in roughly 33% of patients. Likewise, using SCH66336 in STI-resistant chronic or accelerated-phase CML, 2/12 patients achieved hematologic response with evidence of increased peripheral blood myeloid maturation in several others.

New Disease Settings

Despite encouraging signs of clinical activity in patients with advanced hematologic malignancies, it is likely that the use of FTIs as single agents will not elicit large-scale, durable responses in the setting of relapsed or refractory disease, where complicated and redundant signaling networks and chromosomal aberrations provide multiple mechanisms by which malignant cells survive. New settings in which single agent FTI therapy should focus on states where the tumor burden is at a minimum and where, at least in theory, clonal evolution may not have occurred. The minimal residual disease state following remission induction and consolidation therapies offers a fertile testing ground for FTIs. To date, post-remission chemotherapy for AML in elderly patients or those with other poor-risk features (including MDS/AML and treatment-related AML) has not prolonged disease-free or overall survival. In a related scenario, the ability of FTIs to prevent the progression of clonal disorders from premalignant conditions to fully-transformed states should be studied as well. Several hematologic malignancies might be appropriate diseases in which to test this latter concept, for instance, high-risk MDS or MPDs which have defined rates of progression to full-blown AML.

The full development of FTI therapy will require the design and testing of rational combinations of FTIs with cytotoxic, biologic and immunomodulatory agents in both the laboratory and the clinic. In particular, the combination of FTIs with other signaling inhibitors is based on the rationale that malignant hematopoietic cells are governed by a diverse and often redundant array of signaling networks, thereby nullifying the ability of a single pharmacologic agent to abrogate the cellular proliferative and survival processes. The combination of agents that block components of signal transduction at multiple levels could provide a potent strategy for the suppression of cell cohorts whose survival is driven by those pathways.

SELECTED REFERENCES

1. End DW. Farnesyl protein transferase inhibitors and other therapies targeting the Ras signal transduction pathway (1999) Invest New Drugs 17:241-258.
2. Rowinsky EK, Windle JJ, Von Hoff DD. Ras protein farnesyltransferase: a strategic target for anticancer therapeutic development (1999) J Clin Oncol 17:3631-3652.
3. Lancet JE, Karp JE. Farnesyltransferase inhibitors in hematologic malignancies: new horizons in therapy (2003) Blood 102: in press
4. Karp JE, Lancet JE, Kaufmann SH, et al. Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leuekmias: a phase I clinical-laboratory correlative trial (2001) Blood 97:3361-3369
5. Harrousseau JL, Stone R, Thomas X, et al. Interim results from a phase II study of R115777 (ZARNESTRA) in patients with refractory and relapsed acute myelogenous leukemia (2002) Proc Am Soc Clin Oncol 21:265a (abstract)
6. Lancet JE, Gojo I, Gotlib J, et al. Tipifarnib (ZARNESTRA™) in poor risk previously treated AMl and MDS: interim results of a phase II trial (2003) Blood 102; in press (abstract)
7. Kurzrock R, Sebti SM, Kantarjian HM et al. Phase I study of a farnesyl transferase inhibitor, R115777, in patients with myelodysplastic syndromes (2001) Blood. 2001;98:623a.
8. Kurzrock R, Cortes J, Rybak ME et al. Phase II study of R115777, a farnesyltransferase inhibitor, in myelodysplastic syndrome (2001) Blood 98:848a.
9. Cortes J, Holyoake TL, Silver RT et al. Continuous Oral Lonafarnib (Sarasar™) for the Treatment of Patients with Advanced Hematologic Malignancies: A Phase II Study (2002) Blood 100:793a.
10. Cortes J, Kurzrock R, O'Brien SM et al. Phase I Study of a Farnesyl Transferase Inhibitor (FTI), BMS-214662, in Patients with Refractory or Relapsed Acute Leukemias [abstract]. Blood. 2001;98:594a.
11. Cortes J, ALbitar M, Thomas D, et al. Efficacy of the farnesyltransferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies (2003) Blood 101: 1692-1697
12. Gotlib J, Loh M, Vattikuti S et al. Phase I/II study of ZARNESTRA™ (farnesyltransferase inhibitor [FTI] R115777, Tipifarnib) in patients with myeloproliferative disorders (MPDs): Preliminary Results (2002) Blood 100:798a-799a.

Targeted Toxin Therapy in AML

A.E. FRANKEL
Wake Forest University School of Medicine, Medical Center Dr. Hanes, Winston-Salem, USA

Diphtheria toxin has been genetically modified to replace the receptor-binding domain with AML selective ligands (DT_{388}GMCSF, DT_{388}IL3, and DT_{388}IL3K116W) and to change the furin-cleavage site to a urokinase-specific cleavage sequence (DTU2GMCSF). These recombinant toxins have been expressed in Escherichia coli, purified from inclusion bodies by washing, denaturation, refolding, dialysis, anion exchange chromatography, size exclusion chroma-tography, and, in some cases, polymyxin B affinity chromatography. Two of these proteins (DT_{388}GMCSF and DT_{388}IL3) have been produced under CGMP and completed monkey toxicology studies, and DT_{388}GMCSF was tested in a phase I clinical trial of refractory AML patients. The major clinical findings with DT_{388}GMCSF were immunogenicity limiting the number of cycles to one or two and liver toxicity limiting dose escalation. Remissions were observed in 4/38 patients including a complete remission lasting one year. An animal model of the DT_{388}GMCSF-induced liver toxicity has been generated. Damage to GMCSF receptor positive Kupffer cells triggers secondary hepatocyte injury with nitrotyrosine deposition. We are evaluating DTU2GMCSF, DT_{388}IL3 and DT_{388}IL3K116W as second generation AML recombinant toxins which should have increased potency and reduced liver toxicity. Results of further toxicology studies with DT_{388}GMCSF and clinical studies with DT_{388}IL3 will be presented.

PKC 412 FLT3 Inhibitor Therapy in AML: Results of a Phase II Trial

R. M. STONE[1], D: J. DE ANGELO[1], I. GALINSKY[1], E. ESTEY[2], V. KLIMEK[3], W. GRANDIN[1], D. LEBWOHL[4], A. YAP[4], P. COHEN[4], E. FOX[1], D. NEUBERG[1], J. CLARK[1], D. G. GILLILAND[1], and J. D. GRIFFIN[1]
Dana-Farber Cancer Institute, Boston[1], MA; USA, MD Anderson Cancer Center, Houston, TX[2]; USA Memorial Sloan-Kettering Cancer Center, New York, NY[3]; USA; and Novartis, Basel, Switzerland[4]

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The oncogenes that cause AML may represent relevant target molecules for purposes of the development of less toxic and more specific therapies in AML. One such potential target is the class III receptor tyrosine kinase FLT3, which is mutated and activated in about 30% of all patients with AML.[1] The mutations involve either an internal tandem duplication of between 3 and 33 amino acids in the juxtamembrane region (in about 25% of AML patients) or a point mutation in the activating loop (about 7% of patients). Patients with mutations in FLT3, particularly ITD mutations, have a worse prognosis, with lower rates of complete remission, and lower overall survival.[2].Because PKC412 (N-benzoylstaurosporine) was developed originally as a protein kinase-C and vascular endothelial growth factor receptor inhibitor, a phase I trial with this agent in solid tumors was conducted which demonstrated the feasibility of giving doses up to 75 mg orally three times a day.[3] Subsequently, this compound was found to specifically and potently inhibit the growth of leukemic cells lines rendered factor independent by transfection with either an ITD or an activating loop mutation of FLT3.[4] Moreover, mice with the fatal activated FLT3-induced-myeloproliferative syndrome survived longer due to administration of PKC412.[5] We therefore conducted a phase II proof-of concept trial of PKC412 in patients with advanced AML and MDS (relapsed/refractory disease or not a chemotherapy candidate, no BMT within the previous 2 months, ECOG performance status 0-2 and no hydroxyurea within 7 d of starting the study drug or thereafter) whose cells were documented to have an activating mutation of FLT3. PKC412 was administered at a dose of 75 mg orally 3 times a day until disease progression or severe non-hematological toxicity occurred. In the event of grade 3 or 4 non-hematological toxicity drug was held; if the toxicity resolved to grade 1 within one week, it was restarted at 50 mg PO TID.

Standard NCI criteria were used for CR and PR, and an additional category, significant clinical benefit, was defined as a greater than 2 log reduction in the absolute number of peripheral blood blasts compared with baseline, lasting for at least 4 weeks. Twenty eligible patients (11 relapsed, 6 refractory, 3 previously untreated) were enrolled and received at least 2 weeks of study drug. The median age was 62 (range: 29-78). A median of 3 prior regimens (range of 0-5 regimens) had been given. 5 had AML evolving from a prior myelodysplastic syndrome (2) or after chemotherapy for another neoplasm (3). 11 had a normal karyotype. Blasts from 18 of the 20 patients had an internal tandem duplication mutation of between 6 and 33 amino acids in length (median 16 amino acids) and two had a D835Y point mutation. Toxicity was limited to grade ½ nausea and vomiting in 11 patients, variable myelosuppression thought mainly disease-related, and two patients sustained fatal pulmonary events of unclear etiology on day 15 (an autopsy in one of these disclosed non-specific pathology). 14/20 patients (70%) experienced at least a transient 50% reduction in peripheral.blasts, with 7 of these displaying major clinical benefit (table 1), with a median duration of response of 90 days (range 63-330 days).

One such patient (Figure 1) had drug stopped on day 48 due to a grade IV infection, but went on to display CR status (except for a slightly hypocellular marrow) at day 165. He then relapsed on day 210, was retreated on a compassionate basis and responded for another 90 days.

The drug is 99% per cent protein bound. Pharmacokinetic studies measuring total drug suggested that drug levels were lower (and free levels assumed to be hovering near the IC50) in non-responders (Figure 2). The explanation for progression remains unclear, although plasma levels routinely declined after 2 weeks. The ratio of a phosphorylated FLT3 to total FLT3, a measure of target inhibition, declined in 3/5 responding patients.

We therefore demonstrated that an orally administered FLT3 tyrosine kinase inhibitor, PKC 412, lead to significant clinical responses in advanced AML of a similar magnitude (35%) to that observed with imatinib in myeloid blast crisis of CML[6], a state likely due to additional mutations beyond bcr-abl.

Table 1

Baseline PB blasts/ul	Best response, blasts/ul
110K	0, d 29
65K	0.06 K, d 42
21K	0, d50
5K	0.1K, d22
16K	0, d15
71K	0, d57
46K	0, d51

Figure 1

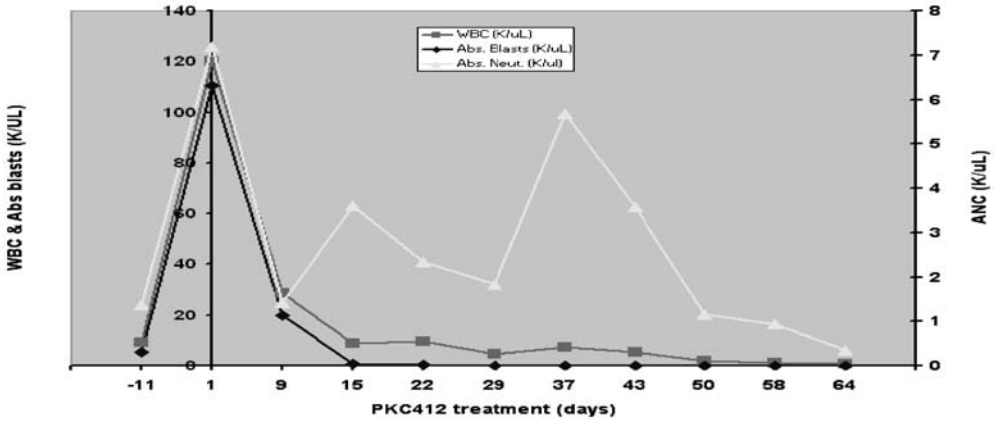
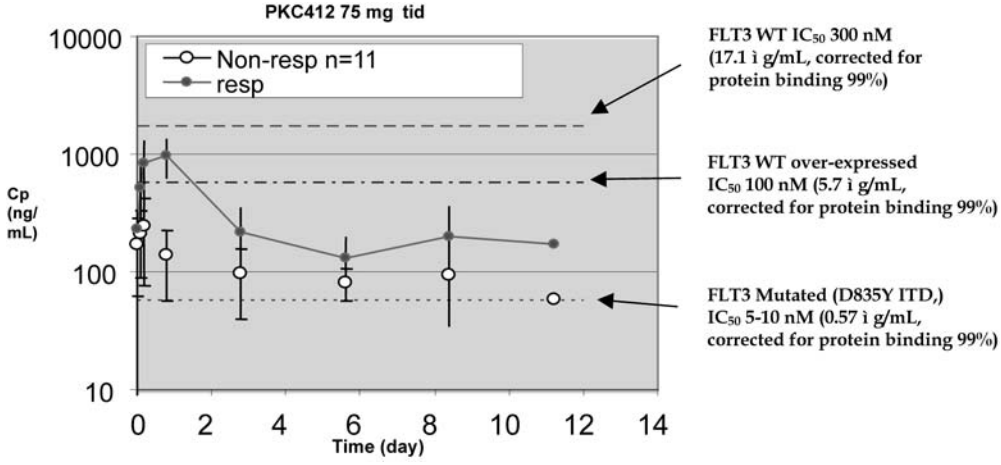


Figure 2



The drug was well-tolerated, but subsequent studies will require close monitoring of patients' pulmonary function; reports of interstital lung damage with other vascular endothelial growth factor inhibitors is a cause for concern in this regard. Because of the multiplicity of pathophysiological pathways in AML (at least including tyrosine kinase-mediated overproliferation and failure of normal hematopoeitic differentiation), it is unlikely that targeting only one such event could lead to frequent and/or prolonged remissions. Nonetheless, by depriving cells of a required survival signal, such as via the inhbition of activated FLT3 in the subset of AMLs harboring such a mutation, apoptosis and reduction in leukemic burden could occur. Whether such a phenomenon accounts for the observed responses in our study remains unclear. Although we did show target inhibition (decrease in FLT 3 tyrosine phsophorylation compared with total FLT3 by Western blotting) in several responding patients, other enzymes potentially critical to the AML phenotype, such as protein kinase C and vascular endothelial growth factor receptor, are also inhibited. Additional strategies to elucidate the mechanism of response currently underway include re-sequencing FLT3 in patients who have lost a response to determine if new mutations, such as those preventing PKC412 binding, are found; and evaluating the drug in patients with AML whose blasts can not be shown to have an activating FLT3 mutation. Another mechanism for loss of response could be pharmacokinetic in that drug levels of PKC412 decline within several weeks. Therefore, studies involving novel dosing strategies, as well as short term use in combination with other agents active in AML, including standard chemotherapy, represent important avenues for clinical research with PKC 412.

References

1. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood 2002;100:1532-42.
2. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752-9.
3. Propper DJ, McDonald AC, Man A, et al. Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C. J Clin Oncol 2001;19:1485-92.
4. Weisberg E, Boulton C, Kelly LM, et al. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. Cancer Cell 2002;1:433-43.
5. Kelly LM, Liu Q, Kutok JL, et al. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. Blood 2002;99:310-8.
6. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001; 344: 1038-1042.

Valproic Acid: An Old Drug Newly Discovered as Inhibitor of Histone Deacetylases

M. GÖTTLICHER
Institute of Toxicology and Environmental Hygiene, Technical University Munich, Germany, and Institute of Toxicology, GSF National Research Center for Environment and Health GmbH, Ingolstädter Landstraße 1, Neuherberg, Germany

Summary

Fusion proteins encoded by several types of chromosomal translocations in promyelocytic leukemia can serve as aberrant transcriptional repressors relying on recruitment of histonedeacetylases (HDACs) into DNA-associated multi-protein complexes. Thus, inappropriate modulation of chromatin structure by HDACs and subsequently repression of gene expression that is critical for myeloid differentiation appear to be major factors in the development of the disease. They identify inhibitors of HDACs as prime candidates for novel anti leukemic drugs. Over the last years several candidate compounds have been introduced into clinical trials and have successfully been used in compassionate use protocols. Amongst them phenylbutyrate served as the first example to establish proof of principle. Novel drugs such as suberoylanilide hydroxamic acid (SAHA) are developed for example by modifications of the microbial HDAC inhibitory compound trichostatin A with a hydroxamic acid as the key structural element. The branched chain carboxylic acid valproic acid (VPA) that is in use as antiepileptic drug over decades was also discovered to inhibit HDACs and preferentially class I HDACs. HDAC inhibition is likely to mediate the teratogenic side effects of VPA but not the antiepileptic activity. In contrast to other HDAC inhibitors VPA also induces proteasomal degradation of HDAC2. None of the currently available compounds may be the optimum HDAC inhibitory drug but each of them may serve to answer urgent questions concerning the concept of HDAC inhibition in the treatment of malignant diseases.

Prominent questions are i) whether and by which mechanisms HDAC inhibition can be expected to affect a malignant disease not only in the early stage but also at later stages that have acquired additional genetic defects, ii) which forms of cancer in addition to myelocytic leukemia respond to HDAC inhibition, iii) by which markers those susceptible forms could be identified and iv) which individual HDACs are the most critical isoenzymes to address in treatment of malignant diseases.

Chromatin modification by acetylation

Organization of DNA into chromatin including nucleosome formation on histone proteins as well as higher orders of structure established for example during transient transcriptional regulation or heterochromatic gene silencing is a prerequisite without that ordered propagation and reading of the genetic information in eucaryotic cells would appear to be impossible. Histones and particularly histones H3 and H4 are highly susceptible to modifications. The amino-terminal tails of these core histones appear not to contribute to nucleosome structure in an ordered fashion amenable to crystallization and X-ray diffraction (25). However, they are susceptible to multiple modifications including acetylation of ε-amino groups in lysine residues, methylation, ubiquitination and phosphorylation and thereby apparently constitute a 'histone code' which is thought to affect, both, structure of the chromatin by ionic interactions between nucleosomes and affinity for proteins recognizing and binding to the modifications in chromatin (2).

Histone acetylation and deacetylation are the most transient modes of modifications that may precede more stable modifications such as histone methylation and DNA methylation (2). Modifications depend on recruitment of multi-protein complexes with catalytically active components to selected sites of chromatin by sequence-specific transcription factors such as nuclear hormone receptors and also members of the Myc- or AP-1 protein families (5). Histone acetylation is controlled by histone acetyl transferases and histone deacetylases (HDACs). Histone acetyl transferases in many cases are intrinsic activities of transcriptional coactivators such as the CBP/p300 protein (23). HDACs constitute a family of individual proteins grouped in three subfamilies, e.g. class I, class II and Sir2- like HDACs, that are recruited into larger multi-protein complexes for example with transcriptional co-repressors such as N-Cor/SMRT or mSin3 (1, 16). Consistent with the transient nature of histone modification by acetylation both catalytic activities are recruited to individual target genes by transcription factors. The family of nuclear hormone receptors for steroids, retinoids and other ligands constitutes the prime example for an exchange between multi-protein complexes with either histone acetyltransferases or HDACs depending on activation status of the nuclear receptor (5). The histone acetylation status at a given genetic locus appears to contribute substantial to transient regulation of activity of gene expression from this locus (2, 9, 12). Thus, efficiency of chromatin immune precipitation with antibodies directed against acetylated forms of histones reflects transcriptional activity of many genes. Application of chemical inhibitors of histone deacetylases to shift the balance between acetylated and deacetylated histones induces transcriptional activity of many genes but there are many more genes actively transcribed in mammalian cells that do not respond to HDAC inhibition and little is known why only a few percent of the transcribed genes respond to HDAC inhibition. Although it is tempting to assume that acetylated histones facilitate active gene expression from a certain gene locus due to prevention of strong ionic interactions between neighboring nucleosomes this may not be the complete story. Also other proteins may be susceptible to acetylation and deacetylation by HDACs and p53 may just be one example (27).

Aberrant transcriptional repression in malignant diseases

Several forms of promyelocytic leukemia establish model cases that indicate a prominent role of HDAC dependent aberrant transcriptional repression for the development of the disease. Some of the chromosomal translocations found in the established disease such as the PML-RAR protein or the AML-ETO protein code for aberrant transcriptional repressors (8, 13, 18). They are thought to repress target genes that are essential for the differentiation process of myeloid cells and whose lack of expression confers a block in differentiation. Despite the apparent requirement of one of the chromosomal translocations for disease development there are two major questions, e.g. whether the expression of the fusion protein alone suffices to induce leukemia, and whether indeed the aberrant transcriptional repression function is required.

Whether fusion proteins such as PML-RAR or AML-ETO suffice to induce leukemia has been studied by transgenic expression in murine cells or the mouse. Retroviral expression of PML-RAR in precursor cells induces leukemia at high efficiency. However, mono- or oligo-clonality of leukemic cells and a delay of several weeks before disease onset suggest the need for additional genetic lesions (19). Expression of AML-ETO induces leukemia in mice only when co-expressed together with an activated mutant form of the receptor tyrosine kinase TEL (7). Thus, clearly other defects in addition to the chromosomal translocation have to be acquired. The question whether it is solely the aberrant transcriptional repression function of the fusion proteins may find an answer with the use of inhibitors of HDACs and careful analysis of their biological effects. At least in the case of PML-RAR the fusion protein recruits additional enzymatic activities such as a DNA-methyltransferase activity (4) that as well or even more stably may contribute to chromatin remodeling at gene loci that are relevant for myeloid differentiation. The latter considerations also raise the question whether one might expect the consequences of the fusion protein to be reversible by interfering with HDAC activities. The preliminary use of HDAC inhibitors supports this view. One might, however, also consider that alterations acquired in addition to histone deacetylation such as DNA methylation or additional mutations are not reversible.

Myeloic leukemia appears to be a model case in which aberrant transcriptional repression as an essential step in pathogenesis and therapeutic interventions directed against aberrant chromatin modifications can be studied. However, the concept may prove useful for other forms of malignant diseases and carcinomas. In carcinomas there is usually none of the chromosomal translocations described. Aberrant function of the transcriptional repression machinery may, however, also play a role since HDAC2 in contrast to other HDACs is found at elevated expression levels in many tumor cell lines compared to primary cell from corresponding normal tissue (26).

Drugs for therapeutic intervention

Since aberrant transcriptional repression depending on HDACs appears to be essential for the development of promyelocytic leukemia and other malignant diseases it had been obvious to attempt interfering with HDAC activity though it was not clear from the beginning that 'normal' cells in the organism would tolerate such interference surprisingly well. There are currently many drugs developed by academic groups and the pharmaceutical industry. This article will not cover all the compounds but focus on those that serve as model compounds for different aspects concerning HDAC inhibitory drugs.

Trapoxin and trichostatin A are the prominent microbial toxins that are valuable HDAC inhibitors in cultured cells but of limited use in vivo. Butyrate and phenylbutyrate have been known for long to affect histone acetylation status but they appear also to affect other chromatin remodeling enzymes such as (13). Nevertheless phenylbutyrate was used first to successfully treat a patient who suffered from a retinoid resistant acute promyelocytic leukemia (24). Suberoylanilide hydroxamic acid (SAHA) is chemically related to the microbial toxin TSA (10, 17) and has completed a phase I clinical trial and is in phase II trials (11). Leukopenia, thrombocytopenia and hypotension appear as dose limiting toxicities. Among a total of 29 patients two suffering from lymphoma and 2 from bladder cancer showed objective tumor regression under treatment. The cyclic tetrapeptide depsipetide shows dose limiting toxicities of fatigue, nausea, vomiting, thrombocytopenia and cardiac arrhythmia (22). The phase I trial indicated partial (3 patients) and complete (1 patient) responses in patients suffering from cutaneous T-cell lymphoma (21).

Valproic acid has been used in therapy of epilepsy for decades and induction of differentiation in cancer cells had been described (3). Only recently it became apparent that VPA preferentially inhibits the catalytic activity of class I HDACs (6, 20). In addition it induces proteasomal degradation of HDAC2 by transcriptional induction of the E2 ubiquitin conjugating enzyme Ubc8 (14). Despite the preferential interference with only a subset of HDACs by inducing degradation of HDAC2 and inhibiting the catalytic activity of remaining classl but not of class II enzymes VPA potently induces differentiation and apoptosis in PML-RAR expressing murine myeloblasts as well human leukemic blasts as well as carcinoma cell lines (6). The hitherto exploited sedative and antiepileptic activity is not mediated by HDAC inhibition since derivatives of VPA such as valpromide may be even more potent sedative agents without any

HDAC inhibitory activity (6). Rather, in a series of VPA related compounds HDAC inhibition segregates with teratogenicity and VPA like TSA for comparison had been shown to interfere with proper embryonic development also in Xenopus (20). The lack of serious toxicity even during long-term treatment suggests that HDAC inhibition *per se* at least with compounds that preferentially affect HDAC2 and other class I HDACs could be tolerated quite well by the adult organism. The sedating activity of VPA that is not linked to HDAC inhibition would in this case appear most likely as the dose-limiting toxicity.

Questions and hopes for therapeutic intervention

Initial results in phase I and phase II clinical trials as well as compassionate use of HDAC inhibitors support the hope that HDAC inhibitors will become a tool in therapy of cancer. Those forms of cancer will have to be identified that are susceptible to treatment with HDAC inhibitors and predictive markers of response will have to be developed. Questions concerning the concept will have to be solved. How is a manifest tumor that usually has acquired several genetically or epigenetically fixed alterations expected to respond? Even if aberrant transcriptional repression had been essential during development of the disease it is not necessarily required during later stages of the malignant disease. And even if the ultimate tumor is still susceptible, could one expect reduction of tumor cell load or just slowing of their proliferation? Depending on the tumor either concept may apply since many cells respond to HDAC inhibitor treatment with a p53 independent up-regulation of the p21 cell cycle inhibitor and others undergo apoptosis (15, 17). Also, it is not clear yet whether a tumor cell responds to HDAC inhibitors because the drug reverses one of the events that are essential for maintenance of the transformed phenotype of the cancer cell or whether HDAC inhibition induces a set of cellular responses, including for example induction of the p21 cell cycle inhibitor, to which tumor cells happen to be more susceptible than normal cells. Understanding the mechanisms behind the responses will help to define the HDAC isoforms that may be the most promising targets of therapeutic intervention. Such knowledge could substantially influence the development of future generations of HDAC inhibitory drugs. Decisions will be made easier whether HDAC inhibitors should be used during a limited time period such as conventional cytostatic drugs or whether they should be taken life-long to repress a potential minimal residual disease. Despite many open questions the promising results from initially published studies support the hope that HDAC inhibitors will not become the 'magic bullet' but will find their place among other general and specialized concepts of cancer therapy.

References

1. Alland, L., R. Muhle, H. Hou, Jr., J. Potes, L. Chin, N. Schreiber-Agus, and R. A. DePinho. 1997 Nature 387:49-55.
2. Berger, S. L. 2002 Curr Opin Genet Dev 12:142-8.
3. Blaheta, R. A., and J. Cinatl, Jr. 2002 Med Res Rev 22:492-511.
4. Di Croce, L., V. A. Raker, M. Corsaro, F. Fazi, M. Fanelli, M. Faretta, F. Fuks, F. Lo Coco, T. Kouzarides, C. Nervi, S. Minucci, and P. G. Pelicci. 2002 Science 295:1079-82.
5. Glass, C. K., and M. G. Rosenfeld. 2000 Genes Dev 14:121-41.
6. Göttlicher, M., S. Minucci, P. Zhu, O. H. Krämer, A. Schimpf, S. Giavara, J. P. Sleeman, F. Lo Coco, C. Nervi, P. G. Pelicci, and T. Heinzel. 2001 Embo J 20:6969-78.
7. Grisolano, J. L., J. O'Neal, J. Cain, and M. H. Tomasson. 2003 Proc Natl Acad Sci U S A 100:9506-11.
8. Hiebert, S. W., B. Lutterbach, and J. Amann. 2001 Curr Opin Hematol 8:197-200.
9. Jenuwein, T., and C. D. Allis. 2001 Science 293:1074-80.
10. Kelly, W. K., O. A. O'Connor, and P. A. Marks. 2002 11:1695-713.
11. Kelly, W. K., V. M. Richon, O. O'Connor, T. Curley, B. MacGregor-Curtelli, W. Tong, M. Klang, L. Schwartz, S. Richardson, E. Rosa, M. Drobnjak, C. Cordon-Cordo, J. H. Chiao, R. Rifkind, P. A. Marks, and H. Scher. 2003 Clin Cancer Res 9:3578-88.
12. Kouzarides, T. 2000 Embo J 19:1176-9.
13. Krämer, O. H., M. Göttlicher, and T. Heinzel. 2001 Trends Endocrinol Metab 12:294-300.
14. Krämer, O. H., P. Zhu, H. P. Ostendorff, M. Golebiewski, J. Tiefenbach, M. A. Peters, B. Brill, B. Groner, I. Bach, T. Heinzel, and M. Göttlicher. 2003 Embo J 22:3411-20.
15. Lagger, G., D. O'Carroll, M. Rembold, H. Khier, J. Tischler, G. Weitzer, B. Schuettengruber, C. Hauser, R. Brunmeir, T. Jenuwein, and C. Seiser. 2002 Embo J 21:2672-81.
16. Laherty, C. D., W. M. Yang, J. M. Sun, J. R. Davie, E. Seto, and R. N. Eisenman. 1997 Cell 89:349-56.
17. Marks, P., R. A. Rifkind, V. M. Richon, R. Breslow, T. Miller, and W. K. Kelly. 2001 Nat Rev Cancer 1:194-202.
18. Melnick, A., and J. D. Licht. 2002 Curr Opin Hematol 9:322-32.
19. Minucci, S., S. Monestiroli, S. Giavara, S. Ronzoni, F. Marchesi, A. Insinga, D. Diverio, P. Gasparini, M. Capillo, E. Colombo, C. Matteucci, F. Contegno, F. Lo-Coco, E. Scanziani, A. Gobbi, and P. G. Pelicci. 2002 Blood 100:2989-95.
20. Phiel, C. J., F. Zhang, E. Y. Huang, M. G. Guenther, M. A. Lazar, and P. S. Klein. 2001 J Biol Chem 276:36734-41.
21. Piekarz, R. L., R. Robey, V. Sandor, S. Bakke, W. H. Wilson, L. Dahmoush, D. M. Kingma, M. L. Turner, R. Altemus, and S. E. Bates. 2001 Blood 98:2865-8.
22. Sandor, V., S. Bakke, R. W. Robey, M. H. Kang, M. V. Blagosklonny, J. Bender, R. Brooks, R. L. Piekarz, E. Tucker, W. D. Figg, K. K. Chan, B. Goldspiel, A. T. Fojo, S. P. Balcerzak, and S. E. Bates. 2002 Clin Cancer Res 8:718-28.
23. Utley, R. T., K. Ikeda, P. A. Grant, J. Cote, D. J. Steger, A. Eberharter, S. John, and J. L. Workman. 1998 Nature 394:498-502.
24. Warrell, R. P., Jr., L. Z. He, V. Richon, E. Calleja, and P. P. Pandolfi. 1998 J Natl Cancer Inst 90:1621-5.
25. Wolffe, A. P., and J. J. Hayes. 1999 Nucleic Acids Res 27:711-20.
26. Yang, W. M., Y. L. Yao, J. M. Sun, J. R. Davie, and E. Seto. 1997 J Biol Chem 272:28001-7.
27. Zeng, L., Y. Zhang, S. Chien, X. Liu, and J. Y. Shyy. 2003 J Biol Chem 278:24594-9.

G3139, a BCL-2 Antisense Oligo-nucleotide, in AML

G. MARCUCCI, W. STOCK, G. DAI, M. I. KLISOVIC, K. MAHARRY, T. SHEN, S. LIU, D. A. SHER, D. LUCAS, J, A. ZWIEBEL, R. A. LARSON, M. A. CALIGIURI, C. D. BLOOMFIELD, K. K. CHAN, M. R GREVER, and J. C. BYRD

Division of Hematology-Oncology, Department of Medicine [GM, MAC, MIK, TS, SL, DL. CDB, JCB, MRG, KM] and Division of Pharmaceutics, College of Pharmacy [GD, KKC], the Comprehensive Cancer Center and The Ohio State University, Columbus, OH, Divison of Hematology/ Oncology, University of Chicago, USA [WS, DAS, RAL], National Cancer Institute, Bethesda, MD, USA [JAZ]

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Despite many advances made in the management of acute myeloid leukemia (AML) in the past three decades, only 30 to 40% of adult patients are cured of their disease following intensive chemotherapy treatment (1-3). Further, although cure can be achieved with allogeneic stem cell transplantation (SCT), including in patients who fail initial treatment, many are not candidates for this aggressive strategy, underscoring the need for novel therapeutic approaches that could improve the current clinical results.

Most treatment failures in patients with AML are related to development of chemoresistance in leukemic blasts (5). Defects in pathways of apoptosis contribute significantly to inducing resistance to a variety of chemotherapeutic agents. Bcl-2 is a potent inhibitor of caspase-mediated apoptosis (6). In recent clinical studies, abnormal expression of Bcl-2 was proven to be predictive of poor response to treatment and adverse clinical outcome in patients with a variety of hematologic malignancies, including AML (7). Based upon these data, we and others, therefore, have hypothesized that down-regulation of Bcl-2 might ultimately induce a low apoptotic threshold and restore chemosensitivity in otherwise resistant leukemic cells. To validate this strategy, we have combined G3139, an 18-mer phosphorothioate oligodeoxynucleotide (ODN) antisense designed to bind to the first six codons of the human Bcl-2 mRNA, with intensive chemotherapy regimens in the treatment of acute leukemia. Antisense therapy is generally intended as a therapeutic approach that modifies the expression of a specific gene by inhibiting translation of its messenger RNA (mRNA). The antisense ODNs are sequences of 16 to 29 bases of single-stranded DNA that hybridize to specific mRNA targets by Watson-Crick base pairing, resulting in disruption of their functions. Once the hybridization has occurred, the ODN-mRNA duplex becomes a substrate for intracellular ribonuclease H that catalyzes mRNA degradation, while allowing the ODN to recycle for another base-pairing event with the next target mRNA molecule. The net result of this process is a sustained decrease in target mRNA translation and, ultimately, a reduced level of the corresponding target protein synthesis. Preclinical and clinical studies showed that when administered alone or in combination with chemotherapy, G3139 down-regulated Bcl-2 expression in vitro and in vivo, resulting in a significant increase in tumor cell apoptosis (8,9).

We initially conducted a phase I dose escalation study of G3139 with an intensive salvage chemotherapy, FLAG [fludarabine, cytarabine and G-CSF] in patients with refractory or relapsed AML and acute lymphoblastic leukemia (ALL) (10,11). G3139 was delivered as a continuous intravenous infusion (CIVI) on days 1 to 10 at a dose of 4 mg/kg/d for the first four cohorts of patients, and at a dose of 7 mg/kg/d for the fifth cohort of patients. Both fludarabine (dose level 1: 15 mg/m^2 IV over 0.5 hour) and cytarabine (dose level 1:1,000 mg/m^2 IV over 4 hours) were given on days 6 to 10, with 25% increments at each subsequent dose level to achieve the full dose of FLAG administered to the fourth and fifth cohorts of patients. G-CSF was started at day 5 and at a dose of 5 μg/kg/d in all cohorts and continued until the absolute neutrophil count was >3,000/μL for two consecutive days or >10,000/μL for one. Twenty patients (13 females and 7 males) were enrolled. The median age was 56 years. Seventeen patients had AML, 5 with primary refractory disease, 8 in first relapse, and 4 in subsequent relapses. Three patients had ALL, 2 with refractory Philadelphia chromosome-positive disease, and 1 with t(5;14)(q31;q32) relapsed disease and hypereosinophilia. Of the 20 patients, 9 received high-dose cytarabine (HiDAC) with previous treatments, 1 had autologous SCT, and 1 matched unrelated donor (MUD) SCT. The median time to relapse from the initial treatment for relapsed patients was 7 months (range 3 to 21 months). The median number of previous treatments was 2. Hematologic toxicities were similar to those expected with FLAG alone. Median time for neutrophil recovery from start of chemotherapy (i.e., day 6) was 23 days (range 8 to 38 days); median time for platelet recovery (≥50,000) was 39 days (range 21 to 56 days). Common adverse effects included fever, nausea, emesis, hypocalcemia, hypophosphatemia and fluid retention. They were manageable and non-dose limiting.

Pharmacokinetic analysis performed with an HPLC-UV method demonstrated that a steady-state plasma concentration (Css) was achieved rapidly and remained so until the end of G3139 infusion. The mean Css of 3.19±1.29 μg/ml (range 1.59–5.69 μg/ml) for the 4 mg/kg dose was significantly lower than the Css of 5.47±2.16 μg/ml (range 2.67–8.38 μg/ml) for the 7 mg/kg dose (p=0.023). The linearity in pharmacokinetics with the administered dose of G3139 was also reflected by a dose-dependent difference in the areas under the curve (AUC) of patients who received 4 mg/kg/d compared to those who received 7 mg/kg/d ($p<0.05$). The mean $t_{1/2}$ values were 0.63±0.33 hr (range 0.36–1.80 hr) and 0.52±0.23 hr (range 0.33–1.13 hrs) for the 4 and 7 mg/kg/d doses, respectively. Complete responses (CRs) were noted in 6 patients (5 AML and 1 ALL); for two of them CRs persisted for 18.8 and 12.7 months. Three additional patients (2 AML and 1 ALL) achieved an incomplete remission (IR) characterized by no evidence of disease (NED) but failure to recover normal neutrophil and/or platelet counts. Responses were seen at all dose levels. Of the 9 responders, four were >60 years. To fully evaluate the efficiency of G3139 in down-regulating its target, Bcl-2 mRNA levels were measured in bone marrow (BM) samples collected before treatment and at day 5 of the G3139 infusion, prior to FLAG initiation. Of the 12 patients, 9 (75%) showed down-regulation of Bcl-2 mRNA following G3139 administration (range 6.5 to 75.7% decrease in Bcl-2 transcripts).

Based on these encouraging results, we embarked on a study of G3139 in combination with cytarabine and daunorubicin ("7+3") in high-risk previously untreated AML (12,13). We targeted patients of age 60 and older, stratified depending on the presence of de novo or secondary (i.e., post-myeloproliferative or myelodysplastic clonal disorders or post–chemotherapy) AML. Two dose levels of daunorubicin, 45 (level 1) and 60 (level 2) mg/m^2/d IV, d 4–6), with same dosing of G3139 (7 mg/m^2/d CIVI, days 1–10) and cytarabine (100 mg/m^2/CIVI, days 4–10) were studied. Patients who achieved CR received two consolidation courses with G3139 (7 mg/m^2/d CIVI, days 1–8) plus high-dose cytarabine (2,000 mg/m^2/d, days 4–8). To date, 26 patients (median age 67) have been enrolled: 10 with de novo (3 at level 1 and 7 at level 2) and 16 with secondary AML (6 at level 1 and 10 at level 2). Of the 26 patients, 22 have completed the treatment program and are evaluable. Of the 22, 9 patients had normal cytogenetics, 6 intermediate and 7 poor-risk abnormal karyotypes (14). No unexpected or dose limiting toxicities have been observed in the treated patients. Ten patients (45.4%) achieved CR (4 with de novo and 6 with secondary AML). Two patients showed no evidence of disease but failed to maintain normal WBC and platelets for at least 4 weeks after induction. Of these responders, 5 were enrolled in level 1 and 7 were enrolled in level 2. With a median follow-up of 7.2 months, 2 patients treated at level 1 have relapsed at 8.1 and 13.5 months. Of the 10 non-responders (NR) (4 with de novo and 6 with secondary AML), 3 were enrolled in level 1 and 7 were enrolled in level 2. Seven of the 10 received a second induction without improvement of their disease status or additional toxicity.

As part of the correlative studies, we developed a novel fluorogenic ELISA-based assay with a detection limit of 50 pM capable of measuring intracellular concentration (IC) of G3139. Following 72 hours of G3139 CIVI, the median IC of G3139 was 5.62 pmole/mg (95% C.I.=1.24-29.02) in CR patients (n=5) vs. 1.84 pmole/mg (95% C.I.=0.33-14.32) in NR patients (n=5). Bcl-2 mRNA levels were measured in BM samples by Real Time RT-PCR at baseline and following 72 hours of G3139 CIVI, prior to initiating chemotherapy. Bcl-2 down-regulation was observed in 12 (66.70%) of 18 patients analyzed to date. The median Bcl-2 down-regulation at 72 hours of G3139 CIVI appeared more pronounced in the CR patients (71.89% of the pretreatment Bcl-2 level; 95% C.I.=40.40-147.89) than in NR patients (96.95% of the pretreatment Bcl-2 level; 95% C.I.=77.74-203.17). Rates of spontaneous apoptosis measured following 72 hours of G3139 CIVI increased by a median of 33.33% (P=0.16) in unmanipulated BM mononuclear cells (MNCs) (n=9) and by a median of 100.99% (P=0.063) in CD34-positive blasts (n=6) as compared to the respective pretreatment levels. The rate of spontaneous apoptosis following 72 hour of G3139 CIVI was significantly higher in CD34-positive blasts than in unmanipulated BM MNCs (P=0.025), suggesting a potential preferential activity of the antisense on the blast cell population.

These initial studies suggest that G3139 in combination with intensive chemotherapy regimens is a feasible strategy in AML with no additional toxicity observed. Detectable levels of IC of G3139 can be routinely achieved, and these are likely to lower the apoptotic threshold in AML cells. Down-regulation of Bcl-2 occurs in more than 60% of the patients treated with G3139, and appears more pronounced in responder than in non-responder patients. Based on these encouraging results, the combination of G3139, cytarabine, and daunorubicin at the 60 mg/m^2 dose is now being tested in a randomized phase III CALGB trial (10201) for untreated elderly AML.

References

1. Bloomfield C and Caligiuri M,: Molecular Biology of Leukemia (pp 2389-2404), in De Vita V, Jr, Hellman S, Rosenberg S (eds): Cancer: Principle and Practice of Oncology. Philadelphia, PA, Lippincott Williams & Wilkins, 2001
2. Lowenberg B, Downing JR, Burnett A: Acute myeloid leukemia. N Engl J Med 341:1051, 1999
3. Estey EH: Therapeutic options for acute myelogenous leukemia. Cancer 92:1059, 2001
4. Marie JP: Drug resistance in hematologic malignancies. Curr Opin Oncol 13:463, 2001
5. Reed JC: Dysregulation of apoptosis in cancer. J Clin Oncol 17:2941, 1999
6. Wang HG, Reed JC: Mechanisms of Bcl-2 protein function. Histol Histopathol 13:521, 1998

7. Campos L, Rouault JP, Sabido O, Oriol P, Roubi N, Vasselon C, Archimbaud E, Magaud JP, Guyotat D: High expression of Bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 81:3091, 1993
8. Cotter FE, Johnson P, Hall P, Pocock C, al Mahdi N, Cowell JK, Morgan G: Antisense oligonucleotides suppress B-cell lymphoma growth in a SCID-hu mouse model. Oncogene 9:3049, 1994
9. Waters JS, Webb A, Cunningham D, Clarke PA, Raynaud F, di Stefano F, Cotter FE: Phase I clinical and pharmacokinetic study of Bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma. J Clin Oncol 18:1812, 2000
10. Estey E, Thall P, Andreeff M, Beran M, Kantarjian H, S OB, Escudier S, Robertson LE, Koller C, Kornblau S, et al.: Use of granulocyte colony-stimulating factor before, during, and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colony-stimulating factor. J Clin Oncol 12:671, 1994
11. Marcucci G, Byrd JC, Cataland SR, Fisher DB, Lucas D, Chen KK, Young D, Didier LA, Bloomfield CD, Balcerzak SP, Frankel SR, Kraut EH, Grever MR Caligiuri MA: Clinical and biological activity of Genasense (G3139, Genta, inc.), a bcl-2 antisense, in refractory or relapsed acute leukemia : a Phase I Study. Blood 101:425, 2003
12. Marcucci G, Stock W, Zwiebel JA, Cataland SR, Dai G, Moran M, Lucas D, Liu S, Klisovic MI, Kefauver C, Green M , Chan KK, Frankel SR, Grever MR, and Byrd JC: Clinical activity Of Genasense™ (GNS, Oblimersen Sodium), a pro-apoptotic to Bcl-2 oligonucleotide, in combination with daunorubicin and cytarabine: A Phase I study in previously untreated elderly acute myeloid leukemia. American Society of Hematology 2003 meeting, San Diego CA
13. Marcucci G, Dai G, Klisovic MI, Shen T, Liu S, Sher DA, Lucas D, Whitman SP, Vukosavljevic T, Zwiebel JA, Frankel SR, Grever MR, Stock W, Chan KK and Byrd JC: Pharmacologic and biologic assessment of Genasense™ (GNS, Oblimersen Sodium), a pro-apoptotic to Bcl-2 oligonucleotide, in combination with daunorubicin and cytarabine: A Phase I study in previously untreated elderly acute myeloid leukemia. American Society of Hematology 2003 meeting, San Diego CA
14. Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD; Cancer and Leukemia Group B (CALGB 8461): Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 100:4325, 2002

New Designs for Phase II Trials: Application to a Trial of Targeted Therapies vs. Chemotherapy in Patients Age > 60 with AML/High-Risk MDS

E. H. ESTEY
Dept. Leukemia, Box 428, U.T. M.D. Anderson Cancer Center, 1515 Holcombe, Houston, Tex 77030, USA

Currently, a major focus of clinical research in AML is investigation of various new "targeted" therapies, hereafter abbreviated "TT". The appeal of these TT is two-fold. First, they are postulated to be more leukemia cell specific than more conventional therapy, i.e. chemotherapy ("CT"). Second, it is very plausible that their use will be associated with less treatment-related mortality ("TRM") than CT, with death defined as treatment-related if it occurs within two months of initiation of treatment.

The great majority of TTs (and CTs) are investigated using the same phase II methodology that was employed 30 years ago. In this paper, we will contend that such methodology is inadequate. In particular, it ignores the need for comparative trials, the need to focus on multiple outcomes and to account for multiple courses of treatment, and the fundamental issue of which regimen to select for the phase II trial in the first place. These considerations, especially the first two are of considerable importance to patients. After detailing these problems, we describe a new phase II design that we hope to use to address them. The design will be employed in a trial formally comparing TT and CT in patients age 60 and older with untreated AML or high-risk MDS.

Need for Comparative Trials

The great majority of TTs are tested in single-agent phase II trials. This practice reflects the conventional phase II → phase III paradigm. Specifically, most phase II trials are "exploratory" or "observational" and designed to establish activity, with the idea that comparative trials (phase III) should only be conducted after activity has been observed. **A fundamental problem with this formulation is that phase II trials are inherently comparative. In particular, patients are vitally interested in whether a particular therapy/strategy is superior to another.** It is well-known that sophisticated patients often spend many hours on the internet and consult a variety of physicians in hopes of identifying "the best therapy", the term "best" obviously implying comparison. The comparative nature of phase II trials is also implicit in the designs governing their conduct, since these designs specify minimally acceptable response rates (1). Derivation of these rates necessarily entails comparison with other available therapies.

Although phase II trials are thus inherently comparative, the current emphasis on single-arm, non-randomized phase II trials provides a very unreliable basis for treatment comparison (2). This lack of reliability arises from "treatment-trial" confounding ,which arises because the effects of latent (unobserved) variables have a substantive impact on response to treatment, and the distribution of these variables usually vary a great deal between trials. Differences between the response rates of separate trials that are due to such latent variables are called "trial effects." Latent variables may include supportive care, physicians, nurses, institutions, or unknown patient characteristics. Differences between response rates that are due to the treatments are called "treatment effects," and these are the primary focus of clinical trials. However, with single arm trials, only the combined effect of the trials and the treatments can be estimated, and it is impossible to determine how much of this combined effect is due to actual treatment differences. Indeed, it appears logically inconsistent that the need to avoid confounding trial and treatment effects is addressed by randomizing in phase III, yet is ignored in the evaluation of phase II data that determines whether the phase III trial will be conducted in the first place. These considerations emphasize the desirability of randomization among various treatments and strategies in the early stages of their development.

Need to Monitor Multiple Outcomes

It is commonly accepted that adaptive monitoring of clinical trials is highly desirable. Specifically, interim analyses can lower the risk that future patients will receive a therapy that has already been shown to be ineffective/toxic in the initial patients entered on the trial. Adaptive monitoring of most TT trials is limited to interim analyses of response rate, with response defined so as to include "minor response" (MR), which typically is observed much more commonly than CR(see for example 3-9). Attention to MR is important because its presence indicates that the TT has activity and thus might be worthy of further investigation. However, the relationship, if any, between MR and survival or "quality of life"(QOL) generally is unknown. **Thus, making response the sole focus of interim analyses overlooks the reality that patients are likely to be concerned with response only to the extent that it is known to lead to longer survival and/or a better QOL.** This is particularly true in AML or high-risk MDS, given the very short life expectancy of patients with these conditions. Indeed, because formal "stopping rules" in trials of TTs are based on response and not survival, a scenario in which all patients on a trial "respond" but nonetheless die sooner than might be expected if given standard CT could ,in principle, lead to erroneous continuation of the trial. Because death rates in TT trials are typically monitored informally and on an *ad hoc* basis, such trials often have very undesirable "operating characteristics"(OCs).OCs include quantities such as the probability of early termination (PET) if a treatment arm is truly associated with a higher mortality rate, or the

probability of selecting a given treatment if it is truly superior to others, as well as expected sample size (2).

While it is thus desirable to formally monitor death in trials in AML or high-risk MDS, survival time usually cannot be fully assessed until several months after entry onto such a trial , while response (MR or CR) can. Because, however, appreciable numbers of older(e.g. age >60 years) patients with AML or high-risk MDS die within a few months of presentation (e.g. 35% at 2 months if given CT), *some* information about survival is available early on. It follows that it might be reasonable to formally monitor both response and survival several months after beginning a given therapy. Accrual into a treatment arm would stop if either the death rate was too high or the response rate too low. It is possible that a high early death rate might transform into a low later death rate. Similarly, a low response rate might have no effect on survival. However, it is unlikely that patients would accept such possibilities, which should be assessed retrospectively.

It might also be desirable to formally monitor QOL. For example, at interim analysis, if one treatment/strategy is equivalent to another with respect to both response and death ,but is clearly superior with respect to QOL, it might be reasonable to divert future patients to this treatment/strategy. These considerations underscore the desirability of including mechanisms for multiple-outcome monitoring in trials in AML or high-risk MDS.

Need to Consider Strategies as well as Treatments

Monitoring multiple outcomes simultaneously, ideally based on desirable trade-offs between these outcomes, is an example of a "multiplicity". A second type of multiplicity arises from the reality that a given patient with AML or high-risk MDS typically receives multiple treatment regimens. **It is quite plausible that administration of one therapy may affect the outcomes with a subsequent therapy.** For example, because "targeted therapies" may affect multiple targets, a therapy directed at target "X" may also "down (up) regulate" target "Y", thereby influencing response to a future therapy aimed at Y. Furthermore. debate often revolves around whether a less toxic, but potentially less "curative" therapy, should be given a chance to fail before use of more toxic and hence putatively more curative therapy. For example, patients age 60+ with AML or high-risk MDS might have less TRM if given TT first, and CT only should TT fail. We will call this strategy (TT,CT). However the reverse approach might be preferable if CT's anti-leukemia effect is significantly greater than TT's, outweighing any reduction in TRM with the (TT,CT) strategy. In all of these cases, the issue is evaluation of a multi-course treatment strategy, rather than a particular treatment. The specific question is which is the preferable sequence of treatments. Conventional statistical designs for TT trials regard each therapy as a distinct entity, thus paying little formal attention to the issue of the sequence in which two or more therapies are administered. It follows that new designs should address this issue.

Application to a Trial in AML/High-Risk MDS

It is widely accepted that current CT is unsatisfactory for older patients with untreated AML or high-risk MDS. Indeed, it is unclear whether, in many such patients, current therapy improves the natural history of the untreated disease. It follows that for many patients the only rational therapy is that which can conceivably be viewed as "investigational." Such investigational therapy can be divided into CT and TT. As noted above, although TT might produce less TRM than CR, it might also have less anti-leukemia effect. Specifically, TT might be less likely to produce CR. This is important because it has been demonstrated that, at least with CT, responses short of CR have little effect on survival(10). It might be contended that patients would be served best by a strategy in which they receive TT first, followed by CT only if TT fails. This approach however ignores the possibility that, given the natural history of untreated AML in older patients, the condition of patients treated with such an approach may have deteriorated to such an extent as to severely reduce the probability of success with CT. Thus, we view the benefit/risk ratio with a TT first approach as plausibly equivalent to that with a CT first approach. This motivates randomization between the two approaches. Furthermore, the reasoning outlined in the previous paragraph led us to propose to randomize patients among the two general strategies (CT,TT) vs. (TT,CT).

The trial includes clofarabine + ara-C as the CT and three TTs: TT1=PKC412+low-dose ara-C(LDAC) , TT2=R115777+LDAC and TT3=decitabine 10 mg/m2 daily X 10. Note that the TTs represent combinations with a "CT". In fact, the future is likely to see combinations of various TTs or of TT with CT. The latter combinations blur the distinction between TT and CT. However, we prefer to refer to R115777+LDAC and PKC412+LDAC as "TT." This preference reflects the fact that LDAC is likely to produce lower TRM rates than CT regimens that use higher doses of ara-C, such as "3+7" or, very likely, than clofarabine+ara-C. However, LDAC is known to have less anti-leukemia activity than "3+7",and,most likely, than clofarabine + ara-C. It is plausible, however, that the combination of LDAC with TT will maintain low TRM rates, thus qualifying as a "TT", certainly in the minds of patients, while improving efficacy. Because CT has been our standard treatment, we will for convenience refer to clofarabine + ara-C as "S" and the TTs as E1, E2,and E3.The trial is conducted in two stages.

Stage 1

90 patients are randomized fairly among the 4 treatments {S,E1,E2,E3} using dynaic allocation (11) to balance on age (<70 vs. >69, with 70 being the median) and cytogenetics (normal vs. abnormal, with the rare cases of t(8;21 or inv(16) excluded from the trial). Patients are evaluated 60 days after beginning a course of S or E. Patients responding after receiving the therapy given on the first course continue to receive that therapy for an additional 3 courses. A patient failing S in the first course is randomized among E1, E2, and E3 for the second course. A patient failing E1, E2,or E3 on the first course must receive S on the second course because it is considered unacceptable to give TTs in both courses. Response is defined as either CR or as "major hematologic improvement" as specified by the NCI" Working Group" (12). The following figure illustrates the treatment assignment algorithm.

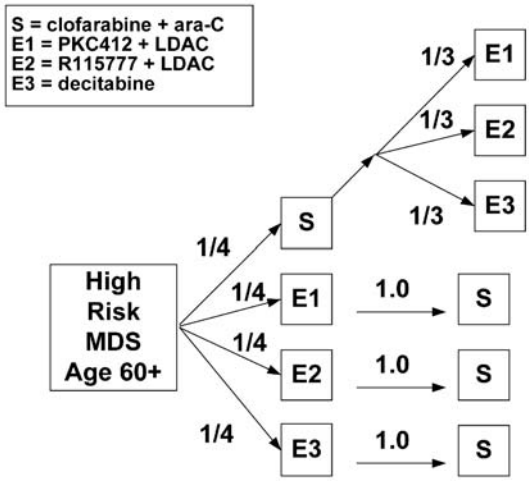

Figure 1

There are thus 6 distinct two-course strategies (S,E1), (S,E2), (S,E3), (E1,S), (E2,S), and (E3,S). (Figure 1)

After completion of the first stage(90 patients randomized to a first course), a monitoring rule is applied within each prognostic group (younger /normal cytogenetics, younger/abnormal cytoge-netics, older/normal cytogenetics, older/abnormal cytogenetics), to drop any treatment *strategy* that is comparatively inferior. The rule says that if, after the first stage is completed, it is highly likely that strategy (*s*,*t*) is more successful than strategy (*u*,*v*) in prognostic subgroup **Z**, then drop (u,v) in that subgroup; the criterion probability 0.95, quantifies the term "highly likely." "Success" is quantified by a utility function, which is based on the overall probabilities of *both* response *and* death with a given strategy. Response, defined as above, and death, yes or no, are scored after completion of the two courses of a given strategy. If both response and death occur, the outcome is considered to be "death". Many different rates of response and death are possible(e.g. 0% response+0% death,50% response+10% death etc).Thus, we define the utility function so as to reduce the probabilities of response and death to a single number by quantifying the trade-off between the likelihood of response and the risk of death. Specifically, the utility is constructed so that all pairs of the probabilities of response and death for which the utility equals a given constant are considered clinically equivalent. The following figure shows a contour plot of our utility function. The point (0.40, 0.35) corresponds to the historical probabilities of response and death after 2 courses of CT (i.e. strategy S,S).This point, as well as all the other points falling on the curve in the figure, is arbitrarily assigned the negative utility – 0.11. Non-negative utilities are considered desirable. For example, the point (0.25,0) and all other points falling on the curve labeled 0 are considered equally desirable and assigned a utility of 0.Higher values for the utility correspond to better overall outcomes. In this way the utility accounts for both response and death in making interim decisions after the first stage is completed.

Stage 2

An additional 90 patients are randomized among S,E1,E2,and E3 in each course as in stage 1, subject to the constraints imposed by dropping any treatment strategies. If strategies have dropped out, the 90 patients will be randomized among the remaining strategies. This will require an additional one year of accrual time. Once 180 patients have been treated and evaluated (approximately 2.3 years after beginning the trial), we will select, for each prognostic group, the 2-course strategy, among those not dropped in that subgroup, for which the Bayesian posterior mean of the utility function is largest. It would also be possible to simply compare treatments rather than strategies since our design not only provides an evaluation of the 6 strategies in each of four prognostic groups, but allows an unbiased comparison of the four treatments CT,TT1,TT2, and TT3 when used as either initial treatment(course 1) or as salvage treatment(course 2).We focus on strategies because, as noted above, it is probably unrealistic to ignore the effect of a given

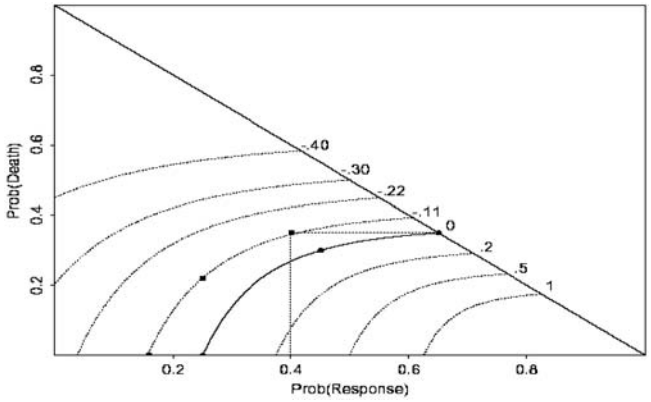

Figure 2

treatment on response independent of the context (strategy) in which it was given. Thus, for example, TT1 would drop out only if the strategies (TT1,CT) and (CT,TT1) were each inferior to its competitors. Note, however, that in the case where all or most patients given, for example, CT on course one die on course one, the posterior distribution of the utility function becomes so unfavorable that all strategies employing CT on course one, (CT, TT1), (CT, TT2), (CT, TT3), drop out.

An alternative method of proceeding would use conventional frequentist (p-value-based) designs with larger sample sizes to address the possibility of false negatives. In a trial which employed a design entirely analogous to the one to be used in the (TT,CT) vs. (CT, TT) trial, the probabilities of correctly selecting the strategy, among the four investigated, which had the highest utility ranged from 0.42 to 0.73 ,depending on prognostic subgroup. These nominally high false negative rates (27–58%) must be compared with the effective rate that would be obtained if, based on a supposedly superior pre-clinical rationale, only one of the 4 strategies was selected for a single-arm phase II trial in the absence of supporting clinical data such as would arise using our design. Assuming that pre-clinical rationale is an unreliable guide to clinical outcome and that, therefore, each of the 4 arms is equally likely to be successful, the effective false negative rate is 75%,corresponding to a power of 25%, and even larger if the single-arm trial is run with early stopping rules. The (TT,CT) vs. (CT, TT) trial is comparing 6 strategies. If based on presumably pre-clinical rationale, only two were investigated ,e.g. (CT, decitabine) vs. (decitabine, CT),the effective false negative rate is 67%. That is, a treatment/strategy not investigated has a false negative rate of 100%. While we acknowledge that pre-clinical rationale is not a completely unreliable guide, as our example suggests it is, we question whether pre-clinical rationale is sufficiently reliable to govern selection of new agents for large, conventionally powered trials with in the absence of clinical data that would be provided by the (TT,CT) vs. (CT, TT) trial**.** It is important to note that our design has the goal of selecting the best treatment, among those not dropped , regardless of the degree of difference between the best and the second best experimental treatment. This is very different from a more conventional design in which the goal is to decide whether the best treatment provides a specified degree of improvement over the others. This type of goal requires a much larger sample size, which, in turn, severely limits the number of treatments that may be studied over time.

References

1. Simon R: Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10:1-10, 1989
2. Estey EH, Thall PF: New designs for phase 2 clinical trials. Blood 102:442-448, 2003
3. Kurzrock R, Sebti SM, Kantarjian HM, Wright J, Cortes JE, Thomas DA, Wilson E, Beran M, Koller CA, O'Brien S, Freireich EJ, Talpaz M. Phase I study of a farnesyl transferase inhibitor, R115777, in patients with myelodysplastic syndrome. Blood 98: 623a, 2001. (abstract)
4. Kurzrock R, Cortes J, Ryback ME, Thibault A, Faderl S, Estey E, Garcia-Manero G, Giles F, Thomas D, O'Brien S, Talpaz M, Kantarjian H. Phase II study of R115777, a farnesyltransferase inhibitor, in myelodysplastic syndrome. Blood 98: 848a, 2001. (abstract)
5. Stone RM, Klimek V, DeAngelo DJ, Nimer S, Estey E, Galinsky I, Neuberg D, Yap A, Fox E, Gilliland DG, Griffin J. PKC412, an oral FLT3 inhibitor, has an activity in mutant FLT3 AML: a phase II clinical trial. Blood 100:86a, 2002. (abstract)
6. Stone RM, Klimek V, Deangelo D, Galinsky E, Fox S, Nimer E, Estey E, Yap A, Gilliland DG, Griffin J. Oral PKC412 has activity in patients (pts) with mutant FLT 3 acute myeloid leukemia (AML): a phase II trial. Proc Am Soc Clin Oncol 22:563, 2003. (abstract)
7. Smith D, Levis M, Brown P, Russell L, Hellriegel E, Dauses T, Allebach J, Small D. Single agent CEP-701, a novel FLT3 inhibitor, shows initial response in patients with refractory acute myeloid leukemia. Blood 100:85a, 2002. (abstract)
8. Roboz G, List A, Giles F, Rae P, Dugan M, Greenberg J, Osama S, Schuster M, Laurent D, Feldman E. Phase I trial of PTK787/ZK 222584, an inhibitor of vascular growth factor receptor tyrosine kinases, in acute myeloid leukemia and myelodysplastic syndrome. Blood 100:337a, 2002. (abstract)
9. Giles FJ, Stopeck AT, Silverman LR, Lancet JE, Cooper MA, Hannah AL, Cherrington JM, O'Farrell AM, Yuen HA, Louie SG, Hong W, Cortes JE, Verstovsek S, Albitar M, O'Brien SM, Kantarjian HM, Karp JE: SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes. Blood 102:795-801, 2003
10. Lopez G, Giles F, Cortes J, Pierce S, O'Brien S, Kantarjian H, Estey E. Sub-types of resistant disease in patients with AML, RAEB-t, or RAEB who fail initial induction chemotherapy. Blood 98: 329a, 2001. (abstract)
11. Pocock SJ, Simon R: Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31:102-115, 1975.
12. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, Lowenberg B, Beran M, de Witte TH, Stone LRM, Mittelman M, Sanz GF, Wigermans PW, Gore S, Greenberg PL: Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood 96:3671-3674, 2000.

Main Session IV

Genetic Classification of Acute Myeloid Leukemia (AML)

T. HAFERLACH, S. SCHNITTGER, W. KERN, W. HIDDEMANN, and C. SCHOCH
Laboratory for Leukemia Diagnostics, Dept. of Internal Medicine III, University Hospital Grosshadern, Ludwig-Maximilians-University, Munich, Germany

Abstract

In 50–60% of patients with acute myeloid leukemia (AML) acquired clonal chromosome aberrations can be observed after metaphase banding analyses. The cytogenetic results at diagnosis provide the most single important parameter for determining prognosis so far. Numerous recurrent karyotype abnormalities have been described in AML. These findings on the chromosomal level were followed and supplied by molecular studies that have identified genes involved in leukemogenesis. Even more, molecular markers such as MLL partial tandem duplications (MLL-PTD) or FLT3 length mutations (FLT3-LM) were found to characterize specific subtypes of AML and completed the genetic marker profile. The identification of specific chromosomal abnormalities or molecular markers and their correlation with cytomorphological features, immunophenotype as well as clinical outcome led to a new understanding of AML as a heterogeneous group of distinct biological entities. The importance of cytogenetic and molecular genetic findings in AML for classification and for the understanding of pathogenetic mechanisms is increasingly appreciated in clinical context and was translated also into the new WHO-classification of AML that uses cytogenetic abnormalities as a major criterion.

Introduction

Several parameters provided by cytomorphology, immunophenotyping and especially cytogenetics are needed to classify acute myeloid leukemias (AML) into biological entities in order to establish the diagnosis and to understand the pathogenesis as well as to develop specific treatment approaches. The discovery of specific chromosomal abnormalities has supported the view that leukemia is a genetic disease on the cellular level and also guided the way to mapping and cloning of genes which are involved in the leukemic process. Using animal models the pathogenetic role of disrupted and deregulated genes has been proven. Furthermore, the karyotype of the leukemic blast has been shown to be the most important, independent prognostic parameters in AML [17]. In genetically defined subgroups of AML, i.e. AML M3 with t(15;17) or AML with complex aberrant karyotype, treatment decisions and strategies are already based on the respective genetic results. Therefore, it is the aim for the future to characterize each AML on the genetic level for better understanding of pathogenesis and for individual treatment approaches.

Pattern of Cytogenetic Abnormalities in AML

The incidence of abnormal karyotypes in AML has been reported to be 55% to 78% in adults and 77% to 85% in children [3, 9, 13, 19, 29, 45, 63]. However, a substantial proportion of patients show no chromosome abnormalities. Furthermore, a considerable proportion of cytogenetically normal patients display submicroscopic gene alterations that can only be detected by molecular methods. For instance, approximately 6% of adult AML patients with normal karyotype display a partial tandem duplication within the MLL gene and nearly 40% demonstrate a length mutation within the FLT3 gene [7, 46, 47].

So-called primary chromosome abnormalities are distinguished from secondary chromosome abnormalities. Primary chromosome aberrations are frequently found as the sole karyotypic abnormality and are often specifically associated with a particular AML subtype. These primary abnormalities are assumed to play an essential role in the early stages of leukemogenesis. Secondary chromosome aberrations are rarely or never found alone and seem to play an important role in the progression of the disease. Although less specific than the primary changes, secondary aberrations nevertheless demonstrate non-random features with distribution patterns that appear to be dependent on the primary abnormality [18]. In contrast to primary aberrations which are often balanced rearrangements as translocations or inversions, secondary aberrations are genomic imbalances (gains or losses of whole chromosomes, deletions, or unbalanced translocations).

Primary Chromosome Abnormalities for Genetic Classification

Two major types of primary chromosome abnormalities can be distinguished:

1. balanced structural abnormalities (reciprocal translocations, inversions and insertions) usually leading to a leukemia specific fusion gene, 2. unbalanced aberrations (trisomies, monosomies, deletions, unbalanced translocations and isochromosomes) leading to gain and/or loss of chromosomal material.

The most common balanced chromosomal abnormalities in AML and genes affected are: t(8;21)(q22;q22)/AML1/ETO,t(15;17)(q22;q12)/PML-RARα, inv(16)(p13q22)/t(16;16)(p13;q22) /CBFβ-MYH11, 11q23 abnormalities/MLL-rearrangements. These abnormalities are found in 15% to 25% of all AML cases and are combined in the first step of the new WHO classification system [5, 55, 60].

The most common unbalanced abnormalities are: deletion 5q, monosomy 7, deletion 7q, trisomy 8, deletion 9q, trisomy 11, trisomy 13, trisomy 21.

In 10 to 20% of patients with AML a complex aberrant karyotypes can be found and is associated with a very poor prognosis [1, 2, 9, 13, 63]. The definition of complex aberrant karyotype varies between different study groups. Commonly it is defined as at least three cytogenetic abnormalities. Most patients with complex aberrant karyotype show unbalanced karyotype abnormalities with loss of material of 5q, 7q and/or 17p [53].

The most common cytogenetic aberrations observed in AML and their incidences in children and adults are listed in table 1.

Secondary chromosome abnormalities

Secondary chromosome abnormalities develop in cells already carrying a primary abnormality. They demonstrate in many cases non-random features with distribution patterns that appear to be dependent on the primary abnormality [18, 31]. The most frequent additional aberration observed is trisomy 8 and a deletion of the long arm of chromosome 9 [15, 52]. In 70–80% of patients with t(8;21)(q22;q22) additional aberrations are observed. The most common secondary aberration is the loss of a sex-chromosome. The frequency of secondary chromosome aberrations in patients with t(15;17) is 30–40%, with trisomy 8 and an isochromosome of the derivative chromosome 17 observed most often [4, 18, 51]. Patients with inv(16) show additional chromosome aberrations in 30–50%. Most often observed are trisomies 22, 21 or 8.

Conflicting data on the prognostic impact of secondary aberrations were published. While no influence on prognosis of secondary abnormalities in patients with t(8;21)(q22), inv(16)(p13q22) or t(15;17)(q22;q12) were reported in the large MRC10-trial and some smaller studies [28, 50, 51, 62], a negative prognostic impact of additional abnormalities was published for patients with t(8;21) and t(15;17) in one study each [22, 52]. The European 11q23 Workshop analyzed 125 patients with t(9;11)(p22;q23). Additional chromosome abnormalities did not impair prognosis [64]. This was confirmed by two further studies [6, 60].

Only little is known about the underlying genetic defects in AML displaying a normal karyotype in chromosome banding analysis. Several molecular defects, such as internal tandem duplications of the *MLL* gene (MLL-PTD), length mutations of the *FLT3* gene (FLT3-LM), and point mutations within the *AML1* or *CEBPα* genes have been described in AML with normal karyotypes [33, 35, 36, 46, 48, 66]. These markers have to be investigated for their diagnostic value and especially for follow-up studies with respect to minimal residual disease (MRD) in the near future [25, 49].

Prognostic significance of cytogenetic abnormalities in AML

The karyotype of the leukemic blasts is the most important, independent prognostic parameter in AML [17]. A favorable outcome under currently used treatment regimens was observed in several studies in patients with t(8;21)(q22;q22), inv(16)(p13q22) or t(15;17)(q22;q11-12). Chromosome aberrations with an unfavorable clinical course are -5/del(5q), -7/del(7q), inv(3)/t(3;3) and complex aberrant karyotype. All others, i.e. patients with a normal karyotype and rare chromosome aberrations, are assigned to an intermediate prognostic group.

Another important finding concerning cytogenetic abnormalities and prognosis was that the incidence of distinct chromosome abnormalities varies with age, but the prognosis of defined cytogenetic aberrations is age-independent [20, 56].

Cytogenetics in de novo AML vs. AML after an antecedent hematological disorder vs. t-AML

Based on the history of the patient AML can be subdivided in three categories:

- de novo AML
- AML occurring after an antecedent hematological disorder
- therapy related AML (t-AML) occurring secondary to chemotherapeutic treatment

The incidence of chromosome abnormalities is lower in de novo AML (50%–55%) compared to AML evolving from an antecedent hematological disorder (approximately 75%) and t-AML (>85%). While the pattern of unbalanced abnormalities was similar in the three subgroups and no abnormality specific for one of these groups was identified, within the group of balanced chromosome abnormalities t(8;21), t(15;17), inv(16)/t(16;16) and translocations involving the MLL gene were only found in de novo AML and t-AML but not in AML evolving from an antecedent hematological disorder [59].

In t-AML two subgroups can be separated depending on whether the patient has received alkylating agents or drugs targeting topoisomerase II. Alkylating agents related t-AML are characterized by a preceding myelodysplastic phase, long interval between cytotoxic treatment and appearance of t-AML (36 to 72 months), cytogenetic abnormalities involving chromosomes 5 and 7 and often complex aberrant karyotypes showing a poor response to chemotherapy.

In contrast, t-AML related to therapy with topoisomerase II inhibitors usually present with overt leukemia and with AML M4 or M5 morphology according to FAB-classification after a short latency period (6 to 36 months) to preceeding therapy. They often show balanced chromosome aberrations, primarily translocations involving chromosome bands 11q23 and 21q22 and a more fa-

Table 1: Chromosome abnormalities in AML

abnormalities	fusion genes	FAB-subtype	frequency	
			children	adults
t(8;21)(q22;q22)	AML1-ETO	M2/M1	10–15%	8–12%
inv(16)(p13q22)	CBFb-MYH11	M4eo	6–12%	8–12%
t(15;17)(q22;q21)	PML-RARA	M3/M3v	8–15%	8–10%
t(9;11)(p22;q23)	AF9-MLL	M5a	8–10%	1–2%
t(3;21)(q26;q22)	AML1-EAP/EVI1	–	1%	<1%
t(6;9)(p23;q34)	DEK-CAN	M1/M2	1–2%	rare
inv(3)(q21q26)	EVI?-?	–	<1%	1–2%
t(1;22)(p13;q13)		M7	2%	–
+8 sole		–	1-4%	3–5%
+11 sole		M1/M2	–	<1%
complex		–	6%	10–20%

vorable response to chemotherapy [11, 12, 27, 37-40, 42, 43, 57, 61]. Translocations involving 11q23 predominate following therapy with epipodophyllotoxins, whereas patients with translocations to 21q22, inv(16) and t(15;17) most often have received anthracyclines. Our own data indicate that patients with balanced chromosome aberrations as t(8;21), inv(16), t(15;17) or t(11q23) were significantly younger than patients with other abnormalities (median 45 vs. 60 years) and showed a shorter latency period between the primary tumor and t-AML (30 vs. 81 months) {59}. Compared to de novo AML, the incidence of clonal chromosome abnormalities in t-AML is higher. In 75% to 96%, karyotype aberrations are detected [23, 24, 41, 54, 57]. The spectrum of karyotype aberrations is comparable to *de novo* AML but distribution varies, as 11q23 abnormalities and complex aberrant karyotypes occur more often in patients with t-AML [8, 37, 44, 54, 57]. Overall t-AML respond less well to treatment than their *de novo* counterparts. Recent data show that as in *de novo* AML cytogenetics are also an important prognostic factor in t-AML and if corresponding cytogenetic subgroups are compared according to response, outcome does not differ much [10, 14, 24, 44, 54, 57]. In our own series of 48 cases of t-AML complete remission was achieved in 92% in the favorable group but in only 50% and 31% in the intermediate and unfavorable group, respectively. Median overall survival was 11 months compared to 7 and 1.5 months, respectively. Outcome in t-AML with favorable cytogenetics in our study is worse than in patients with *de novo* AML and favorable cytogenetics. This might be due to the fact that the incidence of uncommon secondary chromosome aberrations was higher in these t-AML compared to *de novo AML* or due to comorbidity [54, 57].

Additional information on genetic abnormalities based on new techniques

Adding fluorescence in situ hybridization techniques and polymerase chain reaction to the set of diagnostic methods a small subset of patients was identified that showed cryptic, microscopically undetectable rearrangements. The absence of chromosome abnormalities in a significant number of AML cases seems to be a genuine phenomenon rather than a failure in there detection as is supported by a study using 24-color-FISH [67]. The FISH techniques complement standard cytogenetics and are especially helpful in deciphering complex or hidden chromosomal rearrangements [21, 30, 32, 34, 53, 65].

While most techniques so far have been performed on the genomic level the introduction of microarray based gene expression profiling will add a large amount of data on the deregulation of genes in AML. This new technique may also prove helpful for the classification of AML and the definition of new entities [16, 26, 58].

Towards a genetic based classification of AML

Following the genetic path proposed by the WHO, further cytogenetic categories such as trisomies, monosomies, deletions and other unbalanced recurring karyotype abnormalities should be considered. Furthermore, molecular genetic defects including MLL-PTD and FLT3-LM and point mutations of genes (i.e. AML1, CEBPα, FLT3) could be included to categorize a larger proportion of AML on a molecular-genetic basis.

Conclusion

Classical cytogenetics using banding techniques is still the gold standard for the genetic classification of AML. These techniques should be performed in each patient with AML at diagnosis as well as at relapse. New techniques such as fluorescence in situ hybridization, polymerase chain reaction and gene expression profiling may add important information to a more sophisticated subgrouping of AML [16, 26, 58].

A hierarchical classification according to primary chromosome aberrations is needed. For clinical use a prognostic grouping for distinct cytogenetic abnormalities is required. In patients with normal karyotype molecular studies will define distinct entities within this probably heterogeneous group.

The aim for the future is to integrate all information obtained by different genetic techniques including also gene expression profiling for a comprehensive biology based classification of AML in order to develop specific treatment approaches for different AML subtypes.

References

1. Arthur DC, Berger R, Golomb HM, Swansbury GJ, Reeves BR, Alimena G, van den Berghe H, Bloomfield CD, de la Chapelle A, Dewald GW, et al (1989) The clinical significance of karyotype in acute myelogenous leukemia. Cancer Genet.Cytogenet. 40:203-216
2. Berger R, Bernheim A, Ochoa Noguera ME, Daniel MT, Valensi F, Sigaux F, Flandrin G, Boiron M (1987) Prognostic significance of chromosomal abnormalities in acute nonlymphocytic leukemia: a study of 343 patients. Cancer Genet.Cytogenet. 28:293-299
3. Berger R, Bernheim A, Ochoa Noguera ME, Daniel MT, Valensi F, Sigaux F, Flandrin G, Boiron M (1987) Prognostic significance of chromosomal abnormalities in acute nonlymphocytic leukemia: a study of 343 patients. Cancer Genetics Cytogenetics 28:293-299
4. Berger R, Le Coniat M, Derre J, Vecchione D, Jonveaux P (1991) Cytogenetic studies in acute promyelocytic leukemia: A survey of secondary chromosomal abnormalities. Genes, Chromosomes & Cancer 3:332-337
5. Brunning RD, Matutes E, Harris NL, Flandrin G, Vardiman J, Bennett JM, Head DR (2001): Acute myeloid leukemia. In: World Health Organozation of Tumors, Pathology & Genetics, Tumors of haematopoietic and lymphoid tissues. ES Jaffe, NL Harris, H Stein, J Vardiman, eds. IARC Press, Lyon, 75-108pp.
6. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PRK, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD (2002) Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 100:4325-4336
7. Caligiuri MA, Strout MP, Schichman SA, et al. (1996) Partial tandem duplication of ALL1 as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. Cancer Res 56:1418-1425
8. Cortes J, O'Brien S, Kantarjian HM, Cork A, Stass S, Freireich EJ, Keating MJ, Pierce S, Estey E (1994) Abnormalities of the long arm of chromosome 11 (11q) in patients with de novo and secondary acute myelogenous leukemias and myelodysplastic syndromes. Leukemia 8:2174-2178
9. Dastugue N, Payen C, Lafage Pochitaloff M, Bernard P, Leroux D, Huguet Rigal F, Stoppa AM, Marit G, Molina L, Michallet M, et al (1995) Prognostic significance of karyotype in de novo adult acute myeloid leukemia. The BGMT group. Leukemia 9:1491-1498
10. Detourmignies L, Castaigne S, Stoppa AM, Harousseau J-L, Sadoun A, Janvier M, Demory JL, Sanz M, Berger R, Bauters F, Chomienne C, Fenaux P (1992) Therapy-related acute promyelocytic leukemia: A report on 16 cases. J Clin Oncol 10:1430-1435
11. Ellis M, Ravid M, Lishner M (1993) A comparative analysis of alkylating agent and epipodophyllotoxin-related leukemias. Leuk Lymphoma 11:9-13
12. Felix CA (1998) Secondary leukemias induced by topoisomerase-targeted drugs.Biochim Biophys Acta 1440:233-255
13. Fenaux P, Preudhomme C, Lai JL, Morel P, Beuscart R, Bauters F (1989) Cytogenetics and their prognostic value in de novo acute myeloid leukaemia: a report on 283 cases. Br.J.Haematol. 73:61-67
14. Gardin C, Chaibi P, de Revel T, Rousselot P, Turlure P, Miclea JM, Nedellec G, Dombret H (1997) Intensive chemotherapy with idarubicin, cytosine arabinoside, and granulocyte colony-stimulating factor (G-CSF) in patients with secondary and therapy-related acute myelogenous leukemia. Club de Reflexion en Hematologie. Leukemia 11:16-21
15. Haferlach T, Bennett JM, Löffler H, Gassmann W, Andersen J, Tuzuner N, Cassileth PA, Fonatsch C, Schoch C, Schlegelberger B, Becher R, Thiel E, Ludwig W-D, Sauerland MC, Heinecke A, Büchner T (1996) Acute myeloid leukemia with translocation (8;21). Cytomorphology, dysplasia and prognostic factors in 41 cases.Leuk & Lymphoma 23:227-234
16. Haferlach T, Kohlmann A, Kern W, Hiddemann W, Schnittger S, Schoch C (2003) Gene expression profiling as a tool for the diagnosis of acute leukemias. Sem Hematol in press:
17. Haferlach T, Schoch C, Löffler H, Gassmann W, Kern W, Schnittger S, Fonatsch C, Ludwig WD, Wuchter C, Schlegelberger B, Staib P, Reichle A, Kubica U, Eimermacher H, Balleisen L, Grüneisen A, Haase D, Aul C, Karow J, Lengfelder E, Wörmann B, Heinecke A, Sauerland MC, Büchner T, Hiddemann W (2003) Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies.J.Clin.Oncol. 21:256-265
18. Heim S, Mitelman F (1986) Secondary chromosome aberrations in the acute leukemias.Cancer Genet Cytogenet 22:331-338
19. Heim S, Mitelman F (1995): In: Cancer Cytogenetics. Wiley-Liss.

20. Hiddemann W, Kern W, Schoch C, Fonatsch C, Heinecke A, Wörmann B, Büchner T (1999) Management of acute myeloid leukemia in elderly patients. J Clin Oncol 17:3569-3576
21. Hilgenfeld E, Padilla-Nash H, McNeil N, Knutsen T, Montagna C, Tschinda J, Horst J, Ludwig W-D, Serve H, Büchner T, Berdel WE, Schröck E, Ried T (2001) Spectral karyotyping and fluorescence in situ hybridization detect novel chromosomal aberrations, a recurring involvement of chromosome 21 and amplification of the MYC oncogene in acute myeloid leukemia M2.Br J Haematol 113:305-317
22. Hiorns LR, Swansbury GJ, Mehta J, Min T, Dainton MG, Treleaven J, Powles RL, Catovsky D (1997) Additional chromosome abnormalities confer worse prognosis in acute promyelocytic leukaemia. Br.J.Haematol. 96:314-321
23. Johansson B, Mertens F, Heim S, Kristoffersson U, Mitelman F (1991) Cytogenetics of secondary myelodysplasia (sMDS) and acute nonlymphocytic leukemia (sANLL).Eur J Haematol 47:17-27
24. Kantarjian HM, Keating MJ, Walters RS, Smith TL, Cork A, McCredie KB, Freireich EJ (1986) Therapy-related leukemia and myelodysplastic syndrome: Clinical, cytogenetic, and prognostic features. J Clin Oncol 4:1748-1757
25. Kern W, Haferlach T, Schoch C, Löffler H, Gassmann W, Heinecke A, Sauerland M-C, Berdel WE, Büchner T, Hiddemann W (2003) Early blast clearance by remission induction therapy is a major dependent prognostic factor for both achievement of complete remission and long-term outcome in acute myeloid leukemia: data from the German AML Cooperative Group (AMLCG) 1992 Trial. Blood 101:64-70
26. Kohlmann A, Schoch C, Schnittger S, Dugas M, Hiddemann W, Kern W, Haferlach T (2003) Molecular characterization of acute leukemias by use of microarray technology. Genes Chromosomes & Cancer 37:396-405
27. Leone G, Mele L, Pulsoni A, Equitani F, Pagano L (1999) The incidence of secondary leukemias.Haematologica 84:937-945
28. Marlton P, Keating MJ, Kantarjian HM, Pierce S, O'Brien S, Freireich EJ, Estey E (1995) Cytogenetic and clinical correlates in AML patients with abnormalities of chromosome 16.Leukemia 9:965-971
29. Martinez-Climent JA (1997) Molecular cytogenetics of childhood hematological malignancies.Leukemia 11:1999-2021
30. Mohr B, Bornhäuser M, Thiede C, Schäkel U, Schaich M, Illmer T, Pascheberg U, Ehninger G (2000) Comparison of spectral karyotyping and conventional cytogenetics in 39 patients with acute myeloid leukemia and myelodysplastic syndrome. Leukemia 14:1031-1038
31. Mrózek K, Heinonen K, Bloomfield CD (2001) Clinical importance of cytogenetics in acute myeloid leukemia.Best Pract Res Clin Haematol 14:19-47
32. Mrózek K, Heinonen K, Theil KS, Bloomfield CD (2002) Spectral karyotyping in patients with acute myeloid leukemia and complex karyotype reveals hidden aberrations, including recurrent overrepresentation of 21q, 11q and 22q.Genes, Chromosomes & Cancer in press:
33. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T, Misawa S (1996) Internal tandem duplication of the FLT3 gene found in acute myeloid leukemia. Leukemia 10:1911-1918
34. Odero MD, Carlson KM, Calasanz MJ, Rowley JD (2001) Further characterization of complex chromosomal rearrangements in myeloid malignancies: spectral karyotyping adds precision in defining abnormalities associated with poor prognosis. Leukemia 15:1133-1136
35. Osato M, Asou N, Abdalla E, Hishino K, Yamasaki H, Okubo T, Suzushima H, Takatsuki K, Kanno T, Shigesada K, Ito Y (1999) Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2aB gene associated with myeloblastic leukemias. Blood 93:1817-1824
36. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, Behre G, Hiddemann W, Tenen DG (2001) Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet 27:263-270
37. Pedersen-Bjergaard J, Andersen MK, Johansson B (1998) Balanced chromosome aberrations im leukemias following chemotherapy with DNA-topoisomerase II inhibitors. J Clin Oncol 16:1897-1898
38. Pedersen-Bjergaard J, Pedersen M, Roulston D, Philip P (1995) Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy- related acute myeloid leukemia. Blood 86:3542-3552
39. Pedersen-Bjergaard J, Philip P (1991) Two different classes of therapy-related and de novo acute myeloid leukemia?Cancer Genet Cytogenet 55:119-124
40. Pedersen-Bjergaard J, Philip P, Larsen SO, Andersson M, Daugaard G, Ersboll J, Hansen SW, Hou-Jensen K, Nielsen D, Sigsgaard TC, Specht L, Osterlind K (1993) Therapy-related myelodysplasia and acute myeloid leukemia. Cytogenetic characteristics of 115 consecutive cases and risk in seven cohorts of patients treated intensively for malignant diseases in the Copenhagen series. Leukemia 7:1975-1986
41. Pedersen-Bjergaard J, Philip P, Larsen SO, Jensen G, Byrsting K (1990) Chromosome aberrations and prognostic factors in therapy-related myelodysplasia and acute nonlymphocytic leukemia. Blood 76:1083-1091
42. Pedersen-Bjergaard J, Rowley JD (1994) The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation.Blood 83:2780-2786
43. Pui C-H, Relling MV, Rivera GK, Hancock ML, Raimondi SC, Heslop HE, Santana VM, Ribeiro RC, Sandlund JT, Mahmoud HH, Evans WE, Crist WM, Krance RA (1995) Epipodophyllotoxin-related acute myeloid leukemia: a study of 35 cases. Leukemia 9:1990-1996
44. Quesnel B, Kantarjian HM, Pedersen-Bjergaard J, Brault P, Estey E, Lai JL, Tilly H, Stoppa AM, Archimbaud E, Harousseau J-L, Bauters F, Fenaux P (1993) Therapy-related acute myeloid leukemia with t(8;21), inv(16), and t(8;16): A report on 25 cases and review of the literature. J Clin Oncol 11:2370-2379
45. Raimondi SC, Chang MN, Ravindranath Y, Behm FG, Gresik MV, Steuber CP, Weinstein HJ, Carroll AJ (1999) Chromosomal abnormalities in 478 children with acute myeloid leukemia: Clinical characteristics and treatment outcome in a Cooperative Pediatric Oncology Group Study - POG 8821.Blood 94:3707-3716
46. Schnittger S, Kinkelin U, Schoch C, Heinecke A, Haase D, Haferlach T, Büchner T, Wörmann B, Hiddemann W, Griesinger F (2000) Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 14:796-804
47. Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Löffler H, Sauerland MC, Serve H, Büchner T, Haferlach T, Hiddemann W (2002) Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subytpe, and prognosis in the AMLCG study and usefullness as a marker for the detection of minimal residual disease. Blood 100:159-166
48. Schnittger S, Schoch C, Kern W, Staib P, Wuchter C, Sauerland MC, Serve H, Büchner T, Haferlach T, Hiddemann W (2000) FLT3 length mutations in AML: Correlation to cytogenetics, FAB- subtype, and prognosis in 652 patients. Blood 96:826a
49. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W (2003) New score predicting for prognosis in PML-RARA-, AML1-ETO-, or CBFB-MYH11- positive acute myeloid leukemia based on quantification of fusion transcripts. Blood 102:2746-2755
50. Schoch C, Büchner T, Freund M, Bartels H, Haferlach T, Löffler H, Sauerland MC, Fonatsch C (1997): Fifty-nine cases of acute myeloid leukemia with inversion inv(16)(p13q22): Do additional chromosomal aberrations influence prognosis? In: Acute Leukemias VI: Prognostic factors and Treatment strategies. T Büchner, W Hiddemann, B Wörmann, G Schellong, J Ritter, U Creutzig, eds. Springer Verlag, Berlin, Heidelberg, 11-16pp.
51. Schoch C, Haase D, Haferlach T, Freund M, Link H, Lengfelder E, Loffler H, Buchner T, Fonatsch C (1996) Incidence and implication of additional chromosome aberrations in acute promyelocytic leukaemia with translocation t(15;17)(q22; q21): a report on 50 patients. Br.J.Haematol. 94:493-500
52. Schoch C, Haase D, Haferlach T, Gudat H, Buchner T, Freund M, Link H, Lengfelder E, Wandt H, Sauerland MC, Loffler H, Fonatsch C (1996) Fifty-one patients with acute myeloid leukemia and translocation t(8;21)(q22;q22): an additional deletion in 9q is an adverse prognostic factor. Leukemia 10:1288-1295
53. Schoch C, Haferlach T, Bursch S, Gerstner D, Schnittger S, Dugas M, Kern W, Löffler H, Hiddemann W (2002) Loss of genetic material is more common than gain in acute myeloid leukemia with complex aberrant karyotype: A detailed analysis of 125 cases using conventional chromosome analysis and Fluorescence in situ hybridization including 24-color FISH.Genes, Chromosomes & Cancer (in press)
54. Schoch C, Haferlach T, Sauerland MC, Staib P, Schnittger S, Grüneisen A, Wuchter C, Lengfelder E, Zühlsdorf M, Ludwig W-D, Büchner T, Hiddemann W (1999) Prognostic significance of chromosome aberrations in therapy-associated acute myeloid leukemia. Blood 94, Suppl.1:273a
55. Schoch C, Haferlach T, Schnittger S, Kern W, Dugas M, Hiddemann W (2001) AML with recurring chromosome abnormalities as defined in the new WHO-classification: Incidence of subgroups, additional genetic abnormalities, FAB subtype and age distribution in an unselected series of 1897 cytogenetically and molecular-genetically analysed AML. Blood 98:457a-(abstr.)
56. Schoch C, Kern W, Krawitz P, Dugas M, Schnittger S, Haferlach T, Hiddemann W (2001) Dependence of age-specific incidence of acute myeloid leukemia on karyotype. Blood 98:3500
57. Schoch C, Kern W, Schnittger S, Hiddemann W, Haferlach T (2003) Karyotype is an independent prognostic parameter in therapy-related acute myeloid leukemia (t-AML): An analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. Leukemia in press:
58. Schoch C, Kohlmann A, Schnittger S, Brors B, Dugas M, Mergenthaler S, Kern W, Hiddemann W, Eils R, Haferlach T (2002) Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. Proc Natl Acad Science 99:10008-10013
59. Schoch C, Schnittger S, Kern W, Dugas M, Hiddemann W, Haferlach T (2003) Acute myeloid leukemia with recurring chromosome abnormalities as defined by the WHO-classification: incidence of subgroups, additional

genetic abnormalities, FAB subtypes and age distribution in an unselected series of 1,897 patients with acute myeloid leukemia. Haematologica 88:351-352
60. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T (2003) AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 102:2395-2402
61. Secker-Walker LM, Moorman AV, Bain BJ, Mehta AB, on behalf of the EU concerted Action 11q23 Workshop (1998) Secondary acute leukemia and myelodysplastic syndrome with 11q23 abnormalities. Leukemia 12:840-844
62. Slack JL, Arthur DC, Lawrence D, Mrozek K, Mayer RJ, Davey FR, Tantravahi R, Pettenati MJ, Bigner S, Carroll AJ, Rao KW, Schiffer CA, Bloomfield CD (1997) Secondary cytogenetic changes in acute promyelocytic leukemia - Prognostic importance in patients treated with chemitherapy alone and association with the intron 3 breakpoint of the PML gene: A Cancer and Leukemia Group B Study. J Clin Oncol 15:1786-1795
63. Swansbury GJ, Lawler SD, Alimena G, Arthur D, Berger R, van den Berghe H, Bloomfield CD, de la Chappelle A, Dewald G, Garson OM, et al (1994) Long-term survival in acute myelogenous leukemia: a second follow-up of the Fourth International Workshop on Chromosomes in Leukemia. Cancer Genet.Cytogenet. 73:1-7
64. Swansbury GJ, Slater R, Bain BJ, Moorman AV, Secker-Walker LM, on behalf of the European 11q23 Workshop (1998) Hematological malignancies with t(9;11)(p21-22;q23) - a laboratory and clinical study of 125 cases. Leukemia 12:792-800
65. Veldman T, Vignon C, Schröck E, Rowley JD, Ried T (1997) Hidden chromosome abnormalities in hematological malignancies detected by multicolour spectral karyotyping. Nat Genet 15:406-410
66. Yu M, Honoki K, Andersoen J, Paietta E, Nam DK, Yunis JJ (1996) MLL tandem duplication and multiple splicing in adult acute myeloid leukemia with normal karyotype. Leukemia 10:774-780
67. Zhang FF, Murata-Collins JL, Gaytan P, Forman SJ, Kopecky KJ, Willman CL, Appelbaum FR, Slovak ML (2000) Twenty-four-color spectral karyotyping reveals chromosome aberrations in cytogenetically normal acute myeloid leukemia. Gens, Chromosomes & Cancer 28:318-328

Subgroup Specific Therapy Effects in AML: AMLCG Data

Th. BÜCHNER, W. HIDDEMANN, W. E. BERDEL, B. WÖRMANN, C. SCHOCH, C. FONATSCH, H. LÖFFLER, T. HAFERLACH, W.-D. LUDWIG, G. MASCHMEYER, P. STAIB, L. BALLEISEN A. GRÜNEISEN, C. AUL, E. LENGFELDER, R. HEHLMANN, W. KERN, H. L. SERVE, R. M. MESTERS, H, EIMERMACHER, N. FRICKHOFEN, J. KIENAST, A. GIAGOUNIDIS, M.-C. SAUERLAND, and A. HEINECKE
German AMLCG, Department of Medicine, Hematology and Oncology, University of Münster, Germany

Major changes in outcome by different intensity treatment have been demonstrated in favorable rather than unfavorable prognostic groups of AML patients. Thus, post-remission high versus standard dose araC produced longer remissions in CBF AML and not in other abnormal karyotypes (1). And autologous transplantation vs chemotherapy alone prolonged the relapse-free survival (RFS) in the good and not in the poor risk group according to karyotype and residual b.m. blasts after the 1st induction course (2, 3).

We addressed subgroup-specific treatment effects by two sequential trials starting in 1992 and 1999 (ongoing) involving 832 and 1094 pts 16–81 years of age with 36% and 50% 60 years of age and older whose diagnoses were de novo AML and in the 1999 trial also sAML (after cytotoxic therapy or MDS) and high-risk MDS. By multivariate analysis in both trials we equally identified unfavorable karyotype, age 60+ Y, high serum LDH, and high day 16 b.m. blasts as independent risk factors. Therapeutic regimens were standard dose TAD and high-dose araC/mitox (HAM) for induction, TAD for consolidation, one S-HAM for intensive consolidation and reduced TAD courses monthly during 3 years for maintenance.

In the 1992 trial 832 patients 16–81 (median 54) years of age with de novo AML were randomized upfront to receive standard dose TAD and high-dose araC 3 (1 in 60+ Y) g/m^2×6 / mitoxantrone 10 mg/m^2×3 (HAM) for induction, TAD consolidation and either monthly maintenance by reduced TAD courses for three years or one course of S-HAM with araC 1 (0,5 in 60+ Y) g/m^2×8/mitoxantrone 10 mg/m^2×4 instead of maintenance. S-HAM exhibited considerable myelotoxicity by a median neutrophil and platelet recovery time of 6 weeks. According to intent-to-treat, the relapse-free survival (RFS) at 3 years is 39% in the maintenance and 27% in the S-HAM arm (p=0.012). A combined poor risk group included patients 60+ Y of age or those with LDH ≥700 U or with unfavorable karyotype or with day 16 blasts ≥40% and was associated with a RFS of 23% at 3 years. The good risk group in contrast included patients with documented complete absence of any of the poor risk factors by complete data and showed a RFS of 46% at 3 years (p<0.0001). The rest of the patients amounting to 21% of the population was not classifiable as poor or good risk. In the combined poor risk group the 5 year RFS was 24% in the maintenance arm and 12% in the S-HAM consolidation arm (p=0.0061), while in the good risk group the corresponding RFS was 30% and 47% (p=0.28). When only the 20% good risk patients are excluded the entire rest of patients shows a benefit from maintenance in their RFS (p=0.0005) (fig. 1) and their survival (p=0.0085) (fig. 2) (4).

In the 1999 trial we are currently investigating dose effects of induction treatment on the outcome of patients in different prognostic groups according to multiple risk factors. 1094 patients 16–1 (median 60) years of age have been entering the trial and randomized to either HAM-HAM with high-dose araC 3 (1 in ≥60 Y) g/m^2×6 or TAD-HAM for induction. Both arms are balanced for age </≥60 Y, diagnosis de novo/secondary AML and MDS, karyotype favorable/intermediate/unfavorable, and LDH </≥700 U. Furthermore, the two induction arms are balanced for the upfront randomized G-CSF priming yes or no, and for prolonged maintenance or autologous transplantation. Significant differences in the relapse-free survival (RFS) between the two in-

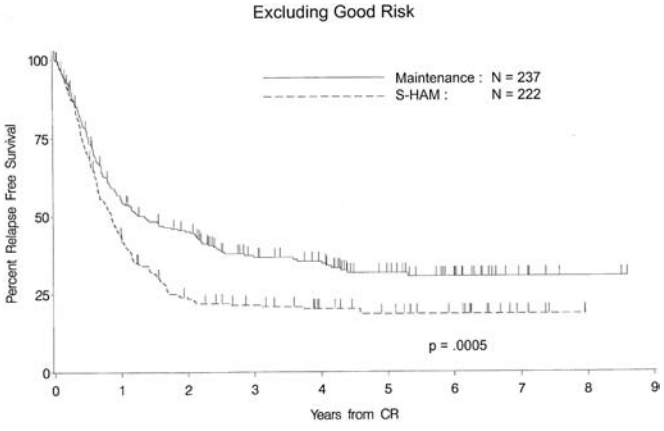

Figure 1: AMLCG 1992 trial: RFS according to maintenance versus S-HAM excluding the 20% patients in the good risk group

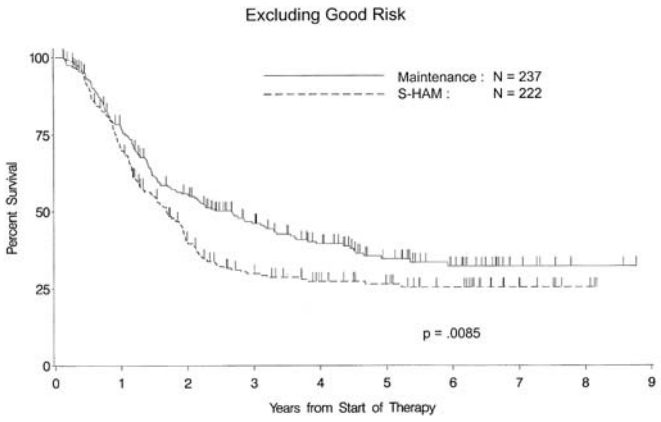

Figure 2: AMLCG 1992 trial: Survival of CR patients according to maintenance versus S-HAM excluding the 20 % patients in the good risk group

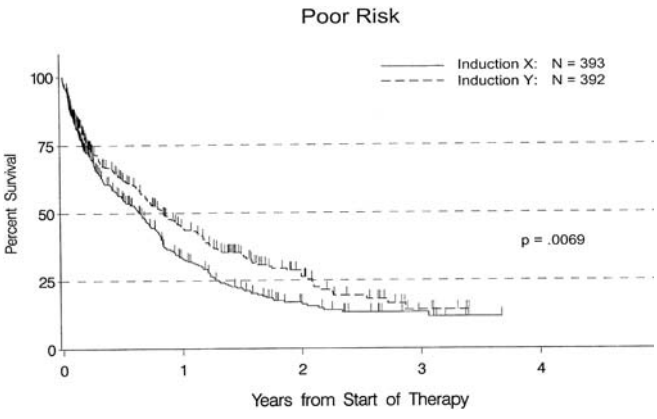

Figure 5: AMLCG 1999 trial: Survival of all patients entering according to induction by TAD-HAM versus HAM-HAM (blinded) in the combined poor risk group

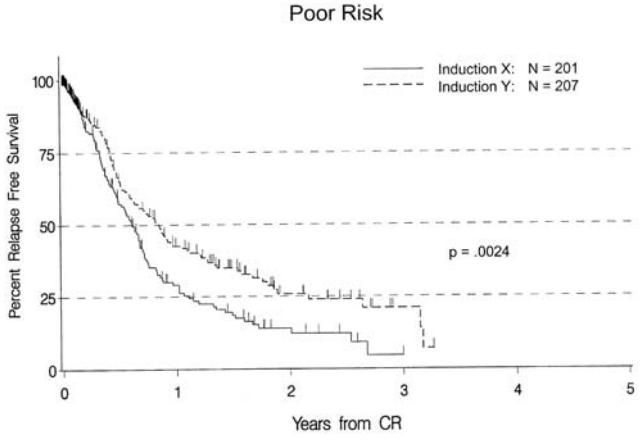

Figure 3: AMLCG 1999 trial: RFS according to induction by TAD-HAM versus HAM-HAM (blinded) in the combined poor risk group

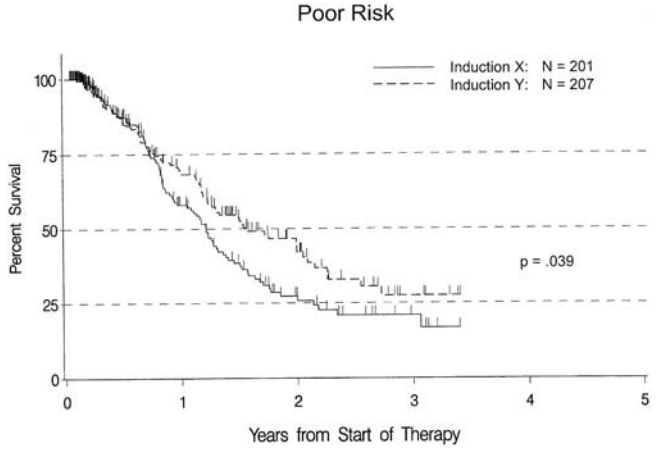

Figure 4: AMLCG 1999 trial: Survival of CR patients according to induction by TAD-HAM versus HAM-HAM (blinded) in the combined poor risk group

duction regimens X and Y (blinded) are seen in older (p=0018) rather than younger patients, and those with high (p=00033) rather than low LDH, while neither patients with favorable nor those with unfavorable karyotype contributing 10% and 17% to the patients show these differences. Based on a multivariate analysis a combined poor risk group was defined including age 60+ Y or unfavorable karyotype or LDH ≥700 U or day 16 bone marrow blasts ≥40%, while in a combined good risk group documented absence of any of the poor risk features by complete data was required. In the combined poor risk group representing 69% of the patients induction X vs Y resulted in a superior RFS (p=0.0024) (fig. 3) while in the combined good risk group (29% patients) this difference in RFS is not seen. Similarly to RFS, differences in favor of induction X versus Y are found in the survival of patients entering and the survival of patients attaining CR in those of 60+ Y (p=0.031/0.034), patients with LDH 700+ U (p=0.0054/.039), and patients in the combined poor risk group (p=0.0069/0.038) (figs. 4 and 5).

We conclude that considering multiple risk factors a poor rather than a good prognosis in patients with AML can be improved by adequate treatment.

References:

1. Bloomfield CD, Lawrence D, Byrd JC et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 58:4173, 1998
2. Burnett AK, Goldstone AH, Stevens RM et al. Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 trial. UK Medical Research Council Adult and Children's Leukaemia Working Parties. Lancet 351:700, 1998
3. Burnett AK. Treatment of acute myeloid leukaemia in younger patients. Best Practice and Research Clin. Haematol. 14:95, 2001
4. Büchner T, Hiddemann W, Berdel WE et al. 6-Thioguanine – AraC – Daunorubicin (TAD) and High-Dose AraC – Mitoxantrone (HAM) for Induction, TAD for Consolidation and either Prolonged Maintenance by reduced monthly TAD versus TAD-HAM-TAD and one course of intensive Consolidation by Sequential HAM in Adult Patients at All Ages with De Novo Acute Myeloid Leukemia (AML). A Randomized Trial by the German AML Cooperative Group. J Clin Oncol (in press)

AML Studies of the East German Study Group – OSHO

D. NIEDERWIESER, U. HEGENBART, C. BECKER, R. KRAHL, W. PÖNISCH, L. UHAREK, H. AL ALI, P. MOOSMANN, C. GENTILINI, U. WEDDING, F. FIEDLER, R. PASOLD, M. FREUND, R. SUBERT, D. KÄMPFE, J. MERAN, M. HEROLD, N. PETER, W. KNAUF, E. KETTNER, W. SCHULTZE, K. DACHSELT, H.H. WOLF, G. SCHOTT, M. SCHULZE, T. ITTEL, M. ASSMANN, N. GROBE, R. HUHN, J. STEGLICH, V. SCHIRMER, C. KLINKENSTEIN, J. DOEPPER, C. WINKELMANN, and W. HELBIG
for the East German Hematology and Oncology Study Group (OSHO), Leipzig, Germany

The East German Study group (OSHO), founded in 1983 in the former East Germany (DDR), has a long tradition in performing clinical studies on patients with AML. To date almost 1500 patients from 36 hospitals have been included in six AML protocols. The strength of the study group has been the previously nationwide, and now in the new German countries still is, the complete consecutive reporting of patients with AML and other hematological malignancies using a centralized reviewing system. The following protocols dealt with AML in younger (<60 a) patients: AML 85, AML 89, AML 91, AML 93 and AML 96. In the first two studies the role of autologous and allogeneic hematopoietic stem cell transplantation was investigated. A superiority of allogeneic HSCT compared to autologous HSCT and conventional chemotherapy has been proven**.** The effect of Granulocytes colony stimulating factor (G-CSF) on neutrophil recovery, on a possible reduction of therapy associated morbidity and mortality, on the feasibility of dose intensification and on relapse was investigated in the AML 91 and 93 studies**.** The results of the AML 91 protocol showed that G-CSF accelerates reconstitution without increasing the relapse rate. AML 93 investigated the role of priming on treatment results showing no difference in CR and DFS.

Starting in 1996 a new study generation was initiated taking into consideration pharmacokinetic measurements of Ara-CTP. Intracellular Ara-CTP formation is saturated at much lower infusion rates than used in HIDAC schedules, probably causing cytarabine accumulation in the plasma and increased toxicity. It was our objective to investigate in a phase II study feasibility and efficacy of intermediate doses of cytarabine (IDAC) delivered at the presumptive saturating infusion rate over a prolonged period of time. Forty previously untreated AML patients with de novo AML received intermediate doses of cytarabine (2 to 4 g/m^2/d) at moderate infusion rates (250 to 667 mg/m^2/h/d) and prolonged infusion over 6 or 8 hours. Cytarabine was applied in combination with an anthracycline on alternate days (day 1,3,5,7). Thirty-two of the 40 patients (80%, 95%CI: 64%–91%) achieved CR after induction treatment. TRM during induction chemotherapy was only 2.5%. After two to four IDAC courses, stem cell harvesting was successful in 71% of the patients eligible for HDCT. After two years 62% (95%CI: 46%–78%) of all patients were alive and 57% (95%CI: 35%–79%) of the patients who entered CR were free of leukemia. Using this experience a phase III study comparing intermediate doses of AraC given in two different schedules in combination with Idarubicin or Mitroxantrone was initiated. In addition the use of autologous, related and in high-risk patients the use of unrelated HSCT was intensified. After recruitment of 360 patients with the novo and secondary AML, the complete remission rate reached 68% (CI 95%; 63%–73%) in both arms with an early death rate of 10% (7%–14%). Multivariate analysis indicated that cytogenetics and presence of prior MDS or secondary AML were significant prognostic parameters influencing response rates, irrespective of treatment allocation. A longer follow up is necessary before definitive conclusions can be drawn with regard to the two treatment modalities. The accrual was terminated in 2003 and more follow up is needed for looking at the long term results.

Along this line, a new AML study (AML 2002) was started in patients below the age of 60 years investigating the role of optimised AraC infusion in comparison to the standard arm of the German AML Intergroup. Furthermore the question of improving the CR rate by using MITO/Flag compared to Mitoxantron/AraC treatment is raised. Finally the role of one in comparison to two consolidation therapies before autologous or allogeneic HSCT is investigated. For patients with unfavorable karyotyp (–5/5q, –7/7q, abnormal (11q23) and complex karyotyp) early allogeneic related or unrelated stem cell transplantation after induction therapy is tested. More than 90 patients have been entered so far in this study.

In elderly patients (over the age 60 years) each patient is registered in the AML 97 study first and, according to the clinical situation, treated according to one of three arms with either curative, palliative or supportive intention. Since March 1998, a total of 432 patients were enrolled in these three protocols (curative 320, palliative 80 and supportive 32 patients). Excellent results have been obtained in the curative arm using an AraC and Mitoxantron combination. CR were obtained in 75,7% (68%–81%) of patients with de novo AML and in 60,0% (51%–68%) in patients with secondary AML with an early death rate of 11,4% (7%–17%) and 17,5% (12%–25%) respectively. Cytogenetics at diagnosis were again the most important prognostic factor for CR ($p<0,0005$, multivariate analysis). 100% of the patients with favourable, 81,2% with normal, 78,1% with other aberrations and only 45,6% with unfavourable karyotyp achieved CR. The event free survival (EFS) at 2 years, however, was 0,19±0,04% for patients with de novo and 0,15±0,05% for patients with secondary AML. OS according to favourable, normal and unfavourable cytogenetics was 52%, 30% and 11% respectively. The median survival for patients treated with the palliative protocol was 52 days and the median survival for patients with supportive care 11 days.

In order to improve postremission therapy in older patients or younger patients with contraindications to conventional transplants, a protocol using allogeneic related or unrelated stem cell transplant was designed and tested within the Seattle consortium. More than 100 patients have been entered in this protocol using Fludarabine and 200 TBI pretransplant in addition to cyclosporine and mycophenolate mofetil posttransplant so far. Sustained allogeneic engraftment was obtained in 95,6% of the patients with related and 93% of the patients with unrelated donors. For patients in CR1 OS of 67%±14% for unrelated and 45%±13% for related transplants was reached. This protocol is now open for all patients over the age of 60 years and will be tested in a randomised fashion.

P-Glycoprotein (Pgp) Modulation in Untreated Acute Myeloid Leukemia (AML): Cancer and Leukemia Group B (CALGB) Trials in Younger and Older Adults

J.E. KOLITZ [1], S.L. GEORGE, M.R. BAER, E.J. LEE, C.D. BLOOMFIELD, and R.A. LARSON
Cancer and Leukemia Group B, Chicago, IL, USA

In 1994, the CALGB began a series of trials for patients (pts) with untreated AML aimed at establishing the toxicity and efficacy of induction chemotherapy regimens incorporating pharmacologic inhibition of Pgp, a transmembrane, ATP-dependent pump encoded by *MDR-1.* The role of Pgp in primary drug resistance and treatment failure in AML has been well documented [1]. Pgp, along with other drug resistance mechanisms, is especially prevalent in AML occurring in older patients and in refractory disease [2] . Furthermore, there are data pointing to a primary role for Pgp with respect to drug resistance in AML stem cells [3].

A phase 3 trial evaluating the effect of cyclosporine A (CsA), a first generation Pgp-inhibitor, in relapsed AML showed a significant advantage of the modulator-containing induction regimen over the chemotherapy regimen given alone with respect to disease-free (DFS) and overall (OS) survival [4]. PSC-833 (P), a non-immunosuppressive CsA analogue with substantially greater *in vitro* potency as a Pgp inhibitor, was chosen for study within CALGB. While these trials were being performed, other investigators established that Pgp-susceptible agents, including the anthracyclines, epidophyllotoxins, and taxanes, had to be dose-reduced by 25%–66% in order to be combined safely with PSC-833, and that greater reductions were needed when Pgp-susceptible agents were used in combination [5, 12]. Dose-limiting toxicities (DLT) generally consisted of mucositis and hepatic dysfunction, especially hyperbilirubinemia. The CALGB trials have evaluated concurrent administration of daunorubicin (D) and etoposide (E), along with ara-C (A) without (ADE regimen) and with P (ADEP) in order to utilize two active agents subject to Pgp-mediated efflux. Furthermore, rather than perform classic phase I trials, parallel phase I trials were performed separately both in older (CALGB 9420) [6] and younger adults (CALGB 9621) [7]. Large numbers of patients were enrolled in successive treatment groups in which D or E was dose-escalated or reduced in an attempt to establish ADE and ADEP induction regimens that were associated with comparable clinical toxicity and efficacy so that subsequent phase III trials could be performed in both age populations. The large number of patients enrolled contributed to a precision in the estimation of the equitoxic doses that could not otherwise be achieved. Finally, a decision was made not to establish pharmacokinetic equivalence between regimens, in part because drug serum levels may not be indicative of intracellular drug concentrations in the presence of modulators of drug efflux.

The 9420 and 9621 phase I induction regimens are outlined in Table 1. D and E are given daily for 3 days by short i.v. infusion; A is given by c.i.v. over 7 days. The P dose is 2.8 mg/kg i.v. loading dose followed by a 72 hour c.i.v. at 10 mg/kg/day overlapping the D and E administration. The table includes the initial doses of D and E and the final doses chosen for phase III testing (in boldface), and the direction of dose escalation and/or de-escalation (arrows), but doesn't include the multiple intervening treatment groups which were evaluated. Altogether, seven dose cohorts were studied in older patients and 15 cohorts in younger patients to derive the appropriate phase III regimens to test.

The phase 3 trial comparing ADE and ADEP in older pts (CALGB 9720) was halted after enrollment of 120 pts because of excess early mortality in pts treated with P [8]. CR rates were 46% for ADE and 39% for ADEP (p=0.008), but median DFS (7 vs. 8 months) and OS (33% at 1 yr) were nearly identical. The observed excess toxicity in that study, coupled with the substantial tolerance for escalated doses of D in younger pts, led to the expansion of 9621 to include 410 pts and the determination of equitoxic phase 3 doses of D for the subsequent comparative study (CALGB 19808) that were 50% higher in ADE and equal in ADEP to those used in older pts in 9720. The E dose used in ADEP in 19808 was reduced by 33% from that used in 9720.

In both phase I studies, mucositis and hepatotoxicity proved dose-limiting in ADEP. Diarrhea was also a DLT in the older pts. In the younger pts, excess cardiotoxicity was not observed in pts receiving higher than conventional doses of D (up to 95 mg/m^2/day).

Outcomes with respect to achievement of complete remission (CR), DFS and OS among pts treated with either ADE or ADEP on CALGB 9420 and 9720 are roughly comparable to historical controls [6]. Among younger pts treated on 9621, the CR incidence in both ADE and ADEP (about 80% in both regimens) has been higher than the prior CALGB experience. In addition, the percentage of pts achieving CR with one induction using ADEP has been over 90% [7 and unpublished data].

Because relapse of AML is likely due to drug resistance in leukemic stem cells, we hypothesized that the benefit of an MDR-modulator would lead to prolonged DFS, and that this would be most apparent in younger pts who characteristically have lower levels of Pgp expression. A trend has emerged from the non-randomized phase I 9621 trial favoring the use of ADEP with respect to DFS in pts ≤45 yrs with a three-fold increase in DFS as compared to ADE, but this remains to be confirmed by analysis of the randomized trial 19808.

Table 1. Induction regimens. Doses are in mg/m^2/day.

	CALGB 9420 (110 pts age ≥60)		CALGB 9621 (410 pts ages 15–59)	
	ADE	ADEP	ADE	ADEP
A	**100**	**100**	**100**	**100**
D	30→**60**	30→**40**	60→95→**90**	40→50→**40**
E	**100**	100→**60**	100→150→**100**	60→**40**
P	0	+	0	+

The phase 3 comparison of ADE and ADEP in younger pts (19808) completed accrual in August, 2003 after 300 pts had been registered, because P was no longer available. Relevant endpoints continue to be tabulated in that trial to see if differences emerge in favor of Pgp modulation during induction, particularly among the younger pts who may be less likely to utilize multiple drug resistance pathways. Additional pts are accruing onto that trial using the ADE induction therapy (doses of 100, 90, and 100 mg/m^2/day, respectively) in order to complete a phase 3 evaluation of post-remission/consolidation immunotherapy with interleukin-2 compared to observation.

An important component of these trials has been *in vitro* measurements of P-modulatable dye ($DiOC_2(3)$) efflux within pre-treatment AML blasts and from samples obtained at relapse. Among pts receiving ADE, those whose pre-treatment blasts exhibited P-modulated dye efflux had significantly lower CR and OS rates than those without such efflux. Despite studying only a small numbers of pts, those with P-modulated dye efflux had a longer DFS (median, 14 months) when treated with ADEP rather than with ADE (5 months, p=0.07) [8]. These findings raise the possibility of individualizing remission induction therapy according to pretreatment measurements of drug resistance.

Although P has become unavailable, third generation Pgp modulators are now entering clinical trials. The CALGB plans to evaluate the pipecolinate derivative VX-710 (biricodar, Incel), a multipotent modulator that inhibits Pgp, Multidrug Resistance Protein (*MRP*) and Breast Cancer Resistance Protein (*BCRP*) and significantly inhibits drug efflux from AML cells [9]; it also lacks pharmacokinetic interactions with drug substrates [10]. Beyond inhibition of drug efflux, pharmacologic blockade of Pgp may enhance apoptosis, a mechanism which can be independently exploited using pro-apoptotic agents [11]. The role of strategies aimed at inhibiting Pgp and other drug resistance mechanisms continues to be defined in the treatment of AML.

References

1. Wuchter C, Leonid K, Ruppert V, et al (2000): Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. Haematologica 85:711-721.
2. Leith CP, Kopecky KJ, Godwin J et al. (1997). Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 89: 3323-3329.
3. te Boekhorst PA, Lowenberg B, van Kapel J et al. (1995). Multidrug resistant cells with high proliferative capacity determine response to therapy in acute myeloid leukemia. Leukemia 9:1025-1031.
4. List AF, Kopecky KJ, Willman CL et al. (2001) Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. Blood 98:3212-3220.
5. Advani R, Saba HI, Tallman MS et al. (1999) Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833 (Valspodar). Blood 93: 787-795.
6. Lee EJ, George SL, Caligiuri M et al. (1999) Parallel phase I studies of daunorubicin given with cytarabine and etoposide with or without the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age or older with acute myeloid leukemia: results of cancer and leukemia group B study 9420. J Clin Oncol 17: 2831-2839
7. Kolitz JE, George, S.L., Dodge, R.K., et. al. (2001) Dose escalation studies of ara-C, daunorubicin and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia < 60 years: final induction results of CALGB 9621. Blood 98: 461a.
8. Baer MR, George SL, Dodge RK et al. (2002) Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B Study 9720. Blood 100:1224-1232.
9. Baer M, Suvannasankha A, O'Loughlin, KL, et. al. (2002) The pipecolinate derivatives VX-710 (biricodar, Incel) and VX-853 are effective modulators of drug efflux mediated by the multidrug resistance proteins P-gly-

coprotein, multidrug resistance protein and breast cancer resistance protein in acute myeloid leukemia cells. Blood 100:67a.
10. Rago RP, Einstein A, Jr., Lush R et al. Safety and efficacy of the MDR inhibitor Incel (biricodar, VX-710) in combination with mitoxantrone and prednisone in hormone-refractory prostate cancer (2003). Cancer Chemother Pharmacol 51: 297-305
11. Pallis M, Turzanski J, Grundy M, Seedhouse C, Russell N. (2003): Resistance to spontaneous apoptosis in acute myeloid leukaemia blasts is associated with p-glycoprotein expression and function, but not with the presence of FLT3 internal tandem duplications. Br J Haematol 120:10091016.
12. Sonneveld P, Burnett A, Vossebeld P et al. (2000) Dose-finding study of valspodar (PSC 833) with daunorubicin and cytarabine to reverse multidrug resistance in elderly patients with previously untreated acute myeloid leukemia. Hematol J 1:411-421.

Treatment of Acute Myeloid Leukemia Younger Adults: The GOELAM Experience

J.-L. HAROUSSEAU
Centre Hospitalier Regional et Universitaire des Nantes, France

In the past fifteen years the GOELAM group has initiated three randomized trials for acute myeloid leukemia (AML) therapy in younger adults (up to 60 years of age).

The GOELAM1 Trial

In the GOELAM1 study (1987-1994) the main question was the comparison of autologous bone marrow transplantation (ABMT) and intensive consolidaton chemotherapy (ICC) as post-remission therapy.

The results of this trial were published in 1997 (1) and updated in 2001 (2).In this trial patients aged 15 to 50 with de novo AML (all FAB subtypes including M3) were eligible. Patients with a previous myelodysplasia for more than 3 months were excluded but patients with an antecedent of unexplained cytopenia could be included. Also excluded were patients with a myeloproliferative disorder in blast crisis, patients who had previously received chemotherapy or radiotherapy, patients with clinical or electrocardiographic signes of heart failure or coronary disease and patients with hepatic or renal failure.

The induction treatment consisted of ARA-C (200mg/m^2/D CI on days 1–7) with either Idarubicin (IDR) (8mg/m^2/D IV on days 1–5) or Rubidazone (RBZ) (200mg/m^2/D IV on days 1–4).A bone marrow aspiration was performed on day 17. If the marrow was hypoplastic and nonblastic, no further treatment was administered. If the marrow was hypoplastic and contained less than 50% blasts, a second induction course was administered with a combination of three days of ARA-C and two days of the anthracycline initially allocated. An alloBMT was offered to all patients aged 40 and younger and with an HLA identical sibling. All other patients aged 51 and younger were assigned to receive a first course of ICC consisting of HD ARA-C (3 g/m^2) given as a 3 hour infusion every 12 hours, days 1–4 combined with either IDR (10 mg/m^2/D, days 5–6) or RBZ (200 mg/m^2/D days 5–6). Bone marrow was harvested after the first course of ICC without any in vitro manipulation. All patients still in CR were randomly assigned to receive a second course of ICC or an autoBMT. The second course of ICC consisted of m-Amsa (150 mg/m^2/D days 1–5) and etoposide (100 mg/m^2/D days 1–5). The conditioning regimen prior to ABMT consisted of busulfan (4 mg/kg/D p.o. for 4 consecutive days) and cyclophosphamide (50 mg/kg/D IV for 4 consecutive days).

Overall results. From November 1987 to May 1994, 517 eligible patients were included by 16 centers. Of the 503 patients evaluable for induction treatment, 366 (73%) achieved CR. With a median follow-up of 4 years, the 4-year actuarial DFS of the 366 patients who achieved CR was 39%. The median overall (OS) survival of the 517 eligible patients was 21 months and the 4-year actuarial survival was 39%. The outcome was not significantly different between the two induction treatment arms (IDR or RBZ).

Post remission therapy. Eighty-eight patients under the age of 41 who had an HLA identical sibling but only 73 patients underwent an alloBMT according to the protocol. Their 4-year DFS was 47% and the 4-year OS was 54%. The actuarial risk of relapse at 4 years was 37%.

We compared the outcome of the 88 patients for whom the intention was to perform an alloBMT with the 134 patients up to the age of 40 without an HLA-identical sibling for whom the intention was to perform another type of intensive consolidation therapy. The 4-year DFS was 42% for allo BMT and 40% for intensive consolidation (p=0.54). The 4-year OS was 51% for allo BMT and 52.5% for intensive consolidation (p=0.63).

Comparison of intensive consolidation chemotherapy and autoBMT (updated results). Out of the 237 patients who underwent the first course of ICC, only 164 patients were randomized according to the protocol (86 auto BMT, 78 ICC). With a median follow-up of 80 months, the 6-year DFS and OS were 44% and 52% respectively for the 75 patients who actually underwent auto BMT. For the 71 patients who underwent ICC2 as assigned, the 6-year DFS and OS were 38% and 55% respectively.

Comparison between the two post-remission strategies was made on an intent-to treat basis. There was no significant difference concerning initial prognostic factors between the 86 patients randomly assigned to auto BMT and the 78 patients randomly assigned to ICC. The results are in Table 1.

Table 1. GOELAM1 trial. Comparison between autologous bone marrow transplantation and intensive consolidation chemotherapy. Interet to treat analysis

	ABMT N=86	ICC N=78	p-value
6-year DFS	44%	38%	0.47
6-year OS	50%	51%	0.75
Toxic death rate	13%	3%	
Relapse rate	48%	60%	0.11

We concluded that after a first course of ICC ABMT is not superior to a second course of ICC.

The GOELAM2 Trial

The results of this trial have been published in 2003 (3).For this trial, considering the results of the GOELAM1 protocol, we decided to stop auto BMT. The objective of the GOELAM2 trial (1995–1999) was to improve the results of chemotherapy by using quinine as a p-glycoprotein modulator.

We used quinine for a variety of reasons. Serum from patients receiving conventional doses of quinine by intravenous infusion reverses the MDR phenotype of various tumor cell lines (4). Quinine is safe when combined with mitoxantrone and cytarabine for treating acute leukemia patients (5). This compound does not significantly improve the response rate of refractory and relapsed acute myelogenous leukemias (6) but increases the response of P-glycoprotein-expressing myelodysplastic syndromes to a mitoxantrone-Ara-C combination (7). There was a second randomised question concerning the interest of G-CSF given after consolidation chemotherapy (8). The inclusion criteria were the same as in the GOELAM1 trial except the upper age limit (60 instead of 50 years) and the exclusion of patients with M3 FAB subtype (who were treated with ATRA-containing regimen). The induction treatment was the IDR arm of the GOELAM1 protocol. Patients were randomly assigned to receive or not a continous intravenous infusion of quinine (30 mg/kg/day), starting 12 hours before the first dose of IDR and ending 12 hours after the end of the last IDR infusion. A bone marrow aspiration was performed on day 20. If the marrow contained $\geq$ 20% blasts, patients received a combination of Ara-C (3 g/m^2 on a 3-hour infusion every 12 hours) days 1–4 (total dose 24 g/m^2) and mitoxantrone (12 mg/m^2/d) days 5–6. According to the initial randomization, patients also received quinine at the initially defined dose on days 4 through 6.

Patients in CR after 1 or 2 courses of intensive induction who were aged 45 years or younger with an HLA-identical sibling were proposed allogeneic BMT. The conditioning regimen included total body irradiation (12 Gy, 6 fractions from day -6 to day -4), cyclophosphamide (60 mg/kg/d, day -3 to day –2) and granulocyte colony-stimulating factor (G-CSF) (5 μg/kg/d day –6 to day –1). All other patients received the same 2 courses of ICC as in the GOELAM1 trial. In patients randomly assigned to receive quinine, the MDR-reversing agent (30 mg/kg/day by continuous intravenous infusion) was administered from day 4 to day 6 during ICC1 and from day 1 to day 6 during ICC2. During the first part of the study, patients also were randomly assigned to receive or not receive G-CSF (5 μg/d) from the day after chemotherapy until granulocyte recovery throughout the 2 courses of ICC. As reported previously (8), intermediate analysis demonstrated that G-CSF significantly reduced the duration of neutropenia and hospitalization. Subsequently, G-CSF was administered to all the patients who received ICC.

Bone marrow samples obtained at diagnosis were shipped by express mail to one of 3 reference centers. Blasts cells were separated on fycoll-hypaque and cryopreserved. Frozen samples were distributed among the 3 reference centers were the following evaluations were performed : MDR1 gene expression by RT-PCR, p-glycoprotein function by rhodamine efflux. Moreover quinine serum concentrations and MDR-reversing activity of serum samples were determined.

Overall Results. From February 1995 to January 1999, 425 eligible patients were randomised by 17 centers (213 quinine, 212 control). CR was achieved in 344 patients (81%) with no significant difference between the two arms. While MDR1 gene and p-glycoprotein expression did not significantly influence CR rate in the two groups of treatment, quinine significantly increased the CR rate in 54 patients with rhodamine efflux (83% CR versus 48%, p=0.01). In 106 patients whose blasts did not demonstrate any rhodamine efflux, quinine did not modify the CR rate. The 4-year OS of the 425 eligible patients was 42% and the 4-year DFS of the 344 patients in CR was 43% without significant difference between patients with or without quinine.

Post remission therapy. Allo BMT: An HLA-identical sibling was identified in 82 of 207 patients aged 45 years or younger who achieved CR. Of those patients, 73 received the planned allo BMT. In intention to treat analysis, we compared these 82 patients to 125 patients in the same age category and without an HLA-identical sibling. The 4-year DFS was significantly better in the allo BMT group (58% versus 42% p=0.018) while the benefit on 4-year overall survival did not reach statistical significance (60% vs 48%) (p=0.27).

Impact of quinine. Out of 262 patients assigned to ICC, 231 received the first course and 194 patients received the second course. Quinine did not influence the 4-year DFS (39% versus 38%) nor the 4-year OS (45% vs 47%) (p=0.91). Again, while MDR1 gene and p-glycoprotein expression did not significantly impact DFS or survival in the two groups, quinine appeared to improve the EFS and the OS in patients with rhodamine efflux. However, due to small numbers of patients, the differences did not reach statistical significance.

Conclusion. We concluded that 1) quinine did not improve the outcome althought it improved CR rate in a small subgroup of patients defined by rhodamide efflux. 2) Allogeneic was superior to ICC in this trial.

Table 2. Overall results of GOELAM1 and GOELAM2 trials

	Nb of patients	Age (median)	Inclusion criteria	CR rate	4-year DFS	4-year OS
GOELAM1	517	15–50	all FAB subtype	73%	39% (N=366)	39% (N=517)
GOELAM2	425	15–60	M3 Excluded	81%	43% (N=344)	42% (N=425)

Table 3. Results of allogeneic BMT in the GOELAM1 and GOELAM2 trial (intention to treat analysis)

	DONOR		NO DONOR	
	GOELAM1* N=87	GOELAM2** N=82	GOELAM1* N=134	GOELAM2** N=125
4-year DFS	42%	58%	40%	42%
4-year OS	51%	60%	52.5%	48%

Summary of GOELAM1 and GOELAM2 trials. The overall results of the GOELAM1 and GOELAM2 trials are shown in Table 2. Although the inclusion criteria would have favored GOELAM1 trial (upper age limit 50 instead of 60 and inclusion of M3 FAB subtype) the CR rate was higher in the GOELAM2 trial (83% versus 71%). When restricting the analysis to comparable patients (15-50 years , M3 excluded) the 4-year EFS was 38% in the GOELAM2 trial versus 29% in the GOELAM1 trial (p=0.001). The better outcome in the GOELAM2 trial appears in fact to be related to much better results of allo BMT while there was apparently no improvement for patients without an HLA identical sibling (Table3).

In the ongoing LAM 2001 study two questions are being addressed : comparison of idarubicin and high-dose daunorubicin during induction treatment and comparison of single versus double autologous peripheral stem cell transplantation as post-remission therapy.

References

1. Harousseau JL, Cahn JY, Pignon B et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia: The Groupe Est Leucemies Aigues Myeloblastiques (GOELAM). Blood 1997; 90:2978-2986
2. Harousseau JL, Pignon B Chevallier MP et al. Comparison of intensive chemotherapy and autologous transplantation as post remission therapy in adult acute myeloid leukemia. Update of the GOELAM1 trial, in Acute Leukemias VIII. Ed T Büchner, W Hiddemann, B Wörmann, G Schellong, J Ritter, U Creutzig. Springer 2001; 660-664
3. Solary E, Drenou B, Campos L et al. Quinine as a multidrug resistance inhibitor : a phase 3 multicentric randomized study in adult de novo acute myelogenous leukemia. Blood 2003; 102:1202-1210
4. Solary E, Velay I, Chauffert B et al. Sufficient levels of quinine in the serum circumvent the multidrug resistance of the human leukemic cell line K562/ADM. Cancer 1991; 68:1714-1719
5. Solary E, Caillot D, Chauffert B et al. Feasibility of using quinine, a potential multidrug resistance-reversing agent, in combination with mitoxantrone and cytarabine for the treatment of acute leukemia. J Clin Oncol 1992; 10:1730-1736
6. Solary E, Witz F, Caillot D et al. Combination of quinine as a potential reversing agent with mitoxantrone and cytarabine for the treatment of acute leukemias : a randomized multicentric study. Blood 1996; 88:1198-1205
7. Wattel E, Solary E, Hecquet B et al. Quinine improves the results of intensive chemotherapy in myelodysplastic syndromes expressing P glycoprotein: results of a randomized study. Br Haematol 1998; 102:1015-1024
8. Harousseau JL, Witz B, Lioure B et al.Granulocyte colony-stimulating factor after intensive consolidation chemotherapy in acute myeloid leukemia: results of a randomized trial of the Groupe Ouest-Est Leucemies Aigues Myeloblastiques. J Clin Oncol 2000; 18:780-787

Main Session V

AML in Older Patients: Current Approach of HOVON-SAKK Cooperative Group

B. LÖWENBERG
Erasmus University Medical Center, Rotterdam, The Netherlands

The results of treatment of patients of older age (eg above 60 yrs) with acute myeloid leukemia have remained unsatisfactory in spite of improvements in supportive care, better remission induction chemotherapy programs and the introduction of high dose cytotoxic therapy coupled to hematopoietic stem cell transplantation. The reasons for the overall unsatisfactory outcome relate to unfavorable disease intrinsic features and age related patient factors and co-morbidity interfering with adequate treatment delivery. Future developments clearly depend on a better recognition of the molecular heterogeneity of AML allowing for risk adapted treatment as well as on the introduction of new modalities of therapy. The Dutch-Belgian-German HOVON Cooperative Group in part in collaboration with partner groups has invested in evaluating modified remission induction schedules and post induction regimens. In this presentation an overview will be presented of the HOVON rationale and approach.

Age and the Nature of Acute Myeloid Leukemia

F. R. APPELBAUM and H. M. GUNDACKER
Fred Hutchinson Cancer Research Center and University of Washington School of Medicine, Seattle, Washington, USA

Introduction

The characteristics of acute myeloid leukemia (AML) change with the age of the patient. As we and others have noted, AML in older individuals, when compared to the disease in younger patients, more often is preceded by a myelodysplastic phase, is more likely to have unfavorable cytogenetics, more frequently expresses multi-drug resistance (MDR), and responds less well to chemotherapy.[1, 2] While these categorical conclusions can be made, less is known about the extent of these associations and how they vary by decade of patient age. Thus, in this current study, we took a closer look at how AML changes with patient age.

Methods

Selected clinical and biologic features of AML in 921 patients entered onto four Southwest Oncology Group trials, two dealing with patients age <56 (S9034 and S9500) and two directed at patients age >55 (S9031 and S9333), were analyzed.[3, 4, 5, 6]

Results

Table 1 presents some of the demographics of the 921 patients. The percent of non-white patients entered on studies declined significantly with age. Contrary to expectations, the proportion of patients with de novo presentation of AML did not decrease in the very elderly (i.e. those over age 75) compared to patients age 56-65 and 66-75.

Table 2 presents hematologic characteristics of the disease at diagnosis. As shown, the only differences were a slightly higher white count and percent circulating blasts in younger patients.

Table 3 details the results of cytogenetic studies for the 769 patients with evaluable cytogenetics, first, grouped according to favorable, intermediate, and unfavorable categories, and then for selected subtypes.

As shown in Table 3, with increasing age, the proportion of patients with favorable cytogenetics including t(8;21) and inv(16), drops precipitously, while the incidence of unfavorable cytogenetics, including –5 or 5q and –7 or 7q, climbs. Also as shown in Table 4, multi-drug resistance as measured by MRK16 staining increases with age.

Finally, as shown in Table 5, complete remission rates fall with increasing age, the incidence of disease resistance as the cause of induction failure increases, and the duration of overall survival and disease-free survival shortens. However, the biggest differences are restricted to patients above and below age 55.

Table 1. Demographics

	Age			
	<56	56–65	66–75	>75
Number	382	222	249	68
Sex (% male)	53%	56%	59%	54%
Race (% white)	83%	87%	92%	90%
Presentation (% de novo)	–	78%	76%	81%

Table 2. Hematologic Values

	Age			
	<56	56–65	66–75	>75
Number	381	222	249	68
Hgb (median)	9.1	9	9.1	9.2
WBC (median)	17.7	13.7	10	12.7
PLT (median)	49	49	61	58
% peripheral blasts	39	27	24	26
% marrow blasts	70	61	67	60
% FAB				
M1	21	26	24	25
M2	33	35	32	29
M4	29	20	18	21
M5	9	9	9	10
M6	2	2	4	4

Table 3. Cytogenetic Results

	Age				P#
	<56	56–65	66–75	>75	
Number*	333	183	199	54	
Favorable (%)	20	5	5	4	}<0.0001
I ntermediate	58	55	56	44	
Unfavorable	21	39	39	52	
–5q or 5q	6	15	14	26	<0.0001
–7 or 7q	8	19	18	22	<0.0001
17p	2	9	7	11	=0.0001
t(8;21)	7	4	2	0	=0.0087
Inv (16)	9	2	4	7	=0.0011

*Includes only patients with evaluable karyotypes; # Chi-square tests using age <56 versus age >=56

Table 4. MRK Positive Leukemias

	Age			
	<56	56–65	66–75	>75
MRK+ (%)*	33%	62%	61%	57%

*Analysis restricted to S9333 and S9500: MRK+ is defined by the Kolmogorov-Smirnov D-value measurement of expression.[7]

Table 5. Treatment Outcomes

	Age			
	<56	56–65	66–75	>75
% Complete Response	64	43	38	36
% Resistant Disease	26	36	39	37
Median overall survival (mo.)	23*	9	6.6	4.2
Median disease-free survival (mo.)	18*	7.4	8.3	7.5

*The age <56 group is restricted to S9500 to avoid influence of hematopoietic cell transplantation.

Discussion

Perhaps the most striking finding of this study is that, although there is no obvious influence of age on the presenting hematologic values or morphology of AML, there is a profound effect of age on cytogenetics, the single most important predictor of treatment outcome. In addition, we saw an increase in MDR expression with age, although once patients were over age 55 no further increase was obvious. Finally, as has frequently been noted, response rates and duration markedly declined with age.

Why AML in older individuals is associated with unfavorable cytogenetics, increased MDR and worse clinical outcome is unclear. We, and others, have postulated that AML in younger patients more often results from a limited number of mutational events diminishing the diversity of leukemic subclones and leaving many cell functions, including the apoptotic apparatus, relatively intact. In contrast, AML in the elderly may more often be the result of a string of mutational events leading to multiple leukemic subclones with the opportunity to develop multiple mechanisms of chemoresistance. A slightly different hypothesis is that normal hematopoietic stem cells age, resulting in telomere shortening, increased genetic instability and the accumulation of intracellular damage. The change in the nature of AML with age may, thus, be the result of a leukemic event occurring in an older stem cell.

Whatever the reason, AML in older patients has a worse outcome following treatment. As in many other studies, the differences noted here may, in part, be due to the diminished doses of therapy given to older patients. With better supportive care, the need to lower doses in older patients is diminishing, but even when identical doses of drugs are given, older patients do worse.

The identification of high levels of MDR in AML from older individuals provides a potential target for therapy, and the Southwest Oncology Group currently is exploring initial treatment using cytarabine, continuous infusion daunomycin and cyclosporine, a regimen found to be effective in patients with relapsed disease.[7]

In addition, the use of non-myeloablative hematopoietic cell transplantation in older individuals is being studied in older patients with matched siblings.[8]

Reference List

1. Leith, C.P., Chir, B., Kopecky, K.J., Godwin, J., McConnell, T., Slovak, M.L., Chen, I.-M., Head, D.R., Appelbaum, F.R., Willman, C.L. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group Study. Blood 89: 3323-3329, 1997.
2. Leith, C.P., Kopecky, K.J., Chen, I.-M., Eijdems, L., Slovak, M.L., McConell, .S., Head, D.R., Weick, J., Grever, M.R., Appelbaum, F.R., Willman, C.L. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia. a Southwest Oncology Group study. Blood 94: 1086-1099, 1999.
3. Cassileth, P.A., Harrington,D.P., Appelbaum, F.R., Lazarus, H.M., Rowe, J.M., Paietta, E., Willman, C., Hurd, D.D., Bennett, J.M., Blume, K.G., Head, D.R., Wiernik, P.H. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N. Engl. J. Med. 339: 1649-1656, 1998.
4. Slovak, M.L., Kopecky, K.J., Cassileth, P.A., Harrington, D.H., Theil, K.S., Mohamed, A., Paietta, E., Willman, C.L., Head, D.R., Rowe, J.M., Forman, S.J., Appelbaum, F.R. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood 96: 4075-4083, 2000.
5. Godwin, J.E., Kopecky, K.J., Head, D.R., Willman, C.L., Leith, C.P., Hynes, H.E., Balcerzak, S.P., Appelbaum, F.R. A double-blind placebo-controlled trial of granulocyte colony-stimulating factor in elderly patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group Study (9031). Blood 91: 3607-3615, 1998.
6. Anderson, J.E., Kopecky, K.J., Willman, C.L., Head, D., O'Donnell, M.R., Luthardt, F.W., Norwood, T.H., Chen, I.-M., Balcerzak, S.P., Johnson, D.B., Appelbaum, F.R. Outcome after induction chemotherapy for older patients with acute myeloid leukemia is not improved with mitoxantrone and etoposide compared to cytarabine and daunorubicin: a Southwest Oncology Group study. Blood 100: 3869-3876, 2002.
7. Young IT. Proof without prejudice: use of the Kolmogorov-Smirnov test for the analysis of histograms from flow systems and other sources. J Histochem Cytochem 25:935, 1977.
8. List, A.F., Kopecky, K.J., Willman, C.L., Head, D.R., Persons, D.L., Slovak, M.L., Dorr, R., Karanes, C., Hynes, H.E., Doroshow, J.H., Shurafa, M., Appelbaum, F.R. Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. Blood 98: 3212-3220, 2001.
9. Feinstein, L.C., Sandmaier, B.M., Hegenbart, U., McSweeney, P.A., Maloney, D.G., Gooley, T.A., Maris, M.B., Chauncey, T.R., Bruno, B., Appelbaum, F.R., Niederwieser, D.W., Storb, R.F. Non-myeloablative allografting from human leucocyte antigen-identical sibling donors for treatment of acute myeloid leukaemia in first complete remission. Br. J. Haematol. 120: 281-288, 2003.

Main Session VI

Childhood AML as a Model for Therapeutic Targeting in Cancer: Are we Aiming at the Right Targets?

R. J. ARCECI
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, USA

Improvements in the outcome of children with AML have come about in large part through different approaches to dose intensification of traditional chemotherapeutic agents. The major pediatric cooperative groups developed progressively more dose intensive therapeutic approaches based on either timing or drug exposure. In addition, the role of allogeneic and autologous hematopoietic stem cell transplantation was compared to chemotherapy. The results were similar from all of the studies with event free survival reaching approximately 45 to 50% at five years or greater. In addition, autologous transplantation did not result in an improved overall survival while allogeneic transplantation showed improved disease free and usually event free survival. Subgroup analysis has been hampered by the numbers of patients, thus leaving open the issue of whether allogeneic transplantation from matched family donors is optimally used during CR1 versus CR2 in good risk patients.

A significant effort has also focused on the development of risk assessment in order to stratify different treatment approaches and avoid bone marrow transplantation when possible. Both cytogenetics and response to initial therapy have been determined to provide for such risk group stratification although the outcome for the majority of patients is not able to be accurately defined using these criteria. Molecular characteristics of AML cells, such as mutations in survival factor cytokines, have provided an additional method for predicted outcome in patients. In addition, the in vivo response to therapy as detected by measurements of minimal residual disease has also been demonstrated to be a highly predictive measure of outcome. Prospective studies will be needed to validate such prognostic factors and then test the efficacy of stratified treatment approaches. Thus, no currently available risk group stratification can accurately predict which type of therapy which will result in an optimal outcome in the majority of patients with AML.

The toxicity of such dose intensification treatments significantly limits future attempts to dose intensify conventional therapies. Alternatives have thus focused on the development of more AML specific targeted therapies that minimize deleterious effects to normal tissues. These targeted therapies have included immunotherapeutic antibodies, drug transporter inhibition, signal transduction and survival pathway blockade, chromatin remodeling and transcriptional therapy as well as antileukemic generating vaccines. Importantly, each of these therapeutic approaches needs to be assessed in terms of the critical question of whether they target the self-repopulating leukemic stem cell or only nonrepopulating progeny. To this end, the identification and characterization of the AML stem cell is critical for screening new therapies and their usefulness.

Fundamental and early characteristics of leukemic and solid tumor stem cells include genomic hypomethylation accompanied by specific promoter hypermethylation as well as a relatively quiescent proliferative physiologic state and a limited lifespan due to replicative senescence. Recent work has now linked these phenomena to tumor cell formation as well as providing a potential new approach to treatment based on therapeutic stem cell eradication through replicative senescence. Screening for new agents that are directed at eradicating leukemic stem cells based on such mechanisms may lead to therapeutic approaches that are fundamentally different than currently used cytotoxic treatments and provide more long lasting cures.

MRC Trials in Childhood Acute Myeloid Leukaemia

I.M. HANN*, D.K.W. WEBB*, B.E.S. GIBSONº, and C.J. HARRISON
*Great Ormond Street Children's Hospital and Molecular Haematology Unit, Institute of Child Health, London, UK; ºRoyal Hospital for Sick Children, Yorkhill, Glasgow, Scotland; ¯Leukaemia Research Fund, Cytogenetics Group, Cancer Services Division, University of Southampton, UK

Keywords: MRC, Acute Myeloid Leukaemia; Childhood

Abstract

The modern approach to therapy for acute myeloid leukaemia (AML) in children began in the late 80's and in the MRC series led to a 30% improvement in survival, up to levels of about 50%. Since 1995 the most recent trial AML 12 has taken those figures to two thirds event free survival and similar overall survival. Resistant disease rates remain at 4% overall but the death rate in complete remission has fallen from 11% to 6% despite increasing intensity of therapy, and due to advances in supportive care including nutrition and antibiotics/antifungals. However, although relapse rates have continued to fall, the biggest challenge is to reduce the currently one third relapse rate. We are much better at predicting who is likely to relapse, based mainly on primary resistance to therapy and karyotype. Analysis of 629 out of the last 808 cases in whom cytogenetic testing was successful (78%) has shown very clearly that t(8;21), t(15;17), inv(16) are independent good risk features. Additionally, loss of a sex chromosome in the 8;21 group defines a group which does exceptionally well, with 93% EFS at 5 years. Chromosome 7 abnormalities also remain of independent prognostic significance when age, WHO classification and white cell count are taken into account, with monosomy 7 doing even worse than 7q abnormalities. The current trial MRC AML 15 investigates the role of fludarabine – idarubicin combination therapy in the induction courses and the role of high dose cytarabine during consolidation; the aim being to increase efficacy and reduce toxicity, particularly that involving the heart. New approaches such as targeted antibody therapy will be explored when toxicity data for children permits.

Introduction

Acute Myeloid Leukaemia (AML) in children was a rather depressing disease to treat until the mid-1980's, with a dismal outcome of less than a third cured in all trials series (fig 1). The subsequent improvement has been one of the great success stories of medicine and has been achieved through improved supportive care, especially in the treatment of bacterial and fungal infection, and the tried and tested method of large collaborative randomised clinical trials. Although the latter can occasionally throw up difficulties in interpretation (13) these merely illustrate the need to continue in the same way and hopefully with even greater international collaboration. The only current alternative would be medical prejudice, which gets us nowhere. Improvements in cure rates for AML have involved the use of very high dose pulsed chemotherapy regimens, with a diminishing role for allogeneic bone marrow transplant (allo-BMT) which is currently performed in only one in ten children in first remission treated within the MRC AML trials and in most of the one third whose disease recurs (1,2,10,12). The challenge facing us now is to hone down the use of anthracyclin drugs without compromising efficacy and to investigate new regimens of therapy such as anti-CD33 mylotarg (7) and fludarabine (8). This article will thus summarise the most important findings from the MRC trials over the past 15 years and attempt to address the challenges, which we currently face.

Overall Outcome of Medical Research Council AML 10 & 12 Trials

The results of the MRC AML 10 trial, which ran from 1988 to 1994, have already been published and will only be summarised here and used as a comparison to MRC AML 12 from the 1995–2002 era (table 1) . MRC AML 10 (10) showed clearly that cranial irradiation should only be considered in children beyond infancy with central nervous system (CNS) disease at the outset, because intrathecal therapy with intensive chemotherapy is associated with a very low risk of relapse in this site, without the serious toxicity associated with irradiation. In addition, there was no advantage of thioguanine over etoposide (the latter at a dose of 100 mg/m^2 for 5 days in both induction courses) (6). Previous studies had suggested that one or other drug had specific activities in various subtypes of AML e.g. etoposide in monocytic variants, but there was no evidence for such an effect. Patients were randomised to receive a high dose procedure with autologous bone marrow rescue (ABMT) if they did not have a matched sibling donor, and although those who received the ABMT had a lower risk of relapse, this did not affect overall survival probably because of a higher salvage rate in those who relapsed after chemotherapy alone. The assumption has been made that equivalent effects could be achieved with less damaging chemotherapy regimens (such as, possibly, CLASP in AML 12).

In addition to documenting the lack of overall efficacy of ABMT, the AML 10 trial showed that contemporary allo-BMT protocols did not convincingly improve the outcome for any of the groups of children with AML. The swings and roundabouts effect of BMT, with increased treatment-related mortality (TRM) and reduced numbers of relapses was well demonstrated (1) and subsequently only one in ten children in the UK have been transplanted in first remission, usually those in the poor risk group defined below. This again brings to the fore the need to reduce TRM both with chemotherapy and BMT from often unacceptable levels (9). It was particularly pleasing when we saw the fall in

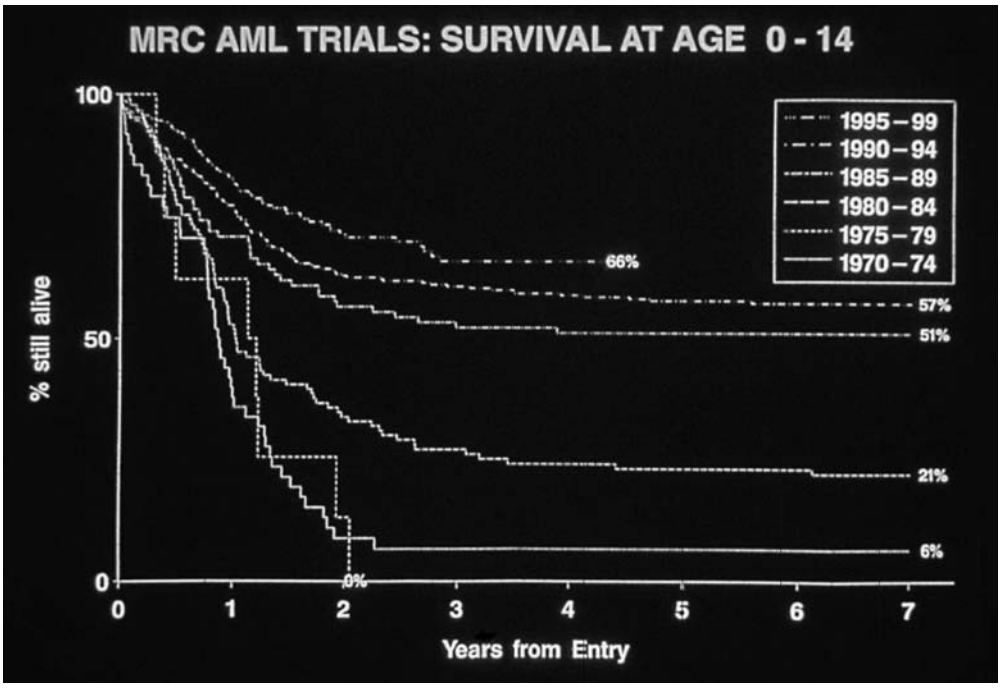

Figure 1. Results of the MRC AML trials for children since 1970. The AML 10 trial was 1988-1994 and AML 12 began in 1995, demonstrating the improvement in outcome over time.

Table 1

Event	MRC AML 10 n=341	MRC AML 12 n=529	P Value
Induction death %	4	4	–
Resistant disease %	4	4	–
Complete remission rate %	92	92	0.9
Death in complete remission %	11	6	0.008
Disease free Survival %	53	63	0.005
Event free survival %	48	58	0.02
Overall survival %	57	68	0.002

Comparison of the last two MRC childhood AML trials. Survival rates are all at 5 years.

Table 2

Event	ADE	MAE	p	4 Courses	5 Courses*	p
Induction death %	3	6	–	–	–	
Resistant disease %	4	4	–	–	–	
Complete remission rate %	92	90	0.3	–	–	
Death in CR %	7	5	0.4	2	1	0.3
Relapse rate	37	29	0.06	33	32	0.9
Disease free survival %	59	68	0.04	65	66	0.8
Overall survival %	64	70	0.1	81	78	0.5

Randomised questions in MRC AML 12
*5 course=CLASP (Cytarabine-asparaginase)
ADE=Daunorubicin, Cytarabine, etoposide
MAE=Mitoxantrone, Cytarabine, etoposide
Survival rates are all at 5 years

deaths during complete remission from 11% in AML 10 to 6% in AML 12 (tables 1 and 2) and currently our quality aim is to maintain that level at 5% or less, and we commend that aim to other groups working within this area.

MRC AML 12 recruited 564 children between April 1995 and May 2002 from 30 centres in the UK, Republic of Ireland, Netherlands and New Zealand. 504 (95%) were randomise between ADE (Danorubicin 50mg/m^2 times 3) and MAE (Mitoxantrone 12mg/m^2 times 3) for the first two chemotherapy courses. Additionally 270 were randomised between a total of four or five courses. The results (figure 1 and tables 1 and 2) were excellent with a complete remission (CR) rate of 92%, induction deaths 4% and resistant disease 4%, deaths in remission 6% and a 5-year relapse rate of 33%. The improvement is only in small part due to reduction in TRM and not at all to the randomised comparisons, with no significant differences between 4 and 5 courses (the additional course being a capizzi-type asparaginase – Cytarabine course, CLASP). This is another demonstration, if it were needed, of the dangers of historical comparisons and the need for randomisation (13). Only 44 matched sibling allo-BMT's, 16 unrelated donor transplants and one other have been performed in first CR. Donor versus no donor comparisons within the standard (77% v 77%) and poor (27% v 58%) risk groups do not show any survival differences, although numbers are small and the recommendation is still to consider BMT in the poor risk group if a good donor can be found, in the hope of a greater anti-leukaemia effect with newer BMT regimens and in the absence of a major breakthrough with other modalities of therapy.

Prognostic Factors and Special Groups

Concomitant with improvements in outcome of treatment has emerged the ability to predict outcome. This has three main advantages the first being that we can tell the families what are the chances of cure. Secondly, we can begin to identify groups, which need greater or lesser or different therapies, and finally this will allow us to gradually begin to understand the underlying biology of the disease and to replace current blunderbuss toxic therapies with hopefully more targeted treatments.

From the outset it became clear that the "old chestnuts" of prognosis were either unhelpful or untrue (11,14). Thus, although it is extremely important to identify groups at particular risk of problems e.g. bleeding with acute promyelocytic leukaemia (APL) and bleeding/leukostasis with high-count monocytic varieties, this did not necessarily lead to better therapeutic options, with the exception of all-trans retinoic acid (ATRA) for APL. The other morphological types show interesting biological associations e.g. Downs' syndrome and megakaryoblastic leukaemia (M7), but otherwise there is no convincing evidence that these lead to more effective altered approaches.

The best indicators of outcome have turned out to be resistance to therapy (e.g. more than 15% blasts after course 1) and karyotype. However, we must never make the leap of faith that would lead one to assume that reductions could safely occur for lower risk patients and increases for higher risk children. There is little evidence to support reductions of therapy in any group at present, the exception being Downs' syndrome wherein we have confirmed a very low relapse risk, increased toxicity with full protocols (4) and probably an equally good response with lower anthracycline doses and Cytarabine-based regimens. MRC trials have not confirmed the poor outcome, which some groups have quoted in relation to infants with AML and M7 subtype (3, 14)

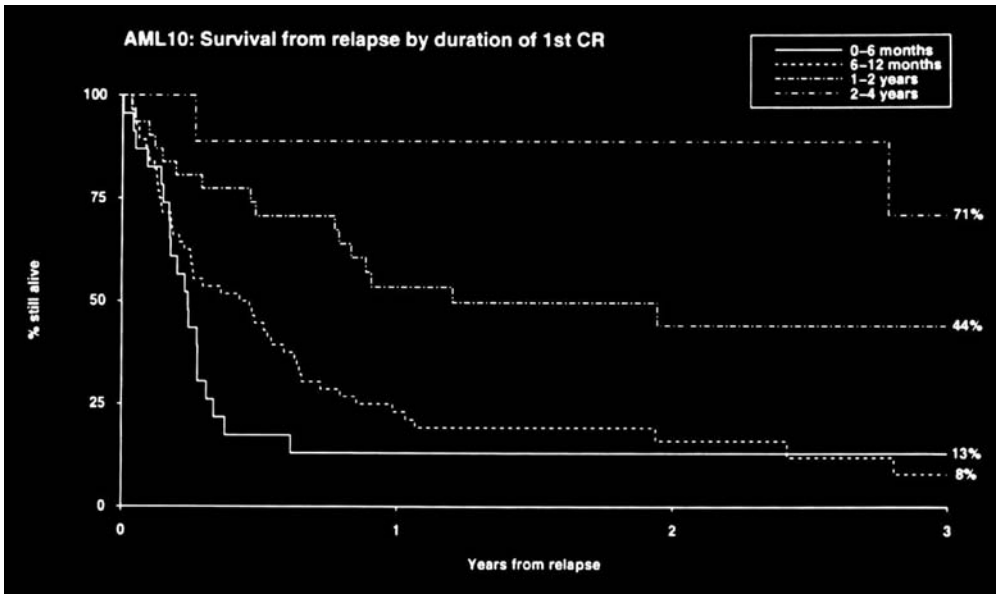

Figure 2. MRC AML 12 trial children. Survival by risk group, showing significantly worse results in the poor risk group.

Table 3

Risk Group	MRC AML 10		MRC AML 12	
	OS from CR	RR	OS from CR	RR
Good	80%	35%	84%	22%
Standard	61%	37%	76%	36%
Poor	34%	39%	39%	50%

MRC AML 10 versus 12 outcome by risk group
OS from CR=survival from complete remission
RR=relapse risk

Table 4

Chromosomal Change	Incidence % n=808
11q23*	15
Trisomy 8	14
t(8;21)	12
t(15;17)	8
Inv(16)	6
Monosomy 7	4.5
Ch5 abnormalities	1.6

Incidence of chromosomal abnormalities in MRC AML 10 and 12 trials.
* t9:11 accounts for 43% of 11q23 abnormalities
Ch=chromosome
Inv=inversion

and would contend that these standard risk patients should continue to be treated within clinical trials. We have also not demonstrated an advantage associated with additional therapy for poor risk groups and these should also continue within current trials investigating ways to achieve greater efficacy.

Chromosomal changes have greatly assisted us in predicting outcome and stratifying therapy (tables 3, 4 Refs 3, 5, 14). Improvements in chemotherapy in the recent past have begun to blur the distinctions between good and standard risk groups, but the poor risk category continues to be at high risk of relapse. The good risk group includes those with t(15;17), t(8;21) and inv(16). The poor risk groups are those with abnormalities of chromosomes 5 and 7 and those with complex (5 or more) chromosomal changes. We have now demonstrated clonal chromosome changes in 629 out of the 808 AML 10 and 12 trial children in whom cytogenetic analysis was successful (78%). Table 4 shows the incidence of the various changes with 11q23 being the most common. Table 5 shows the event free survival (EFS) data. We have confirmed that t(8;21), t(15;17) and inv(16) are good risk features, and for the first time it

Table 5

Chromosomal Change	EFS % 5 Year	P value in univariate analysis versus overall group
t(8;21)	81	0.0001
t(15;17)	78	0.02
Inv(16)	82	0.01
-7	30	0.003
Ch5 abnormalities	25	0.0008
3q abn	47	0.5
11q23 rearr*	67	0.4
+8	62	0.6
Del (9q)	67	0.8
t(6;9)	50	0.3

MRC AML 10 & 12 808 patients (629 clonal changes) outcome versus chromosomal changes.
*=no difference observed between t(9;11) and other 11q23 subgroups.
EFS=event free survival
Ch=chromosome
abn=abnormality
rearr=rearrangement

was shown that within the t(8;21) group a further improvement in outcome was noted in patients with loss of a sex chromosome, leading to a 93% EFS. We have also confirmed the poor outcome associated with chromosome 7 and within that group the monosomy 7 patients have a particularly poor outcome, with 30% EFS at 5 years. A similarly dismal outcome occurred in the small group with chromosome 5 abnormalities (EFS 25% at 5 years). However, we could not confirm a statistically worse outcome associated with 3q abnormalities.

Children with APL continue to have an excellent EFS of 78% at 5 years with anthracycline and ATRA-based AML 12 type therapy and the intention is to continue that approach at present.

We will continue to look at prognostic factors and in AML 15 we will examine the influence of molecular markers such as flt3.

Supportive Care

It is beyond the scope of this short review to mention other than the most important elements of supportive care. That is not to denigrate its vital importance in the management of AML and the constant vigilance which is necessary, to detect the emergence of “new” pathogens and other problems (9). In MRC trials the incidence of TRM has fallen since major emphasis has been placed upon nutritional support and antimicrobial therapy, especially in the youngest patients who now do at least as well as the rest (tables 1 and 2). The difficulties of leukostasis and bleeding remain a problem in the high white count children, but judicious blood product support and early institution of effective chemotherapy has made some inroads. Almost half of the TRM in our trials is due to infection and the biggest problem is with Aspergillus. An ag-

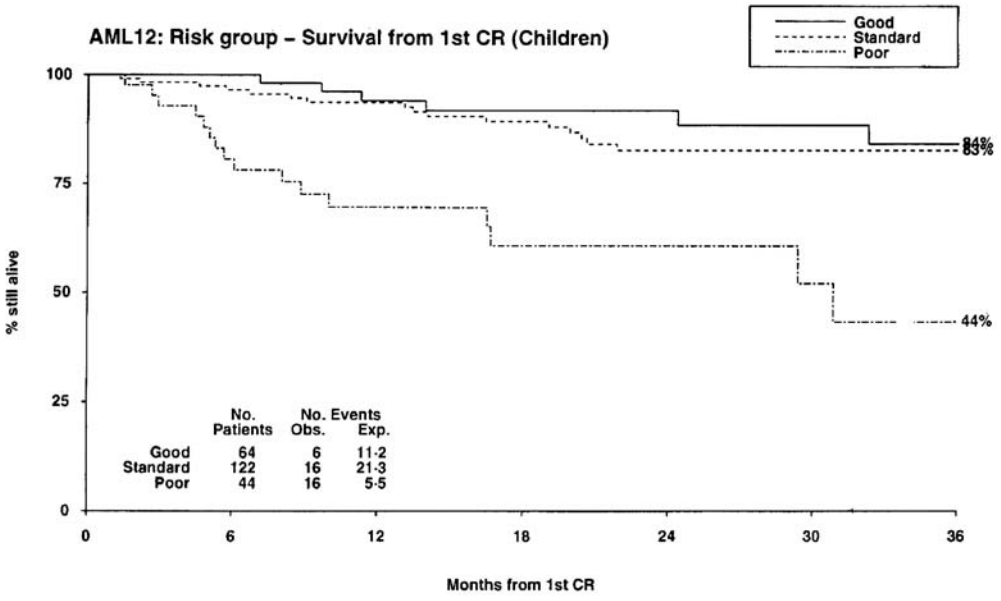

Figure 3. MRC AML 10 trial, children who relapsed. Survival by time from diagnosis to relapse. The earlier the relapse the worse the outcome

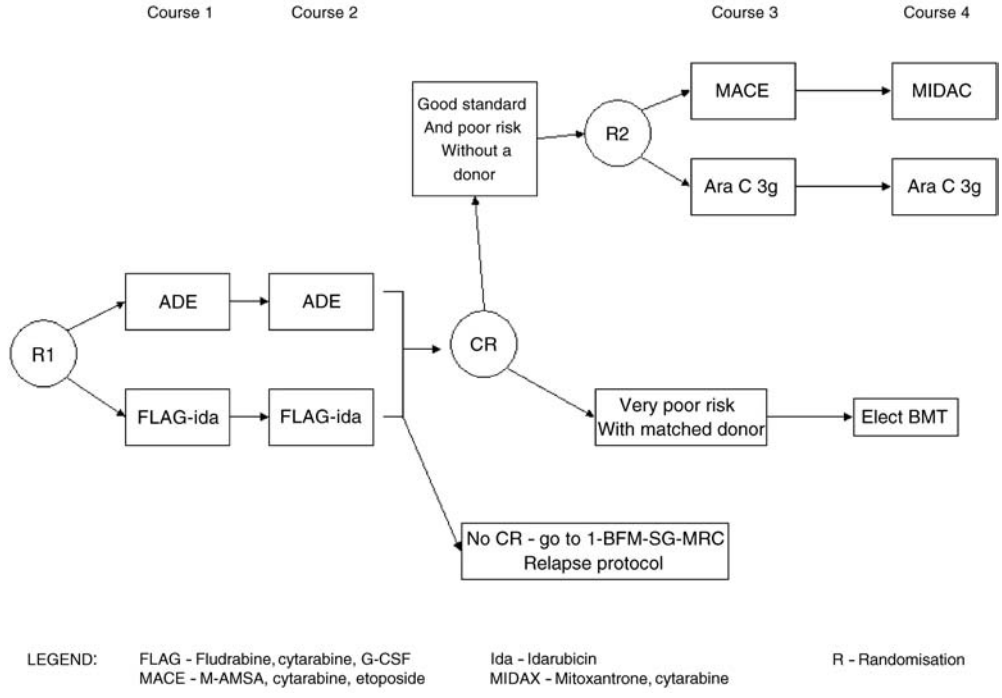

Figure 4. Schema for the AML 15 trial

gressive approach to early diagnosis using CT scans allied to very high dose modern effective antifungals means that the majority of such patients now survive, and this may improve further if combination therapies such as ambisone and caspofungin or variconazole and caspofungin are shown to be effective. It is unlikely that a single agent will do the trick, and trials of combinations are greatly needed. Better diagnostic techniques may possibly be on the horizon and trials of potentially effective prophylactic agents such as the echinocandins, posaconazole etc may prove to be effective; but it will be essential to perform very large blinded randomised trials in high-risk patients, and for extended periods of time. Otherwise we will remain in our current state of ignorance in this area.

Management of Relapse

The collaborative I-BFM-SG-MRC study is the model for future AML trials. There is an absolute need for international trials looking at problems where small numbers preclude a "going it alone" attitude; such as relapsed AML. It should not be long before this is translated to first line therapies. Within the MRC trials we have shown (12) that 90% of relapses occur in the marrow and that 70% occur within the first year from diagnosis, and historically approximately two thirds of these can be induced into a second remission. Clearly, the hope is that the latter figure will improve with the addition of newer agents such as fludarabine-daunoxome based regimens; a question addressed in the collaborative trial. It is very clear that the time to relapse is a major indicator of subsequent outcome, at opposite poles are those who relapse whilst still receiving therapy who have a 13% CR rate and those who relapse after two years from diagnosis who have an 80% CR rate. This is of course translated into a survival benefit (Fig 3). As might be expected, those patients who are good risk from the outset do better. The role of transplant for these patients requires to be better defined for instance by looking at the use of haplo-bmt in the very high risk children with no good matched sibling or unrelated donor, and evaluating ABMT in the late relapses with no good match. The best available current evidence is that some type of high dose procedure is required in these patients.

The Way Ahead and Conclusions

It would seem that the major current issues that require resolution through randomised trials are as follows:

(1) Can toxicity be reduced especially in relation to the heart?
The first step along the way is to take part in the liposomal Daunorubicin randomised relapse study. The second step is to investigate the use of reduced anthracyclin dose regimens, and this is for instance being done in the MRC AML 15 study (figure 4), which randomly allocates patients to receive the standard MACE and MIDAC consolidation, versus high doses of Cytarabine. In the absence of cardio protective agents that are effective and do not compromise the anti-leukaemic effect of chemotherapy schedules, these approaches show the greatest current promise.
(2) We need to reduce the relapse rate, especially in the highest risk group of patients.
AML 15 addresses the question as to whether FLAG-based regimens fulfil their initial promise, which was registered in very challenging situations such as relapsed and resistant disease. We will also answer the question as to whether or not idarubicin is well tolerated and more efficacious as an induction agent. With regard to newer agents, we hope to include anti-CD33 mylotarg as a randomised alternative when more toxicity data is available, from studies e.g. in relapsed or resistant disease, and to trial new promising agents in the collaborative relapse studies, as they become available. However, the failure of a fifth course of ABMT or CLASP to make a significant clinical difference may indicate that we have reached the ceiling with regard to conventional chemotherapy.
(3) The role of modern BMT approaches requires to be established, especially in the poor risk patients. Measures to enhance the graft versus leukaemia (GVL) effect such as early withdraw of immunosuppression need to go hand-in-hand with efforts to bear down on the current TRM rates which obviate the benefits from reduced relapse rates.

All in all this is a challenging and exciting time!

References

1. Burnett A, Goldstone A, Hann I, Stevens R, Rees J, Wheatley K, Gray RG. (2002). The value of allogeneic bone marrow transplant in patients at differing risk of relapse: Results of the UK MRC AML 10 trial. Brit. J. Haem 118, 385-400.
2. Burnett A, Goldstone A, Stevens RF, Hann IM, Gray RG, Rees J, Wheatley K (1998). Randomised comparison of addition of autologous BMT to intensive chemotherapy for AML: Results of MRC AML 10. Lancet, 351:700-708.
3. Chessells JM, Harrison CJ, Kempski H, Webb DK, Wheatley K, Hann IM, Stevens RF, Harrison G, Gibson BE (2002) clinical features, cytogenetics and outcome in ALL and AML of infancy: report from the MRC. Leukaemia, 16(5), 776-784.
4. Craze JL, Harrison G, Wheatley K, Hann IM, Chessells JM (1999). Improved outcome of AML in Down's Syndrome. Arch Dis Child, 81:32-37.
5. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A. (1998). The importance of diagnostic cytogenetics on outcome in AML from MRC AML 10. Blood, 92:2322-2333.
6. Hann IM, Stevens RF, Burnett A, Goldstone A, Wheatley K, Gray R, Rees J. (1997). Randomised comparison of DAT versus ADE as induction chemotherapy in children and younger adults: Results of the MRC AML 10 trial. Blood, 87:2311-2318.
7. Kell W, Burnett A, Chopra R, Yin J, Clark RC, Rohatiner A, Culligan D, Hunter A, Prentice AG, Milligan DW. (2003). Simultaneous administration of mylotarg with intensive chemotherapy in induction and consolidation in younger patients with AML. Blood In Press.
8. McCarthy AJ, Pitcher L, Hann IM, Oakhill A. (1999). FLAG for refractory and high risk relapsed acute leukaemia in children. Med and Ped Oncol, 32:411-415.
9. Riley LC, Hann IM, Wheatley K, Stevens RF. (1999). Treatment-related deaths during induction and first remission of AML in children treated on MRC AML 10. Brit. J. Haem, 106:436-444
10. Stevens RF, Hann IM, Wheatley K, Gray RG. (1998). Marked improvements in outcome with chemotherapy alone in paediatric AML: results of MRC AML 10. Brit. J. Haem, 101:130-140.
11. Webb DKH, Harrison G, Stevens R, Gibson BG, Hann IM, Wheatley K. (2001). Relationship between ago at diagnosis, clinical features and outcome of therapy in children treated in the MRC trials for AML. Blood, 98:1714-1720.
12. Webb DKH, Wheatley K, Harrison G, Stevens RF, Hann IM. (1999). Outcome for children with relapsed AML following therapy in the MRC AML 10 trial. Leukaemia, 13:25-31.
13. Wheatley KE, Clayton D. (2003). Be sceptical about unexpected large apparent treatment effects: the case of an MRC AML 12 randomisation.
14. Wheatley K, Burnett A, Goldstone A, Hann IM, Harrison G, Rees J K H, Stevens RF, Walker H. (1999). A simple, robust and highly predictive prognostic index for the determination of risk-directed therapy in AML, derived from MRC AML 10. Brit. J. Haem, 107:69-79.

Prognostic Relevance of Risk Groups in the Pediatric AML-BFM Trials 93 and 98

U. CREUTZIG, D. REINHARDT, and M. ZIMMERMANN for the AML-BFM Study Group
University Children's Hospital, Department of Hematology/Oncology Münster, Germany

Keywords: AML, risk groups, prognosis

Introduction

Prognosis for children with AML has improved considerably during the last 15 years. This was due to intensification of chemotherapy including SCT. Although some children had been cured by standard AML treatment, prognosis remained poor in others. To improve the prognosis of patients with poor outcome without risking a higher rate of mortality and morbidity in those with favourable outcome, a risk-adapted therapy based on the stratification system resulting from a retrospective analysis of studies 83 and 87 was applied in study 93.

By using morphological criteria based on data of the above named studies, we could define prognostic groups. With more data on response on day 15, the definitions became even more decisive. We could show a strong correlation between a rapid decrease in bone marrow blast cell count during induction and the quality of remission (CR and CCR) [11]. This was also seen by Wells et al. reporting a significant difference in survival in children with less or more than 5% blasts on day 14 in the CCG study [15]. Currently most AML study groups use stratification according to cytogenetic results, and our present analysis corroborates the findings of the preceding studies revealing a high correlation between well-defined morphological and cytogenetic subtypes. We can show that the difference between risk groups may change with intensity of therapy, but their definition remains stable over consecutive trials.

Patients and Methods

The entry criteria for study AML-BFM 93 and -98 included: Newly diagnosed AML patients aged between 0 and 18 years and written informed consent of the patient or parent. Patients with myelosarcoma, secondary AML, myelodysplastic syndromes were excluded (Down's syndrome was excluded from this analysis). All eligible patients of study 93 and those patients of study 98 who had been diagnosed until the end of October 2002 were included. Study 98 is still open for recruitment at the time of writing (11/2003).

Diagnosis

The initial diagnosis of AML and its subtypes was determined according to the FAB classification [1;3]. All initial smears were routinely examined at the University Children's Hospital in Münster and were reviewed by a panel of haematologists including 1–2 external experts (T. Büchner, H. Löffler). The diagnoses of M0 and M7 subtypes always require the confirmation by immunological methods [1;2]. Day 15 bone marrow aspirates were reviewed centrally.

Treatment

The treatment schedule of study AML-BFM 93 is shown in Figure 1. For details see [7]. Briefly, after diagnosis patients were randomised to receive the 8-day induction with either daunorubicin (in the ADE induction) or with idarubicin (AIE induction). After induction, patients were treated according to risk groups (standard risk group: FAB M1, M2 with Auer rods and FAB M4eo with $\leq$5% blasts on day 15 in the bone marrow, FAB M3 independently from blast count on day 15; high-risk group: all others) [11] (Figure 2).

High-risk patients were randomised to receive either HAM (=high-dose cytarabine 3 g/m^2 every 12 h for three days and mitoxantrone 10 mg/m^2 on days 4 and 5) followed by consolidation (=arm "early HAM") or 6-week consolidation followed by HAM (=arm "late HAM"). Standard-risk patients re-

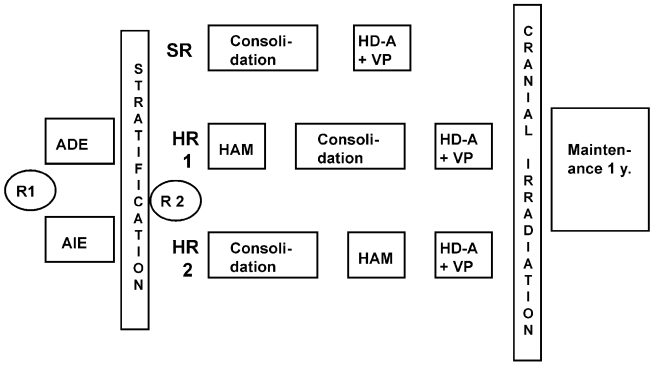

Figure 1: Treatment schedule of Study AML-BFM 93

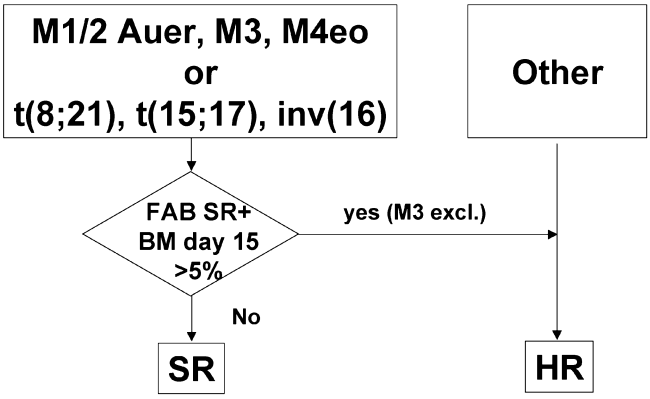

Figure 2: Risk classification by morphology, cytogenetics and response on day 15 in studies AML-BFM 93 and 98

ceived consolidation only, without HAM. Subsequently, all patients were treated with one intensification block of high-dose cytarabine and etoposide followed by cranial irradiation with 18Gy (standard dose in children ≥3 years old) and maintenance with daily thioguanine 40 mg/m^2 orally and cytarabine 40 mg/m^2 subcutaneously ×4 days monthly for a total treatment duration of 18 months. Allogeneic SCT in first CR was recommended in children of the high-risk group only if a sibling donor was available.

In study AML-BFM 98, there were only minor therapy modifications of the overall treatment schedule. Standard risk patients (except FAB M3 and Down's syndrome patients) received the same chemotherapy as high-risk patients. Patients with favourable cytogenetics were stratified to the standard risk group like patients with favourable morphology.

Patients were randomly assigned to either the standard six week consolidation phase or to a two-cycle therapy (cytarabine, idarubicin block=500 mg/m^2 per day continuous infusion cytarabine ×4 days together with idarubicin 7 mg/m^2 per day on day 3 and 5, and high-dose cytarabine 1 g/m^2 every 12 hours for 3 days combined with mitoxantrone 10 mg/m^2 per day on day 3 and 4). The cumulative dose of anthracyclines within the two cycles was similar to that the one given in the consolidation course. In addition, patients without initial CNS involvement were randomised to receive cranial irradiation of either 12Gy or 18 Gy.

In the former AML-BFM studies 83 and 87 adriamycin or daunorubicin were given instead of idarubicin in a cumulative dose of 400 and 300 mg/m^2, respectively. High-dose cytarabine combined with etoposide was applied in study 87 after consolidation and not given in study 83 [6;9].

Definition and Statistics

Complete remission (CR) was defined according to the CALGB criteria [5] and had to be achieved by the end of intensification treatment. Early death (ED) patients were those dying before or within the first 6 weeks of treatment. Event-free survival (EFS) was calculated from date of diagnosis to last follow-up or to the first event (failure to achieve remission, early death, resistant leukemia, relapse or death of any cause). Failure to achieve remission was counted as event on day 0. Survival was calculated from date of diagnosis to

Table 1: Pre-treatment clinical data of patients of studies AML-BFM 93 and 98

	AML-BFM 93		AML-BFM 98		AML-BFM 93/98	
	n	%	n	%	n	%
Number eligible patients	471	100.0	430	100.0	901	100.0
Gender (male)	255	54.1	229	53.3	484	53.7
Age <2 years	112	23.8	105	24.4	217	24.1
2-9 years	174	36.9	134	31.2	308	34.2
≧ 10 years	185	39.3	191	44.4	376	41.7
Leukocytes (x10^3/mm^3)						
<20	241	51.2	227	52.8	468	51.9
20-<100	143	30.4	118	27.4	261	29.0
≧100	87	18.5	85	19.8	172	19.1
CNS leukemia	45	10.3*	47	8.7*	92	9.2*
FAB Types						
M0	25	5.3	20	4.7	45	5.0
M1	55	11.6	63	14.6	118	13
(M1 with Auer)	(35)	(7.4)*	(33)	(7.7)*	(68)	(7.5)*
M2	125	26.6	108	25.1	233	25.9
(M2 with Auer)	(94)	(20.0)*	(79)	(18.4)*	(173)	(19.2)*
M3	23	4.9	27	6.3	50	5.5
M4	91	19.3	83	19.3	174	19.3
(M4eo)	(36)	(7.6)	(34)	(7.9)	(70)	(7.8)
M5	101	21.4	92	21.4	193	21.4
M6	16	3.4	11	2.6	27	3.0
M7	31	6.6	24	5.6	55	6.1
other	4	0.8	2	0.5	6	0.7
Karyotypes nd	184	39.1	55	12.8	239	26.5
Cytogenetic favorable**	82	28.6*	100	26.7*	182	27.5*
normal	56	19.5*	98	26.1*	154	23.3*
other	149	51.9*	177	47.2*	326	49.2*
Standard risk	161	34.2	151	35.1	312	34.6
High-risk	310	65.8	279	64.9	589	65.4

nd = no data

* percentage of patients with data

** Definition of favorable cytogenetics: t(8;21), t(15;17), inv16

Table 2: Results in Studies AML-BFM 93 and 98

	AML-BFM 93		AML-BFM 98		Total	
	n	% (SE)	n	% (SE)	n	%(SE)
No. of Patients	471	100	430	100	901	100
Median follow-up of pts. in CCR (years, range)	6.0	(1.1 - 9.7)	2.1	(0.3 - 4.5)	3.5	(0.3 - 9.7)
Early deaths (total)*	35	7.4	14	3.3	49	5.4
Blasts day 15 > 5%	96	25.1**	66	17.7**	162	21.4**
Non-response	49	10.4	36	8.4	85	9.4
CR achieved	387	82.2	380	88.4	767	85.1
Death in CCR (cumulative incidence)	18	3.8 (1)	17	4.0	35	3.9
Relapse (cumulative incidence)	131	28.1 (3)	116	27.0	249	27.6
Lfu in CCR	14	3.0	0		14	1.6
3-year pEFS		53 (2)		51 (3)		53 (2)

* Early deaths are defined as death until day 42

** Percentage of patients with data

Abbreviations: CR, complete remission; CCR, continuous complete remission; lfu, lost to follow-up;

death of any cause or last follow-up. Disease-free survival (DFS) of patients achieving remission was calculated from date of remission to first event (relapse, death of any cause). Survival rates were calculated according to Kaplan-Meier and compared by log-rank test. Toxicities were measured according to the NCI common toxicity criteria.

Univariate analysis was done using the Wilcoxon test for quantitative variables and Fisher's exact test for qualitative variables. When frequencies were sufficiently large, χ^2statistic was used. Computations were performed using SAS (Statistical Analysis System Version 6.12, SAS Institute Inc, Cary, NC).

Results

Patient Characteristics:

Table 1 gives the pre-treatment clinical data of patients, distribution of FAB subtypes, cytogenetic groups and risk groups of the 901 patients of studies AML-BFM 93 (n=471) and AML-BFM 98 (n=430). Patient distribution was similar in both studies.

Treatment results

Overall results: The overall results (per study and accumulated) are shown in **Table 2:** *In study AML-BFM 93*, 387 of 471 (82%) patients achieved remission, 3-year survival, event free survival (EFS), and disease free survival were 61% SE 2%, 53% SE

2% and 64% SE 2%, respectively [7]. Remission rate increased in study AML BFM 98 (88% vs. 82%, p=0.01), however, according to preliminary results pEFS was in the same range as in study 93 (3-year pEFS 51%, SE 3%, p logrank=0.97).

Blast cell reduction on day 15: In study AML-BFM 93 there was a significant better blast cell reduction in the bone marrow on day 15 in patients who were treated according to the idarubicin arm (25 of 144=17% of patients with ≥5% blasts compared to 46 of 149=31% of patients after daunorubicin, pχ^2=0.01). In study AML-BFM 98 which assigned idarubicin induction to all patients the percentage of patients with ≥5% blasts was 18% (66 of 372 patients).

CR rate and DFS were significantly better in both studies in children with a BM blast count ≤5% on day 15 compared to those with >5% blasts (CR 91% vs 80%, p<0.001; pDFS 0.60 SE 0.02 vs 0.49 SE 0.05, p=0.01; (studies 93/98, FAB M3 patients excluded). Figure 3 shows the EFS in comparison to the previous studies 83 and 87. In studies 93/98 the differences between patients with ≥5% or <5% blasts were minor than in studies 83/87, however, still significant.

Cytogenetics: Outcome of 182 patients from studies 93 and 98 with favourable karyotypes [t(8;21), t(15;17), inv16] was significantly better compared to others (other and normal karyotypes, n=480), 5-year pEFS in studies 93/98 with favourable karyotypes was 74%, SE 4% compared to others 41%, SE 3%, p logrank=0.0001.

There was a significant correlation between the risk groups defined by morphology and blast count on day 15 and the groups defined by cytogenetics. **Table 3** shows the results for these risk groups in studies 93/98.

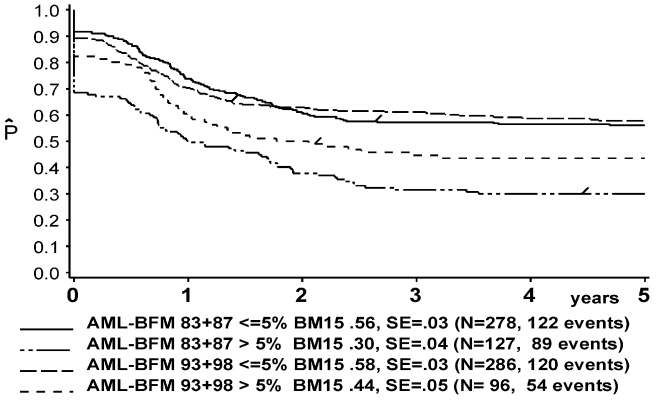

Figure 3: 5-year event free survival in patients of studies 83/87 compared to studies –93/98 according to blast count on day 15

This confirms the results of studies 83/87 with larger numbers of patients. Only high-risk patients with unfavourable cytogenetics show a poor outcome.

High-risk group: In study AML-BFM 93 improvement of prognosis benefited only the 310 high-risk patients (remission rate and 5-year pEFS in study AML-BFM 93 *vs* study 87: 78% *vs* 68%, p=0.07, and 44%, *vs.* 31%, p logrank=0.01). The placing of HAM as the 2nd or 3rd therapy course was of minor importance. However, patients who received the less intensive daunorubicin treatment during induction benefited from early HAM [8].

Standard risk group: In study AML-BFM 98 standard risk patients were treated with the same intensity (with HAM) as high-risk patients. However, interim analysis showed no significant advantage of this treatment intensification. Result remained in the same range as in study 93 and as in the previous studies (Figure 4).

Discussion

In the 70ies, children with AML only rarely survived, whereas nowadays, 50%–0% of children with AML are long-term survivors [7;13;14]. This was achieved mainly by a more intensified chemotherapy and far better supportive care. Nevertheless about 40% of the patients cannot be cured by the help of the current therapy protocols. This might be possible by more intensive treatment including SCT, however, this should be performed without increasing toxicity for those children who can be cured with standard AML treatment. Currently, the SCT related mortality rate after allogeneic HLA identical SCT is about 10% and after unrelated SCT about 20% [12]. The goal of our risk group

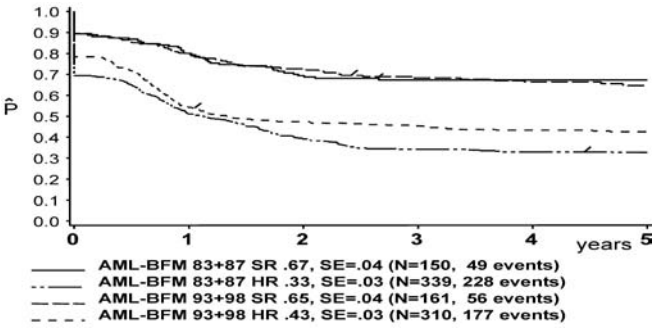

Figure 4: 5-year event free survival in standard (SR) and high-risk (HR) patients of studies 83/87 compared to studies –93/98

Table 3: Correlation between cytogenetics and risk groups in studies AML-BFM 93/98

	Karyotypes favourable		Karyotypes unfavourable	
	N= 182		N= 461	
Risk Groups (defined)	N (%)*	5-year pEFS % (SE)	N (%)*	5-year pEFS % (SE)
SR n = 251	150 (60)	72 (4)	101 (40)	52 (6)*
HR n = 411	32 (8)	84 (7)	379 (92)	39 (3)

*** percentage of standard risk (SR) and high-risk (HR) patients, respectively**

definition was to identify patients who may benefit from a more experimental therapy or from a less toxic treatment in order to reduce the treatment-related mortality and morbidity.

From a biological point of view AML is a very heterogeneous disease not only by morphology but even more by immunophenotypic and cytogenetic features. However, some subtypes of AML are characterized by a significant correlation between morphology, immunophenotypic features and karyotypes [10]. The three subgroups of AML presenting with characteristic morphologic features, FAB M1/ M2 with Auer rods, M3 and M4 with eosinophils implicate a good prognosis. Children with these subtypes have a high response rate and a low rate for early death and relapse. In addition, we could show that the blast count on day 15 is a significant indicator for response and high pEFS (Figure 3). The definition of a standard risk group which comprises about one third (35%) of patients was possible by combining favourable morphological subgroups with blast cell reduction on day 15 and cytogenetic results. As shown in table 3 there is a good correlation between risk groups defined by morphology and blast count on day 15 and cytogenetics. By defining the favourable group by favourable karyotypes only, the group would be smaller (27%) compared to our defined standard risk group.

Patients with favourable cytogenetics and high-risk criteria by morphology are included in our standard risk group since study 98 (Figure 2). Standard risk patients defined by morphology only who have unfavourable cytogenetics mainly have normal karyotypes and will have an intermediate prognosis. Till now, they have remained in the standard risk group, however, in the future, those with FLT3 internal tandem duplication will be shifted to the high-risk group. In an retrospective analysis mainly of patients of the AML-BFM studies it has been shown that FLT3 internal tandem duplication is an independent prognostic factor in childhood AML [16].

In study AML-BFM 83, survival and event-free-survival rate increased compared to the first study AML-BFM 78. The difference between the studies consisted in the introduction of the ADE induction which was not given in study 78. With this intensified induction outcome was significantly better in patients with favourable morphology (most of these patients are now in our standard risk group) [6]. Based on data of study 83 and 87 we defined risk factors, which are still applicable today [11]. Mainly in study 87, we found the prognostic significance of the blast cell reduction on day 15 which allows an even better risk stratification.

In study AML-BFM 93, improvement of prognosis was seen in high-risk patients due to the introduction of HAM as second or third therapy course. In study AML-BFM 98 also standard risk patients have been treated with HAM. P-EFS and p-survival in standard risk patients of studies 93 and 98 were similar to those of studies 87 and 83. These results were not expected, however, they show that it is extremely difficult to increase prognosis in the standard risk group. Analogous observations have been reported from the MRC trial. In the “MRC-good risk group” defined by favourable cytogenetics there was no survival advantage by allogeneic SCT in 1stR compared to chemotherapy only [4;14].

There might be a possibility to increase survival in standard risk patients by new therapeutic approaches like targeted therapy or subgroup directed therapy. A new therapy option could be the application of FLT3 inhibitors in FLT3 positive patients or with tyrosine kinase inhibitors for children with ras mutations or c-kit mutations.

We could show in our studies that prognosis in risk groups may change with intensification of chemotherapy but the prognostic value of the variables used for stratification remained high over studies. This was shown for the factor blast cell reduction on day 15 (Figure 3) but also for the stratification groups defined by combined risk criteria comparing event free survival and also survival in risk groups of studies AML-BFM 83/87 and studies 93 and 98 (Figure 4).

Our data indicate that it was possible to improve prognosis in standard risk patients in the 80ies by intensification of initial chemotherapy. In the last 10 years prognosis could be improved in high-risk patients with more intensive therapy, e.g. high-dose cytarabine courses. This may indicate that these patients might even benefit from further treatment intensification. However, this has to be done without increasing therapy related mortality (early death and death in CCR). To reduce the therapy related mortality which averages out at more than 10% in high-risk patients may be of the same importance.

References

1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C (1991) Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-M0). Br J Haematol 78:325-329
2. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C (1985) Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7). A report of the French-American-British Cooperative Group. Ann Intern Med 103:460-462
3. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C (1985) Proposed revised criteria for the classification of acute myeloid leukemia. Ann Intern Med 103:626-629
4. Burnett AK, Wheatley K, Goldstone AH, Stevens RF, Hann IM, Rees JH, Harrison G (2002) The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: results of the UK MRC AML 10 trial. Br J Haematol 118:385-400
5. Cheson BD, Cassileth PA, Head DR, Schiffer CA, Bennett JM, Bloomfield CD, Brunning R, Gale RP, Grever MR, Keating MJ, Sawitsky A, Stass S, Weinstein H, Woods WG (1990) Report of the National Cancer Institute-sponsored workshop on definitions and response in acute myeloid leukemia. J Clin Oncol 8:813-819
6. Creutzig U, Ritter J, Schellong G (1990) Identification of two risk groups in childhood acute myelogenous leukemia after therapy intensification in the study AML-BFM-83 as compared with study AML-BFM-78. Blood 75:1932-1940
7. Creutzig U, Ritter J, Zimmermann M, Hermann J, Gadner H, Sawatzki DB, Niemeyer CM, Schwabe D, Selle B, Boos J, Kuhl J, Feldges A (2001) Idarubicin improves blast cell clearance during induction therapy in children with AML: results of study AML-BFM 93. AML-BFM Study Group. Leukemia 15:348-354
8. Creutzig U, Ritter J, Zimmermann M, Reinhardt D, Hermann J, Berthold F, Henze G, Jürgens H, Kabisch H, Havers W, Reiter A, Kluba U, Niggli F, Gadner H (2001) Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt- Münster 93. J Clin Oncol 19:2705-2713
9. Creutzig U, Ritter J, Zimmermann M, Schellong G (1993) Does cranial irradiation reduce the risk for bone marrow relapse in acute myelogenous leukemia? Unexpected results of the Childhood Acute Myelogenous Leukemia Study BFM-87. J Clin Oncol 11:279-286
10. Creutzig U, Sperling Ch, Harbott J, Ritter J, Zimmermann M, Löffler H, Riehm H, Schellong G, Ludwig W-D (1995) Clinical significance of surface antigen expression in children with acute myeloid leukemia: Results of Study AML-BFM-87. Blood 86:3097-3108
11. Creutzig U, Zimmermann M, Ritter J, Henze G, Graf N, Löffler H, Schellong G (1999) Definition of a standard-risk group in children with AML. Br J Haematol 104:630-639
12. Hongeng S, Krance RA, Bowman LC, Srivastava DK, Cunningham JM, Horwitz EM, Brenner MK, Heslop HE (1997) Outcomes of transplantation with matched-sibling and unrelated-donor bone marrow in children with leukaemia. Lancet 350:767-771
13. Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, Jonmundsson G, Mellander L, Gustafsson G (2003) Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down’s syndrome: results of NOPHO-AML trials. Br J Haematol 122:217-225
14. Stevens RF, Hann IM, Wheatley K, Gray RG, on behalf of the MRC Childhood Leukaemia Working Party (1998) Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukaemia: results of the United Kingdom Medical Research Council’s 10th AML trial. Br J Haematol 101:130-140

15. Wells RJ, Woods WG, Buckley JD, Odom LF, Benjamin D, Bernstein I, Betcher D, Feig S, Kim T, Ruymann F, Smithson W, Srivastava A, Tannous R, Buckley CM, Whitt JK, Wolff L, Lampkin BC (1994) Treatment of newly diagnosed children and adolescents with acute myeloid leukemia: A Childrens Cancer Group Study. J Clin Oncol 12:2367-2377
16. Zwaan ChM, Meshinchi S, Reinhardt D, Zimmermann M, Harbott J, Radich JP, Huismans DR, Munske L, Podleschny M, Hählen K, Pieters R, Creutzig U, Kaspers GJL, Griesinger F. FLT3 internal tandem duplication (FLT3/ITD) is an independent prognostic factor in childhood AML: Results in 247 children with de novo AML. Leukemia 17[3], 661. 2003.

Maintenance Therapy in Childhood Acute Myeloid Leukemia.

Y. PEREL, A. AUVRIGNON, T. LEBLANC, G. MICHEL, J.-P. VANNIER, J.-H. DALLE, V. GANDEMER, C. SCHMITT, F. MÈCHINAUD, J.-P. LAMAGNERE, Ch. PIGUet, G. COUILLAUD, B. PAUTARD, A. BARUCHEL, and G. LEVERGER for the Group LAME of the French Society of Pediatric Hematology and Immunology (SHIP), France
Centres Hospitalo-Universitaires de Bordeaux, Paris-Trousseau, Paris-St Louis, Rouen, Marseille, Lille, Rennes, Nancy, Nantes, Tours, Limoges and Dijon, France

Key-words: acute myeloid leukemia, childhood, maintenance therapy, drug-resistance

ABSTRACT

Purpose: To determine whether, after very intensive induction and consolidation therapy in childhood AML, further maintenance therapy (MT) confers any advantage.

Patients and methods: Three hundred-nine children with previously untreated AML were registered in the LAME 89/91 protocol. This three-cycle intensive regimen included an induction phase (mitoxantrone plus cytarabine) and, for non-allografted patients, two consolidation courses, one containing timed-sequential high-dose cytarabine, asparaginase and amsacrine. In the LAME 89 study, patients were given an additional MT consisting of mercaptopurine and cytarabine for 18 months. In the LAME 91 trial, patients were randomized to be given or not MT after consolidation therapy.

Results: Out of 309 patients, 276 (90%) achieved a complete remission. The overall survival (OS) and event-free survival at 6 years for all patients were 60%±6% and 48%±6%, respectively. For the complete responders after consolidation therapy, the 5-year OS was significantly better in patients randomized for no further treatment than in patients randomized for MT (81%±13% vs 58%±15%; p=0.04) whilst the 5-year disease-free survival was not significantly different (60%±19% vs 50%±15%; p=0.25). The improvement of OS in MT-patients appeared to be related to a higher salvage rate after relapse.

Conclusion: Over 50% of patients can be cured of AML in childhood. In the context of a very short and drug-intensive regimen, low-dose MT, owing to the lack of improvement in disease control and the worsening of survival, should not be recommended.

Over the past 20 years, the outcome of acute myeloid leukemia (AML) in children has improved substantially. In the eighties, complete remission (CR) was achieved in nearly 90% of patients but event-free survival (EFS) was poor. Myeloablative therapy followed by allogenic bone-marrow transplantation (allo BMT) from an HLA-identical sibling was demonstrated, in our experience, to be the treatment of choice for improving DFS in children with AML in first remission.[6] The major issue was how best to maintain complete remission for patients without an HLA sibling donor. Whereas several groups continued to include low-dose MT[1,2] and others decided to omit it,[5,10] in 1991, our group undertook a prospective randomized trial (LAME 91 protocol), the main aim of which was to assess the efficacy of MT in addition to an intensive induction and consolidation chemotherapy. The main results have been published previously[7] and are now updated and described in a higher number of patients.

Patients and methods

Patients. Inclusion criteria in the LAME 89/91 protocol were as follows: de novo AML with an FAB subtype ranging from M1 to M6, age less than 20 years. Patients with biphenotypic leukemia, secondary AML, and patients with Down's syndrome were not included. 309 children from 18 institutions were registered in the protocol between December 1988 and December 1998.

Induction treatment. Induction therapy was a combination of cytarabine at 200 mg/m^2/d by continuous IV infusion from day 1 to day 7 and mitoxantrone at 12 mg/m^2/d IV from day 1 to day 5. Patients who had more than 20% blasts on day 20 bone marrow aspiration received additional chemotherapy (cytarabine at 200 mg/m^2/d for 3 days and mitoxantrone at 12 mg/m^2/d for 2 days).

Allo-BMT in CR1. Allo BMT was offered to all the 74 patients where an HLA-identical family donor was available. Most of them were conditioned with 16 mg/kg busulfan and 200mg/kg cyclophosphamide.

Postremission therapy. Two consolidation courses were given to patients without an HLA-matched family donor. Consolidation 1 was a combination of etoposide (100 mg/m^2/d IV, 1-hour infusion, from day 1 to day 4), cytarabine (100 mg/m^2/d as a continuous IV infusion from day 1 to day 4), daunorubicin (40 mg/m^2/d IV, 1-hour infusion from day 1 to day 4). Consolidation 2 consisted of cytarabine, 1 g/m^2, 1-hour infusion, administered 12-hourly, on days 1, 2, 7 and 8, amsacrine at 150 mg/m^2/d IV, 1-hour infusion, on days 4, 5 and 6, asparaginase at 6000 U/m^2 on days 3 and 9.

Maintenance therapy. After consolidation chemotherapy, patients were treated with an 18-month maintenance program consisting of continuous oral 6-

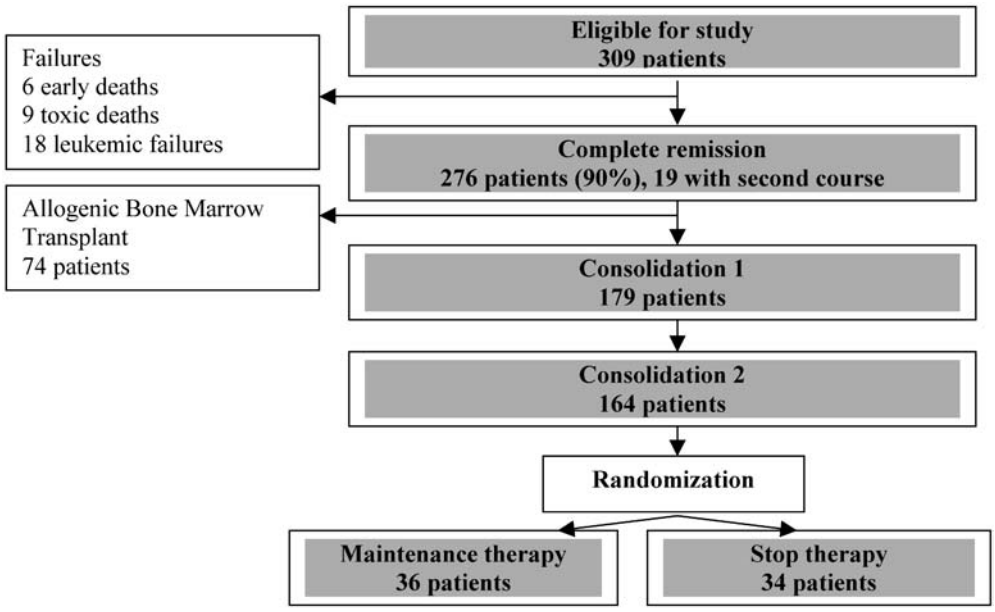

Figure 1. Flow diagram showing patient numbers according to treatment arms

mercaptopurine at 50 mg/m^2/d and monthly pulses of subcutaneous cytarabine at 25 mg/m^2 twice a day for 4 days (LAME 89 protocol). From march 1991 to december 1998, children in first CR were randomly assigned to receive MT or no further therapy after consolidation 2. This randomization was centrally performed, at the time of hematological recovery following consolidation 2.

CNS prophylaxis. CNS prophylaxis was administered to patients with the M4 or the M5 FAB subtypes and to patients with an initial white blood cells (WBC) count higher than 50×10^9/l. These patients were given five doses of intrathecal chemotherapy (IT). Patients with initial CNS involvement were given three additional IT doses and 24 Gy cranial radiation.

Statistical methods. The analysis was performed in February 2001. Event-free survival (EFS), disease-free survival (DFS), and overall survival (OS) were estimated with the Kaplan-Meier method. To study the impact of MT, DFS and OS were also calculated from the day of randomization. For nonrandomized patients, the endpoint was calculated as from the time of hematological recovery following consolidation 2.

RESULTS

Patients characteristics. 309 patients entered the LAME 89/91 protocol; 146 were boys and 163 were girls, with a median age of 6.9 years. 42 children (13%) were under 1 year old. The median of the WBC count at diagnosis was 25.6 x 10^9/l and the distribution of FAB subtypes was as follows : M1 (n=38), M2 (n=87), M3 (n=19), M4 (n=42), M_{4Eo} (n=23), M5 (n=92) and M6 (n=8). 31 children (10%) had initial CNS involvement.

Induction outcome. 276 of the 309 children achieved complete remission (90%), 257 after one course of induction chemotherapy (83%) and 19 after the day 21 reinforcement. The induction toxic death rate was 5% ; there were six early deaths (<8 days), nine toxic deaths and 18 leukemic failures. No clinical prognostic factor was associated with achievement of CR.

Treatment allocation. Patient numbers according to treatment arms of the LAME 89/91 protocol are shown in figure 1. Of the 276 patients in complete remission, 74 were given an allo BMT; all the patients in CR1 with an HLA-identical family donor actually received the scheduled allo BMT. 179 patients were given consolidation 1 and 164 consolidation 2. The treatment-related mortality of the two courses of postremission therapy was 6%.

Potential biases or confounding variables (randomized study). 139 patients were eligible for MT, 70 were randomized and evaluable (36 with and 34 without). If patients of the LAME 89 and/or for whom no further therapy could be administered are excluded, overall compliance of patients for randomization was 68.6%. Table 1 shows the pretreatment characteristics of patients in the two randomized arms.

Global results. The global LAME 89/91 EFS, DFS and OS at six years were respectively 48%±6%, 53%±6% and 60%±6%.

Results of allo-BMT. The DFS was 49%±7% for non-allografted patients and 63%±11% for allografted patients (p=0.05).

Table 1. Patient Characteristics by Maintenance Therapy Randomization

	Randomized patients		p value for homogeneity
Maintenance therapy (no. patients)	MT – (n=34)	MT+ (n=36)	
Median age (years)	5	5.2	0.67
Female/Male	17/17	20/16	0.64
Median WBC 10^9/L	15	24	0.23
FAB subtypes (no. patients)			
M1	4	4	
M2	7	10	
M3	3	2	0.55
M4/M4 eo	5/1	6/5	
M5	12	9	
M6	2	0	
Cytogenetics (no. available)	24	26	
t(15; 17)	2	2	1
t(8; 21)	5	4	0.72
$11q_{23}$ abnormality	6	9	0.46
inv(16)	3	5	0.70
5q-/7q-	0	0	1
Normal	8	6	0.42
Patients needing a second course at day 21	2	1	0.61

Results according to prognostic factors. Outcome and prognostic factors were analyzed in 276 (90%) of 309 LAME 89/91 patients who achieved CR after induction therapy. In multivariate analysis, OS was significantly higher in patients with favorable cytogenetic findings (t(8;21), t(9;11), t(15;17), inv(16) vs others ; p=0.005), in patients with low WBC count (<50×10^9/l vs ≥50×10^9/l; p=0.01), in patients achieving a CR1 with less than 20% marrow blasts on day 20 (p=0.03) and in patients receiving an allo-BMT in CR1 (p=0.03). Gender, age (<1 year old vs ≥1 year old), CNS involvement, FAB subtype (M5 versus others) were of no prognostic significance.

Results according to maintenance treatment. For randomized patients, DFS was 50%±15% with MT and 60%±19% without (p=0.25), and OS was respectively 58%±15% versus 81%±13% (figure 2; p=0.04). One toxic death occurred during MT in a randomized infant and was related to fulminans hepatitis. 31/70 patients relapsed ; MT- randomized patients had a higher likelihood of achieving a second CR than did MT+ patients (11/13 vs 8/18; p=0.03) (table 2).

For the whole population, including randomized and nonrandomized patients, the DFS was 50%±11% for MT+ patients and 63%±12% for MT- patients (p=0.48). The OS was 59%±11% for MT+ patients and 73%±11% for MT- patients (p=0.08). The probability to achieve a second CR was signifi-

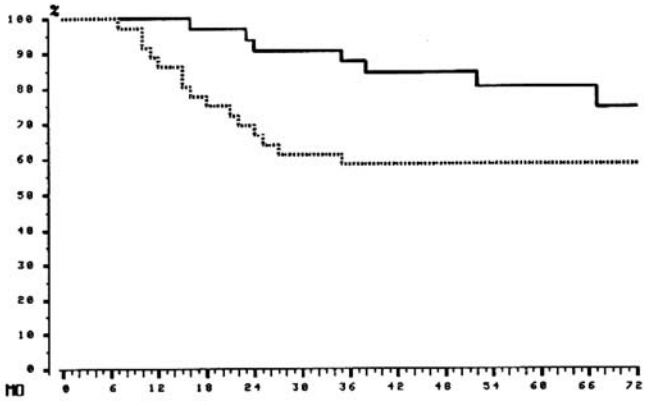

Figure 2. Overall survival comparison of randomized patients with maintenance therapy vs those without maintenance therapy; with time from the day of randomization.
.....OS with MT (n=36), _____ OS without MT (n=34); p=0.04

Table 2. Outcome according to maintenance therapy. Randomized patients

	Randomized patients	
	MT–	MT+
No. of patients	34	36
Relapses	13	18
CR2 achievement	11/13	8/18; p=0.03
Median of CR 2 duration (months)	28	15
Mean and range of CR 2 duration (months)	38 (5–96)	26 (4–75)

cantly higher for MT- patients than for MT+ patients (19/28 vs 14/34; p=0.04).

DICUSSION

With the LAME 89/91 intensive chemotherapy regimen, more than half of the children have been cured. Our results compare favorably with the more recently published pediatric AML studies. [2, 5, 8, 14, 15] Either drug-intensity or each of the three therapeutic phases may have contributed to the improvement in outcome.

Induction therapy. The 90% CR rate is within the range of the best results recently reported (74–92%) from large trial groups. [2, 8, 14, 15] With the combination of a high dose of mitoxantrone plus cytarabine, our intention was to reach a maximum drug-intensity. Intensifying induction therapy has been demonstrated to improve long-term prognosis in AML, irrespective of the postremission regimen, chemotherapy or autograft or allograft. [14]

Allo-BMT in CR1. Children who achieve CR have occult residual disease requiring intensified therapy or allo BMT early in first remission. Overall, superiority of allo BMT in reducing the relapse risk and increasing DFS was suggested in our preliminary LAME 89/91 report, [6] was demonstrated in the CCG experience[15] and is confirmed by these updated results.

Postremission therapy. In the LAME 89/91 protocol, consolidation 2, consisting of an intensive sequentially timed association of high-dose cytarabine, asparaginase and amsacrine, was the most striking phase of the postremission therapy. Dose escalation of cytarabine was used in most of the recent pediatric studies. [5, 8, 10, 12] The CCG 213P trial demonstrated the importance of timing of high-dose cytarabine consolidation. [12] The promising results with a very short, four-course, induction and cyclic intensification therapy in the MRC AML 10 protocol [10] or with the use of only three induction and intensification courses in the LAME 89/91 protocol support the role of drug-intensity.

Maintenance therapy. After standard-dose induction therapy, low-dose MT can improve DFS compared with no further therapy, at least in a subset of patients, both in adults and in children. The BFM protocol has successfully explored this approach ; CR was achieved in 82% of patients with a 5 year EFS at 51%. [2] In our randomized study, MT not only failed to improve the 5 year-DFS and to prevent relapse, but MT+ patients did significantly worse than MT- patients in terms of OS ; the negative predictive value of MT is demonstrated in randomized patients and is also suggested in all patients as treated. Similarly, in the randomized CCG trial 213, a phase of aggressive intensification eliminates the benefit of MT which was, in addition, even demonstrated to be inferior, in terms of survival, to stopping therapy. [13] It is worth noting that the very good long-term EFS for patients with the AML 10 MRC regimen was obtained without any low-dose MT. [10]

In the LAME 89/91 trial, only one death in CR occurred during MT with the result that the difference in terms of OS could not be related to toxicity. The probability to achieve a second CR was significantly better for MT- patients than for MT+ patients both in the randomized study and in all patients as treated. We suggest that the benefit in MT- patients, i.e. a better survival without either a significantly better DFS or a lowered toxic death rate, is related to this higher salvage after relapse.

The mechanism leading to treatment failure in relapsing MT+ patients remains unclear. Acquired drug resistance in the maintenance group might explain the lower CR2 rate. It has been suggested that MDR1 expression in AML blasts could be strongly up-regulated by cytarabine, and that it is correlated with clinical drug resistance. [3] It may be that long-term exposure to low-dose cytarabine will up-regulate MDR1 gene expression and ultimately lead, as in our study, to a higher rate of refractory disease after relapse in MT+ patients. However, cytarabine-induced up-regulation of MDR1 was not confirmed in paired analysis. [11] Mechanisms other than MDR1 might be responsible for the development of clinical drug resistance. [4,9].

Cure rate in AML has improved with intensive induction and postremission therapy with optimized timing. The best results were obtained with the most drug-intensive regimens. Low-dose MT is of no benefit in childhood AML after LAME 89/91 intensive chemotherapy.

Future improvements are unlikely to come from further increases in intensity, the indication of which could be limited to poor-risk leukemia and relapse, according to a risk-directed strategy in AML. Better chemotherapeutic agents, novel therapies, chemotherapy resistance modifiers should be explored.

The following options will be prospectively addressed in the forthcoming LAME protocol:

- to carry on with an intensive regimen including the LAME 89/91 unmodified induction therapy, three consolidations delivered over a short period and no MT
- to match treatment with prognostic factors (cytogenetics, marrow response on day 15)
- to offer an allo-BMT to most patients where an HLA-identical family donor is available
- to determine whether the use of low dose IL2, delivered after intensive induction and consolidation therapy confers any advantage in childhood AML.

References

1. Creutzig U, Ritter J, Zimmermann M and Schellong G. Does cranial irradiation reduce the risk for bone marrow relapse in acute myelogenous leukemia? Unexpected results of the Childhood Acute Myelogenous Leukemia Study BFM-87. J Clin Oncol 1993;11:279-86.
2. Creutzig U, Ritter J, Zimmermann M, Reinhardt D, Hermann J, Berthold F, et al. Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt-Munster 93. J Clin Oncol 2001;19:2705-13.
3. Hu XF, Slater A, Kantharidis P, Rischin D, Juneja S, Rossi R, et al. Altered multidrug resistance phenotype caused by anthracycline analogues and cytosine arabinoside in myeloid leukemia. Blood 1999;93:4086-95.
4. Klumper E, Ossenkoppele GJ, Pieters R, Huismans DR, Loonen AH, Rottier A, et al. In vitro resistance to cytosine arabinoside, not to daunorubicin, is associated with the risk of relapse in de novo acute myeloid leukaemia. Br J Haematol 1996;93:903-10.
5. Lie SO, Jonmundsson G, Mellander L, Siimes MA, Yssing M and Gustafsson G. A population-based study of 272 children with acute myeloid leukaemia treated on two consecutive protocols with different intensity: best outcome in girls, infants, and children with Down's syndrome. Nordic Society of Paediatric Haematology and Oncology (NOPHO). Br J Haematol 1996;94:82-8.
6. Michel G, Leverger G, Leblanc T, Nelken B, Baruchel A, Landman-Parker J, et al. Allogeneic bone marrow transplantation vs aggressive postremission chemotherapy for children with acute myeloid leukemia in first complete remission. A prospective study from the French Society of Pediatric Hematology and Immunology (SHIP). Bone Marrow Transplant 1996;17:191-6.
7. Perel Y, Auvrignon A, Leblanc T, Vannier JP, Michel G, Nelken B, et al. Impact of Addition of Maintenance Therapy to Intensive Induction and Consolidation Chemotherapy for Childhood Acute Myeloblastic Leukemia: Results of a Prospective Randomized Trial, LAME 89/91. J Clin Oncol 2002;20:2774-82.
8. Ravindranath Y, Yeager AM, Chang MN, Steuber CP, Krischer J, Graham-Pole J, et al. Autologous bone marrow transplantation versus intensive consolidation chemotherapy for acute myeloid leukemia in childhood. Pediatric Oncology Group. N Engl J Med 1996;334:1428-34.
9. Steinbach D, Lengemann J, Voigt A, Hermann J, Zintl F and Sauerbrey A. Response to Chemotherapy and Expression of the Genes Encoding

the Multidrug Resistance-associated Proteins MRP2, MRP3, MRP4, MRP5, and SMRP in Childhood Acute Myeloid Leukemia. Clin Cancer Res 2003;9:1083-6.
10. Stevens RF, Hann IM, Wheatley K and Gray RG. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. Br J Haematol 1998;101:130-40.
11. van den Heuvel-Eibrink MM, Wiemer EA, de Boevere MJ, van der Holt B, Vossebeld PJ, Pieters R, et al. MDR1 gene-related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. Blood 2001;97:3605-11.
12. Wells RJ, Woods WG, Lampkin BC, Nesbit ME, Lee JW, Buckley JD, et al. Impact of high-dose cytarabine and asparaginase intensification on childhood acute myeloid leukemia: a report from the Childrens Cancer Group. J Clin Oncol 1993;11:538-45.
13. Wells RJ, Woods WG, Buckley JD, Odom LF, Benjamin D, Bernstein I, et al. Treatment of newly diagnosed children and adolescents with acute myeloid leukemia: a Childrens Cancer Group study. J Clin Oncol 1994;12:2367-77.
14. Woods WG, Kobrinsky N, Buckley JD, Lee JW, Sanders J, Neudorf S, et al. Timed-sequential induction therapy improves postremission outcome in acute myeloid leukemia: a report from the Children's Cancer Group. Blood 1996;87:4979-89.
15. Woods WG, Neudorf S, Gold S, Sanders J, Buckley JD, Barnard DR, et al. A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission. Blood 2001;97:56-62.

Intensified Induction Therapy for Children with AML

W. G. WOODS
The Daniel P. Amos Children's Chair for the AFLAC Cancer Center and Blood Disorders Service, Atlanta, Georgia, USA

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Introduction and Purpose

Acute myeloid leukemia (AML) in both children and adults requires intensive myelosuppressive induction therapy for achieving a remission; and further aggressive post-remission intensification for durable long-term survival. In children improvement in both induction success and long-term outcome have been slow but consistent over the last 25 years.

For induction therapy in AML, the question of whether intensity of treatment effects long term outcome rather than just remission success had not been rigorously tested when the Children's Cancer Group (CCG) Trial 2891 opened in 1989, for newly diagnosed children with AML, myelodysplastic syndrome (MDS) and granulocytic sarcomas. The concept has important implications, as investigators treating patients with AML are often faced with whether or not to continue intensive therapy in the face of profound myelosuppression. Specifically, is the added myelosuppression in such a patient worth the risk of further prolongation of pancytopenia?

To test this hypothesis, children with AML were randomized at diagnosis to either a sequentially timed or standard timed induction approach. The primary end point of this study was patient survival from AML diagnosis.

Patients and Methods

CCG 2891 opened in October in 1989 and closed in April 1995. For the purpose of this report, children and adolescents younger than 21 years of age with blood and marrow biopsy confirmation of the diagnosis of the acute myeloid leukemia M0 to M7, and acute undifferentiated or biphenotypic leukemia with evidence of meyloid differentiation noted on cytologic examination were included. Patients with Down Syndrome, Fanconi Anemia, AML as a second malignant neoplasm, de novo MDS, isolated granulocytic sarcoma, or Ph' positive CML in the chronic phase have been excluded. Within this "de novo" AML subset, 887 patients were eligible and form the basis of this report.

All of the details of the treatment on CCG 2891 have been previously reported in detail [1,2]. Patients at diagnosis were randomized to two induction regimens in which identical drugs and doses were used and administered, with randomization between a "standard timing" and "intensive timing" approach. Patients received a 5-drug cycle of induction therapy administered over four days: dexamethasone, cytarabine, 6 thioguanine, etoposide, and rubidomycin (daunorubicin), or "DCTER." Patients randomized to intensive timing received a second obligatory cycle of DCTER therapy identical to Cycle #1 after a six-day rest, irrespective of marrow or hematologic status. Delays of two to four days were permitted for patients who experienced severe ileus or other life-threatening events with Cycle #1. Patients randomized to standard timing therapy had a bone marrow examination, including biopsy, on day 14. If there was evidence of clearing of circulating blasts in a hypoplastic marrow, indicating a large leukemia cell kill from the first cycle (two-thirds of the patients), Cycle #2 – identical to Cycle #1 – was held until the patients' blood counts recovered or there was clear sign of leukemia progression. The one-third of patients with residual leukemia documented on day 14, defined as more than 40% blasts in a mildly hypocellular to hypercellular marrow, received Cycle #2 at that time.

Four induction cycles were administered to all patients prior to entering the post-remission phase, even for patients achieving remission during the first two cycles. Standard timing induction therapy was closed in May 1993 after a recommendation by the Data Monitoring Committee, with all patients subsequently receiving the intensive timing arm. Furthermore, filgrastim was introduced for all patients during the induction phase at that time. This addition had no overall effect on induction success, post-remission outcome or overall

Table 1: Induction Results on CCG 2891 (N=838*)

Induction Regimen	Remission (%)	Fail (%)	Death (%)
Standard Timing (N=317)	229 (72%)	75 (24%)	13 (4%)
Intensive Timing (N=521)	416 (80%)	57 (11%)	48 (9%)
P Value	0.01	<0.001	0.006

* Excludes 36 patients who withdrew before induction success could be determined, and 13 patients who received 2 standard timing cycles with 2 subsequent intensified timing cycles (crossed over once Standard Timing Arm was closed in May 1993).

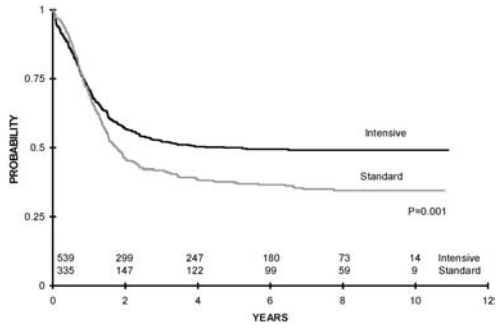

Figure 1 OS from study entry

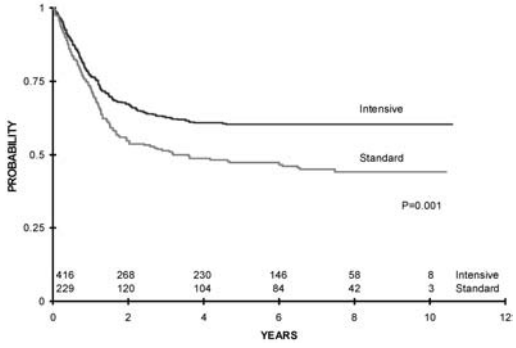

Figure 2 OS from EOI for patients in REM

survival, and results have been reported separately [3]. At the end of induction, patients with five – or six -antigen HLA matched family donors were allocated to allogeneic BMT [3]. The remaining patients were randomized between intensive therapy requiring autologous BMT rescue and non-marrow ablative therapy consisting of four courses of three different chemotherapy regimens, including one with intensively-timed high-dose cytarabine [2].

Data obtained in this study were reanalyzed in August 2001 with a cut-off of February 2001, and with use of several standard methods. All reported comparisons were based on regimens to which patients were allocated, or "intent to treat."

Results

Characteristics of the 887 patients included this study showed no statistical differences between patients randomized to the intensive timing and standard timing arms, including cytogenetics.

Although there were relatively small differences in the overall induction success rate, the reasons for failing to achieve remission were markedly different between the two arms. Patients randomized to the standard timing arm had a leukemia failure rate more than double than those on the intensive timing arm; while those randomized to the intensive timing arm were more than twice as likely to die from complications during induction therapy.

Patients who received intensive timing induction had an overall survival (OS) of 49±4% at 10 years vs. 35±5% for those on the standard timing arm (P=0.001). The advantage of intensive timing induction therapy was seen well past the induction phase (E0I).

Actuarial 0S at 10 years post-induction was 60±5% for those on the intensive timing arm and 44±7% for those on the standard timing arm (P=0.001).

The positive effect of intensive timing therapy on outcome in the post-remission phase was seen for patients allocated or randomized to all three post-remission regimens.

Summary, Conclusion and Brief Discussion

Intensively timed induction therapy for patients with childhood AML markedly improves long-term survival from diagnosis, as well as from achieving remission. Hence, intensity of induction treatment clearly effects long-term outcome rather than just remission success.

Several unanswered questions remain as to the optimum way to intensify therapy for children, adolescents, and adults with newly diagnosed AML. First, as reported by several national cooperative groups in this supplement, comparable long-term results can be seen in patients receiving aggressive induction therapy that is not based on a timing principle. Hence, the method of intensifying therapy may not be as important as the intensification itself.

Table 2: The Effect of Induction on Post-Remission Survival at 10 Years

Induction Program	(N)	Allogeneic BMT	(N)	Autologous BMT	(N)	Chemo-therapy	(N)
Standard Timing	(189)	44±14%	(63)	35±13%	(57)	47±12%	(69)
Intensive Timing	(337)	70±9%	(114)	55±10%	(115)	57±10%	(108)
P Value		0.007		0.02		0.34	

Furthermore, the results in children and adolescents may have direct applicability to young adults with newly diagnosed AML. As previously shown [4], results from CCG 2891 comparing adolescents 16–21 years of age versus children 0–15 show comparable results; while the intensive timing arm was superior to 16–21 year olds treated on the Leukemia Service at the MD Anderson Cancer Center (MDACC) over a several year period of time. At MDACC, 16–21 year olds had a similar survival to patients 21–45 years of age. Based on these results, MDACC is now examining the role of intensive timing induction therapy in young adults with newly diagnosed AML presenting to their institution.

Finally, the CCG 2891 results utilizing intensive timing have been confirmed in two subsequent CCG trials, CCG 2941 (Ref. 5), and CCG 2961, which is reported separately.

References

1. Arthur DC, Barnard DR, Buckley JD, DeSwarte J, Dusenbery K, Gold S, Kalousek D, Kobrinsky N, Lange BJ, Lee JW, Neudorf S, Sanders J, Woods WG (1996) Timed-sequential induction therapy improves postremission outcome in acute myeloid leukemia: A report from the Children's Cancer Group. *Blood* 87:4979-4989.
2. Arthur DC, Barnard DR, Buckley JD, DeSwarte-Wallace J, Dusenbery K, Gold S, Kobrinsky NL, Lange BJ, Neudorf S, Sanders J, Woods WG (2001) A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation and aggressive chemotherapy in children with AML in remission: A report from the Children's Cancer Group. *Blood* 97:56-62.
3. Alonzo TA, Aledo A, Buxton AB, Kobrinsky NL, Lange BJ, Woods WG (2002) Impact of granulocyte colony-stimulating factor use during induction for acute myelogenous leukemia in children: A report from the Children's Cancer Group. *Journal of Pediatric Hematology/Oncology* 24:627-635.
4. Alonzo T, Estey E, Jeha S, Lange BJ, Woods WG (2001) Acute myeloid leukemia (AML) in adolescents and young adults (AYAs): A comparison of outcomes between patients treated on childhood or adult protocols. *Blood* 98:462a-463a.
5. Dindorff P, Lange BJ, Smith F, et al. A pilot study of idarabicin-based intensive timing induction therapy for children with previously untreated AML – CCG Study 2941. In press, *J Clin Oncol* 2004.

Main Session VII

Evolution of BFM Trials for Childhood ALL

M. SCHRAPPE
Department of Pediatric Hematology and Oncology, Hannover Medical School, 30625 Hannover, Germany

Abstract

Up to 80% of pediatric patients with acute lymphoblastic leukemia (ALL) can be cured if intensive therapy is applied. Severe side effects are encountered in all patients of which, however, only the minority is life-threatening. The leading cause of failure in childhood ALL is still recurrence of disease. To reduce the rate of relapses, but also to limit treatment morbidity, the ALL-BFM group has aimed to improve the risk-adaptation of therapy. The most important addition to clinical factors (e.g. age, WBC, extramedullary involvement), and biological characteristics (such as immunphenotype and cytogenetics), was the recognition of early *in vivo* treatment response as the strongest predictor for relapse. The determination of leukemic blasts in peripheral blood after exposure to 7 days of prednisone (PRED) and one dose of intrathecal methotrexate (*prednisone response*) as developed by BFM identified multidrug resistant patients: Such patients had still more than 1,000 blasts per µL at day 8 of therapy (defined as PRED poor responders, 10% of all patients). Prognosis for these was only approximately 35% as compared to approximately 80% in patients with adequate PRED response.

Patient characteristics at relapse reveal that most of them were originally comprised in "good risk" patient subgroups: e.g., in trial ALL-BFM 90, 50% of the relapses were noted in patients with c-ALL even though that group had an EFS of 82% (SE 1%). 70% of the recurrences are found among patients with good response to PRED indicating the lack of specificity in the definition of that subgroup. Therefore, the more refined way of determining *in vivo* response based on the detection of minimal residual disease (MRD) at defined timepoints by identifying clone-specific T-cell receptor- (TCR) or immunglobuline (Ig) gene rearrangements appears to be able to define the patient at high risk to relapse more specifically. In the current ALL-BFM strategy, the high sensitivity of the method is utilized to apply treatment reduction in patients with fast clearance of leukemia. Persistent disease in contrast is an indication for treatment modification and intensification. Logistics and quality controls are demanding but essential for the introduction of this new technology into clinical practice.

Recent developments derived from BFM and other major clinical ALL trials

In childhood ALL, clinical and biological features such as patient age, WBC, organ involvement, immunophenotype and cytogenetics have been used by all major study groups to modulate treatment composition and intensity. Treatment intensity itself turned out to be a major prognostic factor [1–6] The BFM study group has addressed the impact of treatment intensity early on in randomized trials ALL-BFM 76 and 83 conducted between 1976 and 1986 [6, 7]. It was demonstrated that the repetition of (slightly) modified induction therapy was highly efficient in reducing relapses both in high and standard risk patients. The importance of delayed reintensification for standard and intermediate risk patients was confirmed in trials ALL-BFM 86 [1] and CCG-105 [8]. The ALL-BFM Study Group also demonstrated, that in standard risk patients, cranial radiotherapy is an essential component of ALL therapy if not replaced by adequately dosed methotrexate [6, 9]. The prognostic influence of early treatment response was prospectively evaluated in trial ALL-BFM 83, and later utilized in trials ALL-BFM 86 and 90 to identify a group of ALL patients with the highest risk for relapse [1, 10, 11]. Various approaches to determine the early *in vivo* response were developed in the last 15 years, mainly concentrating on the cytomorphological evaluation of blast cell clearance from the peripheral blood and the bone marrow [12–14]. Only recently, more sensitive methods of minimal residual disease detection have been applied systematically to evaluate the *in vivo* response [15–18]. The predictive value of MRD determination, however, depends completely on timing and intensity of therapy [19, 20]. In parallel to recent advances in risk-adaptation of treatment, some important attempts have been made to reduce the toxicity by limiting in particular the components with the potentially most relevant long-term toxicity, e.g. such as radiotherapy.

Prognostic parameters at diagnosis

WBC, age, gender, cytogenetic and immunphenotypic subtypes are the major initial factors determining the risk of relapse [1, 11]. To determine the risk of relapse it is useful to rely on parameters which are uniformly available and not subject to methodological variations. Standard (SR) and high risk (HR) patients have been defined by age/WBC subsets, e.g. in the classification of the National Cancer Institute: SR, age 1-9 years, and WBC <50,000/mm^3; HR, age ≥10 years or WBC ≥50,000/mm^3 [21]. The BFM group prepared results accordingly, thus demonstrating a 6y-EFS of 86±1% for NCI-SR patients (n=1395), and 64±2% for NCI-HR patients (n=724) in trial ALL-BFM 90 (T-ALL patients included). Infants which were not included in the NCI definition were also analyzed: their 6y-EFS was 50±7% [11]. With regard to the risk groups as defined in trial ALL-BFM 90, 6y-EFS was 85±2, 82±1, and 34±3% for SR (n=636), MR (n=1299), and HR (n=243) patients, respectively. Molecular screening for fusion genes and cytogenetics at diagnosis are useful methods to identify some of the high risk patients, but the majority of the patients at risk will not be recognized [22].

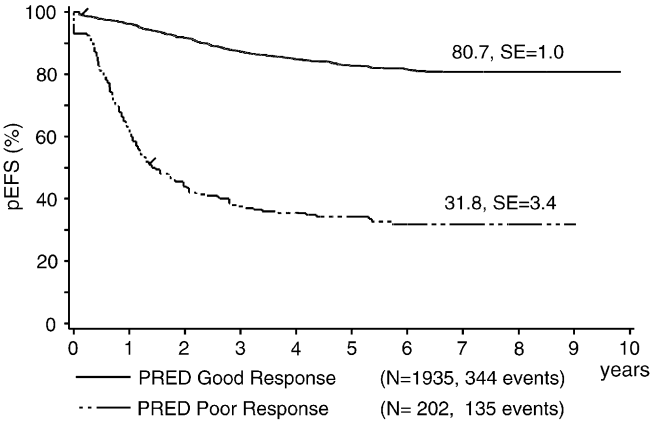

Fig. 1: Event-free survival (at 8 years) in trial ALL-BFM 90 according to prednisone response

Response evaluation: New approach to more specific risk prediction

Early response to prednisone

Response to treatment as measured by blast count in peripheral blood after 7 days of PRED and one IT injection of MTX had prospectively been evaluated in trial ALL-BFM 83 [10]. This study identified a novel prognostic marker that was more predictive of relapse than any other marker used thus far: 8% of the patients formed a small poor risk group that was characterized by the presence of ≥1000 leukemic blasts per µl peripheral blood after the first week of prednisone (PPR). After a median observation time in trial ALL-BFM 83 of 10 years, the probability for event-free survival (pEFS) for patients with PPR is 39%, compared to 66% in patients with adequate response to prednisone (PGR). No other mathematical model nor any other single factor could describe a group of patients that was as large, and had a prognosis of less than 50% pEFS. Reflecting the reliability of this method, 8–10% of the patients have been identified as PRED poor responders (PPR) in all three BFM trials since 1983. In addition, some characteristics have constantly been observed in this patient subset [1, 10] which indicate that this parameter is associated with some well known poor risk features (Tab. 1). The specificity of PPR was high, but the specificity of PGR was limited, as – due to the group size - the majority of relapses (approximately 65%) was observed in the favorable group of patients with PGR (Fig. 1).

Any treatment variation or intensification that was applied in trials ALL-BFM 86 and 90 for patients with PPR did not significantly alter the outcome of this group [11]. In trial ALL-BFM 95, however, significant improvement for patients with PPR has been demonstrated: 56±4% vs. 34±3% in trial ALL-BFM 90 [23]. The AIEOP study group demonstrated in trial AIEOP 9503 that high risk patients defined by PPR had an improved outcome by the repeated use of *Protocol II*, the key reintensification therapy element developed in trial ALL-BFM 76 [7, 24]. Other study groups also utilized the prognostic significance of blast cell reduction in PB or BM [5, 12, 14].

Response in the Bone Marrow

Since 1990, the BM on day 15 of therapy has been routinely evaluated in the central laboratory of the ALL-BFM trial. In contrast to the evaluation of blood smears, conclusive results were prevented occasionally by low cellularity. In addition, the specificity of the BM evaluation was limited: The same number (not percent!) of relapses was observed among patients with a M1, or a M2, or a M3 marrow on day 15. Nevertheless, a M3 marrow – found in 13% of patients – was an indicator of high relapse risk, as 6y-EFS was only 44±5%. When only patients with PRED-GR were analyzed, an M3 marrow was demonstrated in 7.3% of the patients, with an EFS of 54±7%. Failure to achieve remission as shown by a M2 or M3 BM at control time (in BFM, day 33 of induction), was the most adverse prognostic factor in trial ALL-BFM 90, as 6y-EFS was only 11±5% for such patients. This poor risk subset comprises, however, only 2.5% of all patients [11].

Response as assessed by evaluation of minimal residual disease (MRD)

In an effort to provide better specificity by response evaluation and to identify previously unrecognized patients at high risk to relapse several studies have been initiated in the 90s which aimed at a more sensitive, sub-microscopic

Table 1: Outcome by prednisone good-response and prednisone poor-response in specific patient subsets: Data from recent ALL-BFM trials

Patient subsets	Prednisone Good-Response		Prednisone Poor-Response	
	No. of patients	% EFS (SE)[1]	No. of patients	% EFS (SE)[1]
NCI risk group[2]				
Standard risk	1324	87 (1)	48	45 (7)
High risk	564	73 (2)	143	31 (4)
Immunophenotype				
Pro-B ALL	80	68 (6)	19	0
Common ALL	1274	84 (1)	67	46 (6)
Pre-B ALL	338	79 (2)	13	31 (13)
T ALL	180	78 (3)	101	32 (5)
Genetic aberrations				
t(9;22) or BCR/ABL pos.	37	55 (8)	20	10 (7)
Infants				
all infants	78	53 (6)	27	15 (7)
infants with 11q23 rearrangement	17	41 (12)	11	9 (9)
infants t(4;11) or MLL/AF4 positive	9	33 (16)	7	0

[1] Percent event-free survival (standard error) at 6 years; [2] For explanation of NCI risk groups see Smith, M. et al.

monitoring of the leukemic cells during therapy. The two main techniques used are flow cytometry utilizing aberrant immunophenotypes for clone detection, and DNA based PCR technology to identify clone-specific rearrangements of the T-cell receptor and immunoglobuline genes. Both techniques are applicable to approximately 90% of the ALL patients. For the collaborative study of the International BFM Study Group (I-BFM-SG) performed in Germany, Italy, Austria and the Netherlands, the DNA-based clonospecific detection of MRD was chosen due to the high sensitivity of the approach. This technique had been shown to provide a new tool for prognostic evaluation of patients with childhood ALL [25, 26]. This study of four laboratories identified three distinct risk groups on the basis of semiquantitative MRD determination at 5 and 12 weeks of therapy [16]: Two thirds of relapses were found among patients with no or very slow clearance of MRD (this is the newly defined high risk group), but virtually no failures were seen in the large group of patients (40%) with fast clearance of leukemic blasts (considered new low risk group). Another 40% of the patients had residual disease after 5 weeks of induction therapy but only low level or no detectable disease at 12 weeks. These patients are considered intermediate risk [17]. Interestingly, the response kinetics as measured by MRD detection were quite different between patients with B-precursor ALL and T-ALL [27]. For the small but significant patient subset which cleared their leukemic cells to a level of less than 10^{-4} after only 14 days of BFM induction therapy, the recurrence rate was none [18]. A Scandinavian study also found that very fast clearance of blasts as assessed by a slightly different but sensitive DNA-based MRD detection assay is associated with an excellent prognosis [20]. Thus, the MRD studies performed by the I-BFM-SG and the Scandinavian study suggest that treatment reduction might be possible in these newly identified low-risk patients.

Another large study done by the EORTC could also specifically identify high risk patients by DNA-based MRD detection after induction therapy [28]: The sensitivity of that approach was lower, thus, the lack of a MRD signal after induction did not translate into freedom of relapse. Using immunological detection of MRD, a St. Jude study demonstrated the overall prognostic value of MRD but could also not find a group of patients without risk of relapse [15]. This could, however, also be due to the different timing of MRD evaluation during a therapy which is also differently composed as compared to the BFM-based regimen used in the two European studies mentioned above. Remarkable differences are seen in the pattern of MRD levels if the composition and duration of induction therapy differs [19]: If only three drugs are used in induction therapy, the specificity of the post-induction MRD result is low if the BM is analysed also at 5 weeks. Another study which analysed ALL samples for MRD by flow cytometry based on BFM therapy found a strong prognostic impact of persisting MRD signals. The approach was applicable to a high proportion of the patients but also demonstrated that some results from flow cytometry based MRD detection have to be taken with caution due to interaction with regenerating normal but immature lymphocytes [29].

Perspective

All risk parameters have to be investigated for their overall prognostic relevance. This means that identification of a rare biological factor which correlates with a poor prognosis might not be very relevant for the total cohort, as the outcome of the total group will not change much even when patients with that parameter can be treated more adequately. On the other hand, if the unfavorable subset of a given parameter comprises a large number of recurrences, new and more effective therapy might improve overall outcome substantially. In addition, if a parameter such as the high persistent level of MRD is highly specific and is correlated with a dismal prognosis, it is justified, even mandatory, to develop experimental therapies. Such a rational approach in utilizing the specificity of response parameters (including MRD evaluation) has been initiated now by a large multicenter trial of the AIEOP and BFM ALL Study Groups in Germany, Austria and Italy. This study will still include the – successful – principle of over-treatment but it will attempt to limit the over-treatment in a large, well-defined subset of patients. It appears possible that these low-risk patients can be spared some of the toxicity and late effects which were previously and are still currently found in children treated for ALL. A very similar approach is taken by a new trial of the International BFM Study Group in which response in PB and BM at three time points of induction forms the basis for stratification into three risk groups without the use of MRD evaluation.

References

[1] Reiter A, Schrappe M, Ludwig W-D, Hiddemann W, Sauter S, Henze G, Zimmermann M, Lampert F, Havers W, Niethammer D, Odenwald E, Ritter J, Mann G, Welte K, Gadner H, Riehm H: Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994; 84: 3122 - 33

[2] Schorin MA, Blattner S, Gelber RD, Tarbell NJ, Donnelly M, Dalton V, Cohen HJ, Sallan SE: Treatment of childhood acute lymphoblastic leukemia: Results of Dana-Farber Cancer Institute/Children's Hospital Acute Lymphoblastic Leukemia Consortium Protocol 85-01. J Clin Oncol 1994; 12: 740-7

[3] Chessells JM, Bailey C, Richards SM: Intensification of treatment and survival in all children with lymphoblastic leukaemia: results of UK Medical Research Council trial UKALL X. Lancet 1995; 345: 143-8

[4] Pui CH: Childhood leukemias. N Engl J Med 1995; 332: 1618-30

[5] Nachman JB, Sather HN, Sensel MG, Trigg ME, Cherlow JM, Lukens JN, Wolff L, Uckun FM, Gaynon PS: Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med 1998; 338: 1663-71

[6] Schrappe M, Reiter A, Zimmermann M, Harbott J, Ludwig WD, Henze G, Gadner H, Odenwald E, Riehm H: Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Leukemia 2000; 14: 2205 - 22.

[7] Riehm H, Gadner H, Henze G, Kornhuber B, Lampert F, Niethammer D, Reiter A, Schellong G: Results and significance of six randomized trials in four consecutive ALL-BFM trials. Haematol Blood Transfus 1990; 33: 439 - 50

[8] Tubergen DG, Gilchrist GS, O'Brien RT, Coccia PF, Sather HN, Wasserwitz MJ, Hammond GD: Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features: a Childrens Cancer Group phase III trial. J Clin Oncol 1993; 11: 527 - 37

[9] Schrappe M, Reiter A, Riehm H: Prophylaxis and treatment of meningeosis in childhood acute lymphoblastic leukemia. J Neuro-Oncol 1998; 38: 159 - 65

[10] Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein H, Ludwig R, Ritter J, Stollmann B, Henze G: Die Corticosteroid-abhängige Dezimierung der Leukämiezellzahl im Blut als Prognosefaktor bei der akuten lymphoblastischen Leukämie im Kindesalter (Therapiestudie ALL-BFM 83) [The in vivo response on corticosteroid therapy as an additional prognostic factor in childhood acute lymphoblastic leukemia (therapy study ALL-BFM 83). Klin Pädiatr 1987; 199: 151 -60

[11] Schrappe M, Reiter A, Ludwig W-D, Harbott J, Zimmermann M, Hiddemann W, Niemeyer C, Henze G, Feldges A, Zintl F, Kornhuber B, Ritter J, Welte K, Gadner H, Riehm H: Improved outcome in childhood ALL despite reduced use of anthracyclines and of cranial radiotherapy: Results of trial ALL-BFM 90. Blood 2000; 95: 3310 - 22

[12] Gajjar A, Ribeiro R, Hancock ML, Rivera GK, Mahmoud H, Sandlund JT, Crist WM, Pui CH: Persistence of circulating blasts after 1 week of multiagent chemotherapy confers a poor prognosis in childhood acute lymphoblastic leukemia. Blood 1995; 86: 1292-5

[13] Steinherz PG, Gaynon PS, Breneman JC, Cherlow JM, Grossman NJ, Kersey JH, Johnstone HS, Sather HN, Trigg ME, Chappell R, Hammond D, Bleyer WA: Cytoreduction and prognosis in acute lymphoblastic leukemia – the importance of early marrow response: report from the Childrens Cancer Group. J Clin Oncol 1996; 14: 389-98

[14] Gaynon PS, Desai AA, Bostrom BC, Hutchinson RJ, Lange BJ, Nachman JB, Reaman GH, Sather HN, Steinherz PG, Trigg ME, Tubergen DG, Uckun FM: Early response to therapy and outcome in childhood acute lymphoblastic leukemia: a review. Cancer 1997; 80: 1717-26

[15] Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, Rubnitz JE, Rivera GK, Sandlund JT, Pui CH, Campana D: Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 1998; 351: 550-4
[16] van Dongen JJM, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L, Stolz F, Schrappe M, Masera G, Kamps WA, Gadner H, van Wering ER, Ludwig W-D, Basso G, de Bruijn MAC, Cazzaniga G, Hettinger K, van der Does-van den Berg A, Hop WCJ, Riehm H, et al.: Prognostic value of minimal residual disease in childhood acute lymphoblastic leukemia: A prospective study of the International BFM Study Group. Lancet 1998; 352: 1731-8
[17] Biondi A, Valsecchi MG, Seriu T, d'Aniello E, Willemse MJ, Fasching K, Pannunzio A, Gadner H, Schrappe M, Kamps WA, Bartram CR, van Dongen JJM, and Panzer-Grumayer ER: Molecular detection of minimal residual disease is a strong predictive factor of relapse in childhood B-lineage acute lymphoblastic leukemia with medium risk features. A case control study of the International BFM Study group. Leukemia 2000; 14: 1939 - 43
[18] Panzer-Grumayer ER, Schneider M, Panzer S, Fasching K and Gadner H: Rapid molecular response during early induction chemotherapy predicts a good outcome in childhood acute lymphoblastic leukemia. Blood 2000; 95: 790-794.
[19] zur Stadt U, Harms DO, Schluter S, Schrappe M, Goebel U, Spaar H, Janka G, and Kabisch H: MRD at the end of induction therapy in childhood acute lymphoblastic leukemia: outcome prediction strongly depends on the therapeutic regimen. Leukemia 2001; 15: 283-5
[20] Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N, Wesenberg F, Jonsson OG, Forestier E, Schmiegelow K: Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood 2002; 99: 1253-8
[21] Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, Gelber R, Heerema N, Korn EL, Link M, Murphy S, Pui CH, Pullen J, Reamon G, Sallan SE, Sather H, Shuster J, Simon R, Trigg M, Tubergen D, et al.: Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996; 14: 18 - 24
[22] Schlieben S, Borkhardt A, Reinisch I, Ritterbach J, Janssen JWG, Ratei R, Schrappe M, Repp R, Zimmermann M, Kabisch H, Janka-Schaub G, Bartram CR, Ludwig W-D, Riehm H, Lampert F, Harbott J: Incidence and clinical outcome of children with BCR/ABL-positive acute lymphoblastic leukemia (ALL). A prospective RT-PCR study based on 673 patients enrolled in the German pediatric multicenter therapy trials ALL-BFM 90 and CoALL-05-92. Leukemia 1996; 10: 957-63
[23] Schrappe M, Reiter A, Harbott J, Zimmermann M, Henze G, Boos J, Gadner H, Ludwig W.-D., Riehm H: Improved Risk-Adapted Treatment of Childhood ALL with a New Stratification System Based on Early Response, Genetics, Age, and WBC: First Interim Analysis of Trial ALL-BFM 95. Blood 2001; 98: 718a
[24] Aricò, M, Valsecchi MG, Conter V, Rizzari C, Pession A, Messina C, Barisone E, Poggi V, De Rossi G, Locatelli F, Micalizzi MC, Basso G, Masera G: Improved outcome in high risk childhood acute lymphoblastic leukemia defined by prednisone poor response treated with double BFM Protocol II. Blood 2002; 100: 420 - 426.
[25] Foroni L, Coyle LA, Papaioannou M, Yaxley JC, Sinclair MF, Chim JS, Cannell P, Secker-Walker LM, Mehta AB, Prentice HG, Hoffbrand AV: Molecular detection of minimal residual disease in adult and childhood acute lymphoblastic leukaemia reveals differences in treatment response. Leukemia 1997; 11: 1732-41
[26] Seriu T, Erz D, Stark Y, Bartram CR: T cell receptor Ddelta2Ddelta3 rearrangement: a suitable allele-specific marker for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia 1997; 11: 759-61
[27] Willemse MJ, Seriu T, Hettinger K, d'Aniello E, Hop WC, Panzer-Grumayer ER, Biondi A, Schrappe M, Kamps WA, Masera G, et al. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 2002; 99 (12): 4386-93
[28] Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer - Childhood Leukemia Cooperative Group. N Engl J Med 1998; 339 (9): 591-8
[29] Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N, Fritsch G, Gadner H. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 2002; 99 (6):1952-8.

Significance of t(12;21) and High Hyperdiploidy with +4, +10 and +17 (Triple Trisomy) in B-Precursor Acute Lymphoblastic Leukemia

J. B. NACHMAN
Pritzker School of Medicine, University of Chicago, Chicago, IL, USA

Abstract

Both the presence of the T(12:21) and high hyperdiploidy with +4, +10 and +17 (triple trisomy) are favorable prognostic factors in children with NCI standard-risk B-precursor acute lymphoblastic leukemia. The 5-year EFS for both groups is 85%. However, the kinetics of early response and the patterns of treatment failure are markedly different in the 2 groups. Triple trisomy is also a highly significant favorable prognostic factor for NCI high-risk patients. A question remains as to whether more aggressive chemotherapy might further improve outcome for NCI standard-risk ALL patients with T(12:21) or triple trisomy.

Significance of T(12:21) and High Hyperdiploidy with +4, +10, +17 (Triple Trisomy) in B-Precursor Acute Lymphoblastic Leukemia

Introduction

The outcome for children and adolescents with acute lymphoblastic leukemia (ALL) has improved over time. Five-year event-free survival (EFS) for children and adolescents with B-precursor ALL are approximately 70-80% with modern therapy. Treatment intensification has generally lead to better EFS results for children and adolescents with B-precursor ALL. However, it must be borne in mind that 40–50% of patients with B-precursor ALL may be cured with simple anti-metabolite-based therapy. This has led to a search for prognostic factors which might identify both low-risk patients who could achieve cure with minimal therapy and high-risk patients who may require further therapeutic intensification which might include stem cell transplantation. Age and WBC are two critical pretreatment prognostic factors. In addition, both classical and molecular cytogenetic abnormalities in ALL blasts have significant prognostic importance. The presence of the 12/21 translocation (TEL-AML1) or a high hyperdiploid karotype including +4, +10 and +17 (triple trisomy) is associated with a favorable outcome, while the presence of the 9/22 translocation (PH+) or the 4/11 translocation is associated with a poor outcome. Hypodiploidy (<45 chromosomes) is also associated with an adverse outcome. In this paper, we present selected analyses concerning the prognostic significance and outcome for children with T(12:21) and triple trisomy entered on CCG ALL studies between 1995–2002.

Influence of T(12:21) and/or Triple Trisomy in NCI Standard-Risk Patients

Standard-risk ALL includes patients 1–9 years of age and <50,000 WBC. On the CCG-1952 trial for standard-risk ALL, patients received 3-agent induction (vincristine, prednisone and L-asparaginase), CNS prophylaxis with IT methotrexate alone, interim maintenance with vincristine, prednisone, 6MP, MTX, and 2 delayed-intensification courses (dexamethasone, vincristine, L-asparaginase, adriamycin, cytoxan, cytosine arabinoside and 6-thioguanine), followed by maintenance therapy. Patients with an M3 day-14 bone marrow received intensified therapy (augmented BFM). Patients were randomized to IT-MTX versus triple intrathecal therapy (hydrocortisone, ARA-C, MTX) and 6-mercaptopurine or 6-thioguanine. Two thousand one hundred seventy four patients were entered on CCG-1952. Of these, 1009 patients were tested for the T(12:21), and 193 patients were identified as having the translocation. One hundred twenty eight of 942 evaluable patients had triple trisomy. The 5-year EFS for patients with the T(12:21) was 85.3% compared to 79.6% for patients lacking the T(12:21) (P=0.02). The 5-year EFS for triple trisomy patients was 86.1% compared to 79.9% for non-TT patients (P=0.03). Despite the similar excellent EFS results for these 2 cytogenetic subgroups, the kinetics of early marrow response and the pattern of treatment failure were very different in the 2 groups. In the triple trisomy group, 30.9% of patients had an M3 day-7 marrow compared to only 14.5% of patients with an M3 marrow in the T(12:21) group. Patients with triple trisomy have also been shown to have higher levels of MRD at end induction than patients with T(12:21).

The following table shows the type of events occurring in the triple trisomy and T(12:21) groups.

Thus, marrow is the predominant relapse site for T(12:21) patients, while the CNS is the predominant site of relapse for triple trisomy patients. Four hundred forty seven patients had both T(12:21) data and ploidy data avail-

Table 1: Events in T(12:21) and Triple Trisomy Patients

Event	T(12:21) *N=194*	Triple Trisomy *N=129*
M3	12	3
CNS	4	7
Testicular	2	1
Other	1	1
Death in remission	3	0

able. One hundred thirty nine patients had either T(12:21) or triple trisomy, while 308 patients had neither favorable cytogenetic feature. The 5-year EFS for the T(12:21) or triple trisomy group was 84.7% versus 77.6% for the group lacking either favorable cytogenetic feature (P=0.01).

Significance of triple trisomy in NCI high-risk patients

NCI high-risk patients were treated on the CCG-1961 study. Patients were classified as either rapid or slow early responders on the basis of a day-7 bone marrow aspirate (≤25% blasts-rapid response; >25% blasts-slow response). Rapid early responders were randomized to CCG-modified BFM therapy, CCG-modified BFM with a second delayed intensification, augmented BFM, or augmented BFM with a single delayed intensification. Slow early responders received augmented BFM with or without cytoxan/idarubicin in delayed intensification phases. Approximately 5% of patients with evaluable cytogenetics had triple trisomy (N=51). Of these 51 patients, 24 were rapid responders. There have been no events in the 24 rapid responder patients and only 3 of the 27 slow early responders have had events (2 marrow relapses and 1 death in remission). Ninety-five high hyperdiploid patients had +10. There were no events among 52 rapid responder patients and 6 events in the 43 slow early responders (2-death in remission: 4-marrow relapse).

Conclusion

In NCI standard-risk patients, both T(12:21) and triple trisomy predict for excellent outcome. However, the two groups differ significantly with respect to response kinetics and pattern of events. In the NCI high-risk patients, triple trisomy patients also have an excellent outcome, and the outcome is better than that for standard-risk patients with triple trisomy. This raises the issue of whether intensification of treatment might improve the already excellent outcome for standard-risk triple trisomy patients.

Rationale and Design of Total Therapy Study XV for Newly Diagnosed Childhood Acute Lymphoblastic Leukemia

C.-H. PUI[1,2,4], M. V. RELLING[3,4,5], J. T SANDLUND[1,4], J. R. DOWNING[2,4], D. CAMPANA [1,2,4] , and W. E. EVANS [3,4,5]
[1]Dept. of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; [2]Dept. of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; [3]Dept. of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; [4]College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, USA; [5]College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, USA

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Abstract

The current cure rate of 80% in childhood acute lymphoblastic leukemia (ALL) attests to the effectiveness of risk-directed therapy developed through well-designed clinical trials. The ongoing Total Therapy Study XV at St. Jude Children's Research Hospital was designed to further increase cure rate and to improve quality of life. The study consists of intensive systemic and intrathecal therapy but does not include cranial irradiation, irrespective of a patient's risk features. The intensity of postremission consolidation, continuation and reinduction therapy is based on the level of minimal residual disease at the end of induction, as measured by both flow cytometric detection of aberrant immunophenotypes and polymerase-chain-reaction amplification of clonal antigen-receptor gene rearrange-ments. Status of thiopurine methyl-transferase is determined prospectively for treatment modification. Pharmacogenetic, pharmacodynamic, gene expression and proteomic profiling studies of host normal cells and leukemic cells are performed in parallel to elucidate the mechanisms of drug resistance and to advance our understanding of leukemogenesis.

Key words: Acute lymphoblastic leukemia, Cranial irradiation, Methotrexate, Central-nervous-system leukemia, Risk assessment.

Introduction

Despite the cure rate of 80% achieved in some of the most successful clinical trials for childhood acute lymphoblastic leukemia (ALL) to date [1], refractory and relapsed forms of this disease still represent a leading cause of cancer-related deaths in children and pose formidable challenges. Moreover, a substantial proportion of the survivors of childhood ALL encounters long-term complications [2]. Current efforts focus on the optimization of the use of the existing antileukemic agents, early and vigorous assessment of the risk of relapse, and the pharmacogenetics of host normal cells to avoid under- or over-treatment [2]. The goal is to improve not only the cure rate but also the quality of life of the patients. In our current study (Total Therapy XV), emphasis is placed on the reduction of long-term sequelae by limiting the cumulative doses of anthracyclines (100 mg/m^2 in low-risk and 230 mg/m^2 in standard- or high-risk cases) and cyclophosphamide (1 gm/m^2 and 4.6 gm/m^2, respectively), and by omitting cranial irradiation in all patients. Etoposide is given only to a small proportion of the patients (~5%) who will undergo allogeneic hematopoietic stem cell transplantation for high-risk leukemia. Here we briefly describe the rationale of the treatment approach of the study.

Risk assessment

Presenting age, leukocyte count, and leukemic cell genotype are used for risk classification in virtually all contemporary clinical trials [3]. Treatment response to remission-induction therapy is also a well-recognized independent prognostic factor [2, 4]. In this regard, evaluation of treatment response has been significantly refined by the development of methods to measure minimal residual disease, such as flow cytometric detection of aberrant immunophenotypes and polymerase-chain-reaction analysis of clonal antigen-receptor gene rearrangements. These methods are at least 100-fold more sensitive than morphological examination and their specificity is also more superior [5–7].

In study XV, patients are assigned to three risk groups—low, standard- and high-risk (corresponding to standard-, high, and very high-risk categories in other protocols). B-cell precursor cases with age between 1 and 10 years and presenting leukocyte count $<50\times10^9$/L, leukemic cell DNA index ≥1.16, or *TEL-AML1* fusion are provisionally classified to have low-risk ALL, provided that they do not have testicular or central-nervous-system (CNS) leukemia (i.e., CNS3 status), hypodiploidy (<45 chromosomes), *E2A-PBX1* fusion, or *MLL* rearrangement. Patients with *BCR-ABL* fusion (Philadelphia chromosome) are designated to have high-risk disease, and all others including all T-cell ALL cases are provisionally classified to have standard-risk ALL. Final risk status depends on the response to remission-induction therapy. Any patients with 0.01% to 0.99% residual leukemia after completion of 6-week induction therapy are considered to have standard-risk ALL and receive intensive postremission therapy, whereas those with 1% or more residual disease are designated to have high-risk ALL and are candidates for allogeneic hematopoietic stem cell transplantation.

Upfront-treatment with high-dose metho-trexate

We have been studying the pharmaco-dynamics of methotrexate, mercaptopurine, or both in an upfront window-therapy approach (i.e., before conventional remission induction) since 1991 in consecutive Total Therapy studies. Given the same dosage, T-cell leukemic blasts accumulate significantly less methotrexate polyglutamates than B-lineage blasts; among B-lineage cases, non-hyperdiploid blasts accumulate less polyglutamates than hyperdiploid blasts [8]. The findings support the use of increased dose of methotrexate (e.g., 5 gm/m^2) in T-cell and higher-risk B-cell precursor cases [9]. The length of exposure is another important determinant of methotrexate polyglutamate formation and anti-leukemic effects in preclinical in vitro and in vivo models, with longer exposures at equal extracellular concentrations resulting in greater polyglutamylation and effects [10, 11]. Because findings from cell lines might not apply to patients, we are conducting an upfront window study in which patients are stratified and randomized to receive methotrexate at a dose of 1 gm/m^2 administered over 24 hours or the same dose over 4 hours. While both schedules should yield equivalent systemic exposure (i.e., the area under the plasma concentration-time curve), we will determine if methotrexate polyglutamate accumulation and pharmacodynamics differ between the two treatment groups and if the optimal duration of treatment differs between major ALL subtypes. Gene expression and proteomic profile studies will also be performed before and after methotrexate treatment to extend our previous observations that leukemic cells of different molecular subtypes share a common pathway of genomic response to the same treatment, and that the changes in gene expression are treatment-specific [12]. Additional studies may identify specific molecular target(s) or signaling pathway(s) for improved therapy.

Remission induction

Four days after methotrexate treatment (or immediately after diagnosis in patients who do not receive upfront methotrexate), remission induction therapy begins with daily prednisone (40 mg/m^2 for 28 days), weekly vincristine (1.5 mg/m^2 for four doses), weekly daunorubicin (25 mg/m^2 for two doses), and thrice weekly E coli asparaginase (10,000 units/m^2 intramuscularly for 6 doses). Patients with 5% or more residual leukemia in bone marrow after 2 weeks of induction are given three additional doses of asparaginase. Subsequent induction therapy consists of cyclophosphamide (1,000 mg/m^2) on day 26, mercaptopurine (60 mg/m^2 per day) on days 26–39 and cytarabine (75 mg/m^2) on days 27–30 and 34–37. Upon recovery of hematopoietic function, bone marrow is performed to determine remission status and the presence of minimal residual disease.

Consolidation/Reintensification therapy

Consolidation therapy consists of high-dose methotrexate and age-adjusted triple intrathecal therapy with methotrexate, hydrocortisone and cytarabine (every other week for 4 doses) and daily mercaptopurine (50 mg/m^2 per day) for 8 weeks. The dosage of methotrexate depends on the risk classification of patients, since higher dose (i.e., 5 gm/m^2) is needed to improve outcome of T-cell and standard-/high-risk B-cell precursor ALL [9] and lower dose (2.5 gm/m^2) is adequate for low-risk B-cell precursor cases [13]. As we have shown that individualized dose based on the clearance would improve outcome by avoiding low or excessive system exposure [13], the dose is targeted to achieve a steady-state concentration of 65 μM or 33 μM, respectively. Reintensification therapy with high-dose cytarabine, etoposide, dexamethasone, and asparaginase is given to only high-risk cases following consolidation therapy to maximize leukemic cell kill before allogeneic hematopoietic stem cell transplantation. In this regard, high levels of minimal residual leukemia conferred a poor outcome even in the setting of allogeneic stem cell transplantation [14–17].

Continuation therapy (120 weeks for girls and 146 weeks for boys)

In the first 20 weeks of continuation therapy, low-risk cases receive daily mercaptopurine (75 mg/m^2) and weekly methotrexate (40 mg/m^2) with pulses of daily mercaptopurine (75mg/m^2), dexamethasone (8 mg/m^2 per day in three divided doses for 5 days) and vincristine (2 mg/m^2) given every 4 weeks; standard-risk cases receive daily mercaptopurine (50 mg/m^2), weekly E coli asparaginase (25,000 units/m^2), and doxorubicin (30 mg/m^2) plus vincristine (1.5 mg/m^2) every three weeks. All patients receive reinduction therapy twice (weeks 7–9 and weeks 17–20) during the first 20 weeks of continuation therapy. Reinduction therapy in low-risk cases consists of dexamethasone (8 mg/m^2 on days 1–8 and days 15–21), vincristine (1.5 mg/m^2 weekly for 3 doses), asparaginase (10,000 units/m^2 thrice weekly for 9 doses) and doxorubicin (30 mg/m^2 on day 1). In standard-risk cases, reinduction therapy consists of dexamethasone and vincristine (doses same as those in low-risk cases) as well as asparaginase (25,000 units/m^2 on days 1, 8 and 15), plus doxorubicin (30 mg/m^2 on days 1 and 8) in the first course, or high-dose cytarabine (2 gm/m^2 every 12 hours for four doses on days 15 and 16) in the second course. In this regard, two recent studies showed that double reinduction therapy improved outcome of cases with standard (or intermediate)-risk and high-risk ALL [18,19]. Preliminary result suggested that the interrupted use of dexamethasone reduces the risk and severity of osteonecrosis [9] but it is not certain if this schedule would compromise the antileukemic efficacy of this agent.

The remaining continuation therapy in low-risk cases consists of daily mercaptopurine (75 mg/m^2) and weekly methotrexate (40 mg/m^2), interrupted by pulse therapy every 4 weeks (up to week 100) with dexamethasone (8 mg/m^2 per day in 3 divided doses for 5 days), vincristine (2 mg/m^2) and mercaptopurine (75 mg/m^2 per day for 7 days). In standard-risk cases, the remaining continuation therapy consists of 3 drug pairs given in 4-week blocks: mercaptopurine (75 mg/m^2 daily for 7 days) plus methotrexate (40 mg/m^2 per week) in the first and second weeks, cyclophosphamide (300 mg/m^2) plus cytarabine (300 mg/m^2) in the third week (to be replaced by mercaptopurine and methotrexate after week 67), and dexamethasone (12 mg/m^2 per day in 3 divided doses for 5 days) plus vincristine (2 mg/m^2) in the fourth week (to be replaced by mercaptopurine and methotrexate after week 100).

The dosages of mercaptopurine and methotrexate are tailored to the limits of tolerance (as indicated by the total leukocyte and absolute neutrophil counts) but caution is taken to avoid overzealous escalation of dosages leading to the interruption of chemotherapy which has been associated with an inferior outcome [20]. Methotrexate is given intravenously to ensure compliance [21]. Thiopurine methyltransferase phenotype and genotypes are determined prospectively in all patients. The dosage of mercaptopurine is reduced in those with low enzyme activity to avoid excessive hematopoietic toxicities and to decrease the risk of therapy-related cancers [22, 23].

CNS-directed therapy

Although cranial irradiation is the most effective CNS-directed therapy, its use has been limited to patients at high risk of CNS relapse in contemporary clinical trials because of the associated neurocognitive sequelae, endocrinopathy, and second tumors [2, 3]. In our recent study of long-term survivors, prior treatment with cranial irradiation was associated with low employment rate, low marriage rate (in women), and an excess of late mortality due to second cancer [24]. In fact, the cumulative risk of second tumor exceeded 20% at 30 years after initial remission. In Study XV, we will determine if cranial irradiation can be omitted in all patients and replaced by risk-directed systemic and intrathecal therapy. Because traumatic lumbar puncture at diagnosis increased the risk of CNS relapse [25, 26], we routinely perform this procedure under deep sedation or general anesthesia, transfuse patients with thrombocytopenia (i.e., <100×10^9/L), and instill age-adjusted intrathecal cytarabine immediately after collection of cerebrospinal fluid [27]. Moreover, only the most experienced clinicians perform the procedure because experience is one of the most important determinants of a successful lumbar puncture [28].

Age-adjusted triple intrathecal chemotherapy with methotrexate, hydrocortisone, and cytarabine is given on day 19 and at the end of remission induction (coinciding with the start of consolidation therapy with high-dose methotrexate). Because of their increased risk of CNS relapse, additional intrathecal therapy is given on days 8 and 26 of remission induction in patients with CNS2, CNS3, traumatic lumbar puncture with blasts, T-cell ALL with leukocyte count>50×10^9/L, B-cell precursor ALL with leukocyte count>100×10^9/L, or the presence of Philadelphia chromosome, *MLL* rearrangement, or hypodiploidy <45 chromosomes [27]. Triple intrathecal chemotherapy is given every other week during consolidation therapy, and then every 8 weeks in low-risk cases and every 4 weeks in standard-risk cases up to one year (or every 4 weeks to the time of transplantation in high-risk cases); those at particularly high risk of CNS relapse (i.e., T-cell ALL with leukocyte count >50×10^9/L, B-cell precursor ALL with leukocyte count>100x10^9/L, presence of Philadelphia chromosome, *MLL* rearrangement or hypodiploidy <45, or CNS3 status) continue to receive intrathecal therapy every 8 weeks beyond the first year until week 96 of continuation therapy.

Preliminary results

From July 2000 to July 2003, 150 patients aged 1 to 18 years were enrolled on the study. Upon the completion of remission induction therapy, 146 (97.3%) of the patients attained complete remission. Of the 4 initial induction failures, 1 was caused by a fatal sepsis and the other 3 were due to refractory leukemia. All 3 refractory cases eventually achieved remission with extended induction/consolidation therapy. Post-remission failures include 3 hematological relapse, and 1 case each of lineage switch, death in remission (from typhlitis) and CNS relapse. Notably, the CNS relapse occurred in a patient with standard-risk ALL who would not have received cranial irradiation if he were enrolled in our previous Total Therapy studies in the 1990s. Event-free survival and overall survival rates (SE) at 2 years are 91.1% (4.0%) and 94.8% (2.9%), respectively.

References

1. Pui C-H, Relling MV, Downing JR (2004) Acute lymphoblastic leukemia. N Engl J Med (in press)
2. Pui C-H, Campana D, Evans WE (2001) Childhood acute lymphoblastic leukaemia-current status and future perspectives. Lancet Oncol 2:597-607
3. Pui C-H, Evans WE (1998) Acute lymphoblastic leukemia. N Engl J Med 339:605-615
4. Gaynon PS, Desai AA, Bostrom BC, Hutchinson RJ, Lange BJ, Nachman JB, Reaman GH, Sather HN, Steinherz PG, Trigg ME, Tubergen DG, Uckun FM (1997) Early response to therapy and outcome in childhood acute lymphoblastic leukemia: a review. Cancer 80:1717-1726
5. Pui C-H, Campana D (2000) New definition of remission in childhood acute lymphoblastic leukemia. Leukemia 14:783-785
6. Szczepański T, Orfão A, van der Velden VHJ, San Miguel JF, van Dongen JJ (2001) Minimal residual disease in leukaemia patients. Lancet Oncol 2:409-417

7. Campana D (2003) Determination of minimal residual disease in leukaemia patients. Br J Haematol 121:823-838
8. Synold TW, Relling MV, Boyett JM, Rivera GK, Sandlund JT, Mahmoud H, Crist WM, Pui C-H, Evans WE (1994) Blast cell methotrexate-polyglutamate accumulation in vivo differs by lineage, ploidy, and methotrexate dose in acute lymphoblastic leukemia. J Clin Invest 94:1996-2001
9. Pui C-H, Sallan S, Relling MV, Masera G, Evans WE (2001) International childhood acute lymphoblastic leukemia workshop: Sausalito, CA, 30 November-1 December 2000. Leukemia 15:707-715
10. Treon SP, Chabner BA (1996) Concepts in use of high-dose methotrexate therapy. Clin Chem 42:1322-1329
11. Gorlick R, Goker E, Trippett T, Waltham M, Banerjee D, Bertino JR (1996) Intrinsic and acquired resistance to methotrexate in acute leukemia. N Engl J Med 335:1041-1048
12. Cheok MH, Yang W, Pui C-H, Downing JR, Cheng C, Naeve CW, Relling MV, Evans WE (2003) Treatment-specific changes in gene expression discriminate *in vivo* drug response in human leukemia cells. Nat Genet 34:85-90
13. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui C-H (1998) Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. N Engl J Med 338:499-505
14. Knechtli CJ, Goulden NJ, Hancock JP, Grandage VL, Harris EL, Garland RJ, Jones CG, Rowbottom AW, Hunt LP, Green AF, Clarke E, Lankester AW, Cornish JM, Pamphilon DH, Steward CG, Oakhill A (1998) Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood 92:4072-4079
15. van der Velden VH, Joosten SA, Willemse MJ, van Wering ER, Lankester AW, van Dongen JJ, Hoogerbrugge PM (2001) Real-time quantitative PCR for detection of minimal residual disease before allogeneic stem cell transplantation predicts outcome in children with acute lymphoblastic leukemia. Leukemia 15:1485-1487
16. Bader P, Hancock J, Kreyenberg H, Goulden NJ, Niethammer D, Oakhill A, Steward CG, Handgretinger R, Beck JF, Klingebiel T (2002) Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. Leukemia 16:1668-1672
17. Goulden N, Bader P, Van der Velden V, Moppett J, Schilham M, Masden HO, Krejci O, Kreyenberg H, Lankester A, Révész T, Klingebiel T, van Dongen J (2003) Minimal residual disease prior to stem cell transplant for childhood acute lymphoblastic leukaemia. Br J Haematol 122:24-29
18. Aricò M, Valsecchi MG, Conter V, Rizzari C, Pession A, Messina C, Barisone E, Poggi V, De Rossi G, Locatelli F, Micalizzi MC, Basso G, Masera G (2002) Improved outcome in high-risk childhood acute lymphoblastic leukemia defined by prednisone-poor response treated with double Berlin-Frankfurt-Muenster protocol II. Blood 100:420-426
19. Lange BJ, Bostrom BC, Cherlow JM, Sensel MG, La MK, Rackoff W, Heerema NA, Wimmer RS, Trigg ME, Sather HN (2002) Double-delayed intensification improves event-free survival for children with intermediate-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 99:825-833
20. Relling MV, Hancock ML, Boyett JM, Pui C-H, Evans WE (1999) Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood 93:2817-2823
21. Pui C-H, Sandlund JT, Pei D, Rivera GK, Howard SC, Ribeiro RC, Rubnitz JE, Razzouk BI, Hudson MM, Cheng C, Raimondi SC, Behm FG, Downing JR, Relling MV, Evans WE (2003) Results of therapy for acute lymphoblastic leukemia in black and white children. JAMA 290:2001-2007
22. Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, Pui C-H, Evans WE (1999) Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 91:2001-2008
23. Relling MV, Rubnitz JE, Rivera GK, Boyett JM, Hancock ML, Felix CA, Kun LE, Walter AW, Evans WE, Pui C-H (1999) High incidence of secondary brain tumours after radiotherapy and antimetabolites. Lancet 354:34-39
24. Pui C-H, Cheng C, Leung W, Rai SN, Rivera GK, Sandlund JT, Ribeiro RC, Relling MV, Kun LE, Evans WE, Hudson MH (2003) Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. N Engl J Med 349:640-649
25. Gajjar A, Harrison PL, Sandlund JT, Rivera GK, Ribeiro RC, Rubnitz JE, Razzouk B, Relling MV, Evans WE, Boyett JM, Pui C-H (2000) Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. Blood 96:3381-3384
26. Bürger B, Zimmermann M, Mann G, Kühl J, Löning L, Riehm H, Reiter A, Schrappe M (2003) Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. J Clin Oncol 21:184-188
27. Pui C-H (2003) Toward optimal central nervous system-directed treatment in childhood acute lymphoblastic leukemia. J Clin Oncol 21:179-181
28. Howard SC, Gajjar AJ, Cheng C, Kritchevsky SB, Somes GW, Harrison PL, Ribeiro RC, Rivera GK, Rubnitz JE, Sandlund JT, de Armendi AJ, Razzouk BI, Pui C-H (2002) Risk factors for traumatic and bloody lumbar puncture in children with acute lymphoblastic leukemia. JAMA 288:2001-2007

Main Session VIII

The Emerging Genetics of T-cell Acute Lymphoblastic Leukemia: a Fish Tale

A.T. LOOK
Dana Farber Cancer Institute, Harvard Medical School, Boston, USA

Subsets of childhood T-cell leukemias arise from oncogenes activated by antigen receptor gene translocations. Otherwise, little is known about the molecular pathogenesis of this thymic cancer. Here we show that three different T-cell oncogenes (LYL1, HOX11 and TAL1) are often expressed in the absence of chromosomal abnormalities, and that HOX11 activation is significantly associated with a favorable prognosis. Using oligonucleotide microarrays, we identified three distinct gene expression signatures that were indicative of leukemic arrest at specific stages of normal thymocyte development: LYL1+ (pre-T), HOX11+ (early cortical thymocyte) and TAL1+ (late cortical thymocyte). Hierarchical clustering analysis of the microarray findings allowed us to devise a prognostically relevant classification system that accommodated all T-cell cases in this series and integrated oncogene activation and specific chromosomal deletions into emerging multistep molecular pathways of thymocyte leukemogenesis. These results demonstrate a previously undetected molecular heterogeneity among childhood T-cell leukemias, and suggest the ability of gene expression profiling to stratify patients into clinically relevant subgroups.

A new area of research in the laboratory involves the use of a zebrafish genetic system to clarify developmental pathways subverted in human T cell leukemia, which act downstream of the dysregulated expression of master transcriptional regulatory proteins in developing thymocytes. We have generated a model of clonally derived T-cell acute lymphoblastic leukemia in transgenic zebrafish expressing mouse *c-myc* under control of the zebrafish *Rag2* promoter. Visualization of leukemia cells expressing a chimeric transgene encoding Myc fused to green fluorescent protein (GFP) revealed that leukemias arose in the thymus, spread locally into gill arches and retro-orbital soft tissue, and then disseminated into skeletal muscle and abdominal organs. Leukemia cells homed back to the thymus in irradiated fish transplanted with GFP-labeled lymphoblasts. The T-cell leukemias that arise in *Rag2-myc* transgenic fish aberrantly overexpress both Tal1 and Lmo2, indicating that these leukemias faithfully recapitulate the pattern of oncogene expression found in 60% of human T-cell leukemias. This transgenic model provides a platform for drug screens and for genetic screens aimed at identifying mutations that suppress or enhance *c-myc*-induced carcinogenesis. Forward genetic screens in the zebrafish hold promise for the discovery of genetic modifiers, such as tumor suppressor genes, whose mutational inactivation will enhance the rate of tumorigenesis, and genes that encode novel candidate drug targets, whose inactivation will delay or prevent the onset of malignancy.

References

1. Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, Behm FG, Pui CH, Downing JR, Gilliland DG, Lander ES, Golub TR, Look AT. Gene expression signatures define novel oncogenic pathways in T-cell acute lymphoblastic leukemia. Cancer Cell 1:75-87, 2002.
2. Langenau DM, Traver D, Ferrando AA, Kutok JL, Aster JC, Kanki JP, Lin S, Prochownik E, Trede NS, Zon LI, Look AT. Myc-induced T-cell leukemia in transgenic zebrafish. Science 299:887-890, 2003.

The U.S. Trials in Adult Acute Lymphoblastic Leukemia

R. A. LARSON
Department of Medicine and the Cancer Research Center,
The University of Chicago, Chicago, Illinois, USA

Keywords: Adult acute lymphoblastic leukemia, Chemotherapy, Allogeneic transplantation

ABSTRACT

Intensive combination chemotherapy for adults with acute lymphoblastic leukemia (ALL) has resulted in complete remission rates of 70–90%, but overall survival remains at 30–40%. Prognosis depends on clinical features such as age and white blood cell count as well as on biological characteristics such as immunophenotype and cytogenetic or molecular abnormalities. Complex multiagent regimens have evolved to treat distinct subsets of ALL. Different groups of investigators in the United States have been testing various strategies to improve upon these outcomes. These clinical trials include escalation of chemotherapy doses, particularly anthracyclines and antimetabolites, early use of stem cell transplantation, incorporation of monoclonal antibodies into frontline regimens, development of new chemotherapeutic agents, and the use of molecular monitoring as an intermediate endpoint to measure treatment effectiveness.

Intensive combination chemotherapy for adults with acute lymphoblastic leukemia (ALL) has resulted in complete remission (CR) rates of 70–90%, but overall survival (OS) remains at 30–40%. Different groups of investigators in the United States have been testing various strategies to improve upon these outcomes. These clinical trials include escalation of chemotherapy doses, particularly anthracyclines and antimetabolites, early use of stem cell transplantation, incorporation of monoclonal antibodies into frontline regimens, development of new chemotherapeutic agents, and the use of molecular monitoring as an intermediate endpoint to measure treatment effectiveness.

The Cancer and Leukemia Group B (CALGB) hypothesized that early dose intensification of daunorubicin (Dnr) and cytarabine (Ara-C) would improve disease free survival (DFS) and that intensive high-dose intravenous (IV), oral, and intrathecal (IT) methotrexate (MTX) could replace cranial radiotherapy (RT) for central nervous system (CNS) prophylaxis. The CALGB undertook a Phase II study (19802) to examine these issues [8, 9]. Treatment consisted of 6 monthly courses of intensive therapy followed by 18 months of maintenance therapy. Five drugs (cyclophosphamide [Cy], Dnr, prednisone, vincristine, l-asparaginase) were given with G-CSF support for remission induction [3, 4, 10]. In patients <60 years old, the Dnr dose in the induction course (module A) was increased in cohorts from 45 mg/m^2/day on days 1, 2, and 3 (used in prior CALGB ALL studies) to 60 and then to 80 mg/m^2 daily for 3 days. In patients ≥60 years old, Cy was omitted from module A, and Dnr was increased from 30 to 60 mg/m^2/day for 3 days. Consolidation with Cy plus high-dose Ara-C, and CNS prophylaxis with IV, oral, and IT MTX were introduced in the post-remission treatment modules for all patients. No cranial RT was given.

Between January 1999 and January 2001, 163 adults with untreated ALL (FAB L1 and L2) were enrolled on study. The median age was 41 years (range, 16–82); 100 (61%) were male. The median white blood cell (WBC) count at presentation was 10,050/μl (range, 500–348,500). A large proportion, 42 (43%) of 97 centrally reviewed and evaluable cases, had poor risk cytogenetics as defined in prior CALGB studies: 29 with t(9;22), 7 with abnormalities of 11q23, 4 with –7, and 2 with +8. One hundred twenty-seven (78%) of the 163 patients [95% confidence interval (CI), 74–84%] achieved a CR, comparable to CR rates (81–85%) achieved in 3 prior CALGB studies with lower doses of Dnr [3, 4, 10]. By age, the CR rates were 43/47 (91%) for patients <30 years, 65/83 (78%) for patients 30–59 years, and 19/33 (58%) for patients ≥60 years. There were 18 (11%) induction deaths and 18 (11%) induction failures due to refractory ALL. Thirty-nine patients < 60 years received Dnr at 60 mg/m^2; 2 patients (5%) died during induction and 2 (5%) had refractory disease. This was not significantly different when compared to the 8 (9%) induction deaths and 11(12%) with refractory disease among the 91 patients who received Dnr at 80 mg/m^2. Thirteen high-risk patients had an allogeneic stem cell transplant (SCT) in CR1 and 12 more were transplanted after relapse.

With a median follow-up of 2.4 years for survivors, 63/163 (39%) are alive and 44/127 (35%) are in continuous CR. Relapses have occurred in 66 (52%) patients; of these, 8 (5%) had isolated CNS relapses. The median DFS is 1.5 years [95% CI, 1.0–1.9] and the median OS is 1.6 years [95% CI, 1.2–2.4]. Age >60 years impacted adversely on DFS (p=0.02). Of interest, neither poor-risk cytogenetics nor presenting WBC >30,000/μl significantly affected DFS. Both older age (p<0.0001) and adverse cytogenetics (p=0.024) were associated with significantly shorter OS.

In conclusion, intensification of Dnr during treatment of adult ALL is feasible, and the post-remission therapy piloted in CALGB 19802 was generally well tolerated and could be given in the outpatient setting. To date, intensified Dnr has not resulted in an improvement in DFS as has been reported by others. However, very few relapses after 2 years have been noted. CNS prophylaxis without cranial RT has been well tolerated and has not resulted in an increase in CNS relapses. Longer follow-up is needed to determine the potential benefit of intensified Dnr on late relapses. Nevertheless, further Dnr dose escalation seems unlikely to result in significant improvements in outcome. Novel agents that eradicate minimal residual disease (MRD) should be introduced into clinical trials of adult ALL.

Weiss and colleagues at the Memorial Sloan-Kettering Cancer Center have studied induction regimens that contain high doses of mitoxantrone or idarubicin. Their randomized trial comparing high-dose Ara-C (3000 mg/m^2 given once daily by 3-hour IV infusion for 5 days) plus a single dose of mitoxantrone (80 mg/m^2 on day 3) to a standard regimen of Dnr, vincristine, and prednisone (the L-20 regimen) is continuing to accrue newly diagnosed patients. Recently, they reported on the use of a single, high dose of idarubicin (40 mg/m^2) given on day 3 together with 5 days of high-dose Ara-C [12]. Among 29 patients with primary refractory ALL (n=8) or recurrent ALL (n=21), there were 11 (38%) who achieved a CR (95% CI, 20–56%). The treatment was severely myelosuppressive, filgrastim (G-CSF) was routinely used, and there was one treatment-related death.

Additional dose intensification with early use of stem cell transplantation (SCT) continues to be explored in the U.S. by the Eastern Cooperative Oncology Group (ECOG) together with the Medical Research Council of the United Kingdom in ECOG study E2993 [2, 5, 6]. Over 1400 adults <60 years old have been registered on this study so far. The median age is 30 years (range, 14–60). The induction death rate is 5%, and 4% have had resistant disease. Patients ≤50 years old who have an HLA-compatible sibling are assigned to allogeneic SCT in first CR. All other CR patients are offered randomization between autologous SCT and consolidation therapy followed by maintenance for 2.5 years. Matched unrelated donor SCT is an option for Philadelphia (Ph)-positive ALL patients. The CR rate is 84% for Ph+ ALL patients, and their 5 year survival is estimated to be 23% overall. The CR rate is 93% for Ph-negative ALL patients, and their 5-year survival is estimated to be 41%. The study continues to accrue patients, predominantly to answer the randomized autologous SCT question.

The availability of imatinib mesylate has led to several prospective studies in the U.S. for patients with Ph+ ALL. Thomas and colleagues at the M.D. Anderson Cancer Center have added imatinib (400 mg/day) to the first 14 days of each course of their hyper-CVAD regimen [11]. Eight intensive courses are followed by one year of maintenance with imatinib at 600 mg/day plus monthly vincristine and prednisone. In addition, 2 early and late intensifications are given with hyper-CVAD and imatinib. To date, 25 patients have been reported on. Median age was 42 years (range, 19–75). No unexpected toxicities were observed. The toxicity profile was similar to that seen with hyper-CVAD alone. Allogeneic SCT was performed in 10 patients in CR within a median of 3.5 months (range, 1–8 months) from the start of therapy. After a median follow up of only 18 months, there have been 4 relapses and 2 deaths in remission. The 2-year DFS is estimated to be 85%.

The CALGB and the Southwest Oncology Group (SWOG) are enrolling Ph+ ALL patients in first CR onto a study (CALGB 10001) in which 4 weeks of imatinib are given at 400 mg twice per day for 2 months. For those who lack an HLA-compatible donor, filgrastim-mobilized stem cells are then collected for use in autologous SCT. Molecular monitoring using quantitative RT-PCR for BCR/ABL is performed at defined time points as well as for the stem cell product. Imatinib is resumed after recovery from the SCT and continued until the patient's bone marrow specimens are consistently RT-PCR negative.

Two monoclonal antibodies are now being tested in frontline therapy of ALL in the U.S. Investigators at the M.D. Anderson Cancer Center found that patients with CD20-positive ALL had a worse outcome. Therefore, rituximab (375 mg/m^2) was added on days 1 and 8 of each of the first 4 courses of the hyper-CVAD regimen for the half of adult patients with precursor-B ALL

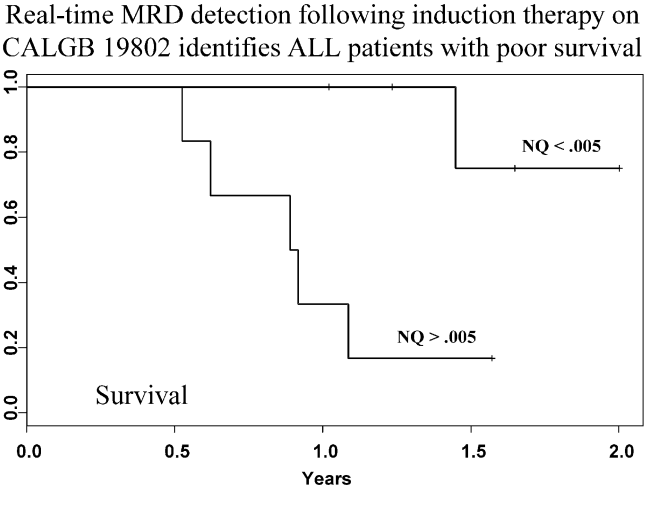

Figure 1

whose lymphoblasts express CD20. The antigen CD52 is detected on about 80% of ALL blasts. The CALGB is testing the monoclonal antibody Campath-1H during post-remission therapy for patients whose lymphoblasts expressed CD52 at diagnosis. In CALGB study 10102, the antibody is given subcutaneously at 30 mg/day three times per week for 4 weeks at a time of minimal residual disease. RT-PCR monitoring for molecular responses as well as Campath pharmacokinetics are being studied.

One important goal of minimal residual disease (MRD) studies in adult ALL is the identification of new prognostic groups that will facilitate optimal post-remission treatment planning. Quantitative real-time (QRT) PCR technology provides precise MRD quantification with excellent reproducibility applicable to large cooperative group studies. The CALGB has performed a pilot study (CALGB 20101) to examine feasibility and clinical relevance of MRD detection using QRT PCR of leukemia clone-specific rearrange-ments of either the IgH and/or TCRγ gene during treatment of 37 adults with newly diagnosed ALL who had enrolled onto a single CALGB treatment study (CALGB study 19802) [7]. For QRT PCR, consensus upstream primer and probe pairs with a patient-specific downstream primer were employed. Results are expressed as a normalized quotient (NQ) of IgH or TCR to GAPDH. The sensitivity of the assay ranges from 1 in 10^4–10^5. At least one clonal marker for MRD analysis was detected in 31 of 37 (84%) patients. Twelve of these 31 patients had blood and bone marrow samples available for MRD detection one month following intensive 5-drug induction therapy. An exploratory analysis was used to identify an optimal cut-point for dichotomizing these patients into 2 groups according to survival. One month after induction therapy, NQ values of >0.005 were associated with both shorter DFS (p=0.02), and a median OS of only 11 months (p=0.01). The 6 patients with NQ values <0.005 had a median age of 27 years (range, 18–51) and lower presenting WBC counts (6800–54,000/μl), and one had a t(9;22) compared to the 6 patients with NQ >0.005 where the median age was 32 years (range, 19–64), the presenting WBC ranged from 5500–80,800/μl, and 2 had a t(9;22). NQ values of paired bone marrow and blood samples showed very good agreement based on the intraclass correlation coefficient (0.895; p<0.001). These results suggest that blood and/or bone marrow MRD detection using QRT PCR of clone-specific IgH or TCR genes is feasible for the majority of adults with ALL (84%) and identifies patients immediately after achievement of CR who are at high-risk of early relapse and poor survival. The CALGB is confirming these promising results prospectively in a larger patient cohort. MRD detection using QRT PCR of IgH or TCR is a novel prognostic marker in adult ALL that may allow clinicians to alter or intensify treatment in first CR.

The most promising new drug for patients with T-cell ALL may be nelarabine (2-amino-9-D-arabinosyl-6-methoxy-9H-purine; compound 506U78). Preclinical studies have demonstrated that immature T-lymphocytes and T-lymphoblasts are extremely sensitive to the cytotoxic effects of deoxyguanosine and its analog, ara-G. Ara-G is difficult to synthesize and poorly water soluble; however, 506U78 is a soluble pro-drug of 9-D-arabinofurano-sylguanine (ara-G), a deoxyguanosine derivative. 506U78 is rapidly demethylated in the serum by adenosine deaminase to ara-G. Phase I studies determined a maximum tolerated dose of 40 mg/kg/day for 5 days in adult patients. The dose limiting toxicity was neurologic, consisting of seizures, obtundation and ascending paralysis. As predicted by preclinical in vitro studies, the highest response rates were observed in patients with relapsed T-cell ALL and T-lymphoblastic lymphoma (LBL). The CALGB and SWOG have completed an intergroup phase II study (CALGB 19801) of 506U78 in patients with relapsed or refractory T-lineage ALL or LBL [1]. In order to decrease the risk of neurologic toxicities, we tested a dosing regimen of 1.5 g/m^2 given IV once per day on an alternate day schedule (days 1, 3, and 5).

Between August 1998 and September 2001, 40 patients were enrolled, 22 with T-ALL and 18 with LBL. Patients with greater than 25% lymphoblasts within the bone marrow were considered to have ALL. The lymphoblasts had to express at least two T-cell antigens. All patients were refractory to at least one induction regimen or were in first or greater relapse after achieving a CR. Patients could not have evidence of CNS disease and had to have a calculated creatinine clearance of greater than 50 ml/min. A maximum of two induction courses was administered. Each cycle was repeated every 21 days. Those patients achieving a CR were allowed to receive an additional 2 courses as consolidation therapy. The median age was 34 years (range, 16-66). There were 33 males and 7 females. Thirty-eight patients were evaluable for response: one patient with LBL was never treated and one patient with ALL withdrew consent after 11 days after achieving CR. Of the 21 evaluable patients with ALL, there were 6 CRs and 2 partial remissions (PR) for a total response rate of 38% (95% CI, 18–62%). For the 17 evaluable patients with LBL, there were 4 CRs and no PRs for a total response rate of 24% (95% CI, 7–50%). The overall response rate (CR+PR) for the 38 evaluable patients was 32% (95% CI, 18–49%). One patient had a seizure and subsequent confusion, which resolved. One patient developed hallucinations but was also receiving narcotics. He was retreated without the recurrence of additional neurologic symptoms. The principal toxicity was marrow suppression. Grade 3 or 4 neutropenia and thrombocytopenia occurred in 43% and 33% of patients, respectively.

The median OS for the 40 patients was 4.6 months (95% CI, 3–10 months). The median DFS for the 10 patients achieving CR was 9.8 months (95% CI, 3–15 months). The one-year OS was 32% (95% CI, 16–47%) and the one-year DFS was 40% (95% CI, 10–70%). These results suggest that 506U78 is well tolerated, and has significant anti-tumor activity in patients with relapsed or refractory T-cell lymphoblastic leukemia/lymphoma. Studies using 506U78 in patients with newly diagnosed T-cell malignancies are warranted.

References

1. De Angelo DJ, Yu D, Dodge RK, Coutre SE, Mitchell BS, Stone RM, Stopeck AT, Larson RA (2002) A phase II study of 2-amino-9-D-arabinosyl-6-methoxy-9H-purine (506U78) in patients with relapsed or refractory T-lineage acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL): CALGB study 19801. Blood 100(Suppl 1): 198a (#743).
2. Goldstone AH, Prentice HG, Durrant J, et al (2001) Allogeneic transplant (related or unrelated donor) is the preferred treatment for adult Philadelphia chromosome positive acute lymphoblastic leukemia. Results from the international ALL trial (MRC UKALL XII/ ECOG E2993). Blood 98(Suppl 1): 856a (#3556).
3. Larson RA, Dodge RK, Burns CP, Lee EJ, Stone RM, Schulman P, Duggan D, Davey FR, Sobol RE, Frankel SR, Hooberman AL, Westbrook CA, Arthur DC, George SL, Bloomfield CD, Schiffer CA (1995) A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood 85: 2025-2037.
4. Larson RA, Dodge RK, Linker CA, Stone RM, Powell BL, Lee EJ, Schulman P, Davey FR, Frankel SR, Bloomfield CD, George SL, and Schiffer CA (1998) A randomized controlled trial of filgrastim during remission induction and consolidation chemotherapy for adults with acute lymphoblastic leukemia: CALGB study 9111. Blood 92: 1556-1564.
5. Rowe JM, Richards SM, Burnett AK, et al (2001) Favorable results of allogeneic bone marrow transplantation for adults with Philadelphia chromosome negative acute lymphoblastic leukemia in first complete remission: results from the international ALL trial (MRC UKALL XII/ECOG E2993). Blood 98(Suppl 1): 481a (#2009).
6. Rowe JM, Buck G, Burnett AK, et al (2003) Induction therapy for adults with acute lymphoblastic leukemia: results of nearly 1400 patients from the International ALL Trial MRC UKALL XII/ ECOG E2993. Blood 102 (Suppl 1): abstract in press.
7. Sher D, Dodge RK, Bloomfield CD, Larson RA, Caligiuri MA, Stock W (2002) Clone-specific quantitative real-time PCR of IgH or TCR gene rearrangements in adult acute lymphoblastic leukemia (ALL) following induction chemotherapy identifies patients with a poor prognosis: Pilot study from the Cancer and Leukemia Group B (CALGB 20101). Blood 100(Suppl 1): 153a (#574).
8. Stock W, Dodge RK, Vardiman JW, Bloomfield CD, Caligiuri MA, Frankel S, Stone RM, Larson RA (2001) Treatment of adult acute lymphoblastic leukemia: phase II trial of dose intensification of daunorubicin and cytarabine followed by high-dose methotrexate and intrathecal methotrexate in place of cranial irradiation (CALGB 19802). Blood 98(Suppl 1): 590a (#2472).
9. Stock W, Yu D, Johnson J, Bloomfield CD, Stone RM, Kolitz JE, Wetzler M, Powell BL, Vardiman JW, Larson RA (2003) Intensified daunorubicin during induction and post-remission therapy of adult acute lymphoblastic leukemia (ALL): results of CALGB 19802. Blood 102 (Suppl 1): abstract in press.
10. Szatrowski TP, Dodge RK, Reynolds C, Westbrook CA, Frankel SR, Sklar J, Stewart CC, Hurd DD, Kolitz JE, Velez-Garcia E, Stone RM, Bloomfield CD, Schiffer CA, Larson RA (2003) Lineage specific treatment of adult patients with acute lymphoblastic leukemia in first remission with anti-B4-blocked ricin or high-dose cytarabine: Cancer and Leukemia Group B Study 9311. Cancer 97: 1471-80.
11. Thomas DA, Faderl S, Cortes J, O'Brien S, Giles FJ, Garcia-Manero G, Keating M, Kornblau S, Andreeff M, Jeha S, Beran M, Verstovsek S, Pierce S, Letvak L, Salvado A, Champlin R, Kantarjian H (2003) Update of the hyper-CVAD and imatinib mesylate regimen in Philadelphia positive acute lymphocytic leukemia. Blood 102 (Suppl 1): abstract in press.
12. Weiss MA, Aliff TB, Tallman MS, Frankel SR, Kalaycio ME, Maslak PG, Jurcic JG, Scheinberg DA, Roma TE (2002) A single, high dose of idarubicin combined with cytarabine as induction therapy for adult patients with recurrent or refractory acute lymphoblastic leukemia. Cancer 95: 581-7.

Risk/MRD Adapted GMALL Trials in Adult ALL

N. GÖKBUGET[1], R. RAFF[2], M. BRÜGGE-MANN[2], T. FLOHR[3], U. SCHEURING[1], H. PFEIFER[1], C.R. BARTRAM[3], M. KNEBA[2], and D. HOELZER[1]
[1]University Hospital, Medical Clinic III, 60590 Frankfurt, Germany; [2]University of Kiel, II. Med. Klinik und Poliklinik, 24116 Kiel, Germany; Ruprecht-Karls-University, [3]Institut für Humangenetik und Anthropologie, 69120 Heidelberg, Germany

Keywords. Acute lymphoblastic leukemia, risk factors, minimal residual disease, study design

Abstract

The German Multicenter Study Group for Adult ALL (GMALL) conducts since 1984 trials with risk adapted study design. The model of conventional prognostic factors comprises now WBC, age, immunophenotype, cytogenetics and molecular genetics. Risk stratification according to these factors allows a highly significant prediction of relapse risk in adult ALL. In the recent GMALL study minimal residual disease (MRD) was added to the risk model. Trials in childhood and adult ALL showed convincingly that MRD is a relevant and independent prognostic factor. It is of particular value in standard risk (SR) patients as defined by conventional factors. In the current GMALL study a risk stratification according to conventional factors is followed by a MRD based stratification in SR patients. Whereas high and very high risk patients receive a stem cell transplantation (SCT) in first CR after induction and first consolidation, SR patients receive cyclic consolidation therapy for one year with MRD monitoring. At the end of the first year a stratification according to course and level of MRD takes place. Treatment is stopped in patients with low risk whereas in high risk patients a SCT is planned. Patients who cannot be allocated to either group are treated as intermediate risk and receive one year of intensified maintenance therapy. Preliminary results show that MRD based risk stratification is feasible and that the treatment recommendations for MRD based risk groups are reasonable. In the future however an earlier identification of high risk patients (after 4 months) will be attempted.

Introduction

In recent adult ALL trials published between 1999 and 2002 a tendency towards higher complete remission (CR) rates of 80–85% and leukemia free survival (LFS) rates of 30–40% could be observed [16] [18]. Intensified consolidation, the extended use of stem cell transplantation (SCT), improvement of supportive care and more experience in participating centers could have contributed to better outcome. Furthermore a number of promising new treatment and management modalities for ALL came up including molecular targeting with BCR-ABL kinase inhibitors, antibody therapy and new approaches in SCT. In addition risk adapted treatment strategies are developed since many years and risk models are continuously amended by new prognostic factors e.g. by evaluation of minimal residual disease (MRD).

The “classic” risk model for adult ALL includes immunophenotype, white blood cell count, cyto- and molecular genetics [19] [16]. Recently it was demonstrated that major subgroups of ALL are characterised by different gene expression profiles as well [29] [10]. Age is another highly significant prognostic factor. In adult ALL prognostic factors are mainly determined at diagnosis. In addition response to treatment is significantly correlated with outcome. This is evaluated as time to achievement of CR - mostly analysed after 2–4 weeks. In childhood ALL even an earlier evaluation of response to a few days of prednisone treatment has a highly significant prognostic impact. This was confirmed recently in adults [1].

Stratification according to conventional risk factors

Many study groups have combined the above mentioned prognostic factors in a risk stratification followed by risk adapted therapy. The German Multicenter Study Group for Adult ALL (GMALL) conducts risk adapted studies since 1984 [19] [13]. In all studies risk stratification took place after an 8 week induction therapy and achievement of CR. The risk model was refined continuously. In the recent GMALL Study 05/93 the following adverse risk factors (RF) were applied: Ph/BCR-ABL, t(4;11)/ALL1-AF4, pro B-ALL, WBC >30000/µL, time to CR more than four weeks. Patients were stratified to four treatment arms: B-precursor ALL with the risk groups standard risk (SR, no RF), high risk (HR, ≥1 RF), elderly (ER, >50 yrs) and T-ALL (independent of RF). Highly significant differences between the risk groups were observed for CR-rate, rate of continuous complete remission (CCR) and survival (table 1) [12]. In the current GMALL study this risk model has been amended by inclusion of immunological subtypes of T-ALL and minimal residual disease (see figure 1).

Role of MRD in risk stratification

The highly predictive value of the above mentioned prognostic factors measured at diagnosis or after induction therapy has been confirmed by many studies. However an assessment of individual response after achievement of CR was not possible until recently. MRD evaluation emerged as a new method to enlighten this so called “black box” of clinical remission. Standard risk (SR) ALL patients are of particular interest for MRD evaluation since in this subgroup no parameters for prediction of relapse are available so far. A high CR rate and long median remission duration is achieved but relapses occur continuously up to 6 years accounting for an overall relapse probability of nearly 50% (table 1) . Other study groups allocate all SR patients with sibling donor to allogeneic SCT in first CR [24]. The GMALL decided to follow an alternative strategy, since overall results with chemotherapy in SR patients were equal to results of SCT. Therefore it seemed inappropriate to allocate SR patients to a treatment procedure with high early mortality and considerable long-term side effects. MRD evaluation emerged as absorbing new method for analysis of individual treatment response in order to identify SR patients with poor response on molecular level which are prone to relapse.

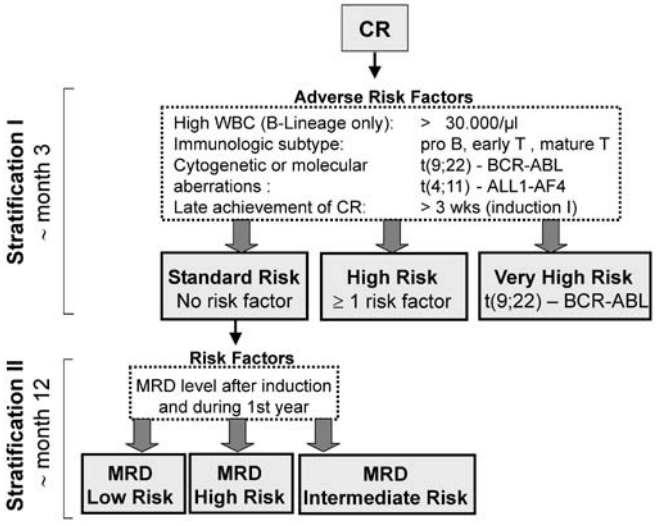

Figure 1: Combined Risk Stratification According to Conventional Factors and MRD in GMALL Study 06/99

Table 1: Outcome according to conventional risk groups in GMALL study 05/93*

	Standard Risk 15–50 years	High Risk 15–50 years	Elderly >50 years	T-ALL 15–65 years
Evaluable	291	352	216	304
CR	87%	85%	70%	86%
CCR at 5 yrs	47%	27%	16%	51%
Med. RD	57	15	17	Nr

Abbreviations: CR,complete remission; CCR, continuous complete remission, Med RD, median remission duration* [12].

Methods for MRD detection

MRD is defined as leukemic cells undetectable by morphologic examination which reaches at best a sensitivity of 1–5 leukemic cells in 100 normal cells (10^{-2}). In ALL several methods fulfill the pre-requisites of reliable MRD techniques such as discrimination between normal and malignant cells, stable markers, reproducible and quantifiable results [26]. MRD evaluation in ALL can be based on detection of leukemia specific constellations of surface markers by flow cytometry (FACS), fusion genes related to specific chromosomal translocations e.g. BCR-ABL in t(9;22) by polymerase chain reaction (PCR) and individual rearrangements of immunoglobulin (IgH, IgK) and T-cell receptor genes (TCR-β,γ,δ) by PCR and recently by real-time PCR. These methods reach in ideal cases a sensitivity of at least 10^{-4}. Overall 90% of the ALL patients have at least one target for MRD evaluation (reviewed in [11] [6] [26]).

Results of MRD studies

In childhood ALL it has been demonstrated convincingly that level and course of MRD are independent prognostic factors [27] [9] [7] [22]. These results were confirmed in the meantime by a considerable number of mainly retrospective studies in adult ALL. There are however some important differences. A very good response as indicated by an early and rapid decrease of MRD already during induction may be associated with a very low relapse risk [5]. However in general the decrease of MRD occurs slower in adults and fewer patients reach a negative MRD status. This applies particularly for patients with low MRD immediately after induction who still show a considerable relapse rate of approximately 50% [2] [23] [28]. Thus in adult ALL MRD analysis immediately after induction provides a good tool for identification of high relapse risk but

Figure 2: Risk adapted treatment decisions in GMALL study 06/99

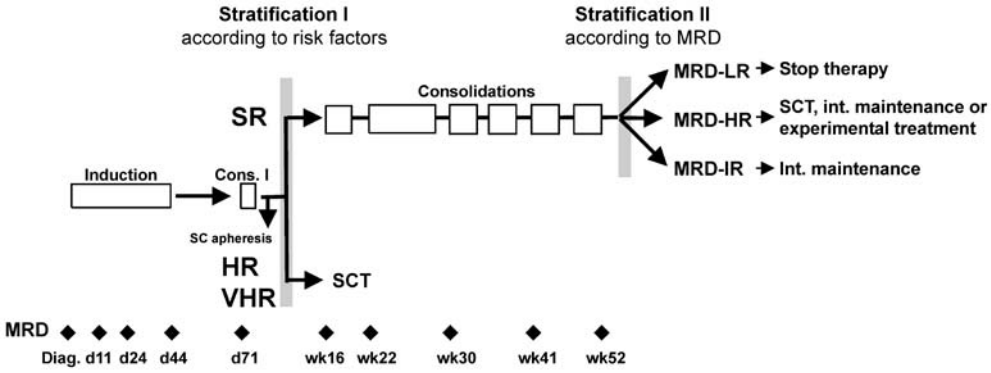

Table 2: MRD based stratification in GMALL study 06/99

MRD risk group	Day 71[a]		Week 16 until week 52[b]
MRD low risk (MRD-LR)	$<10^{-4}$	and	always $<10^{-4}$ negative in week 52
MRD high risk (MRD-HR)	$>10^{-4}$	and	2 times $>10^{-4}$ not negative in week 52
MRD intermediate risk (MRD–IMR)	MRD evaluation not possible Technical pre-requisites not fulfilled[c] Inconclusive course of MRD		

[a] after induction, before first consolidation
[b] during consolidation
[c] Technical pre-requisites: At least 2 clone-specific markers, minimum sensitivity of 10^{-4} material from decisive time-points available

not of those with low risk. The longitudinal course of MRD may be more important in adults compared to children. High MRD at any time-point after induction is associated with a higher relapse risk [8] [4] and the predictive value increases at later time-points (months 6–9) [21].

The predictive value of MRD evaluation depends on the technical quality such as sensitivity (10^{-4} for negative results), number of targets (at least two) and on the frequency of evaluations (3 monthly) in individual patients. In a retrospective study of the GMALL a broad spectrum of target genes (IgH, Igμ and TCR rearrangements) was measured quantitatively with high sensitvity ($<10^{-4}$). A combination of several time-points for MRD evaluation during the first year yielded the highest discriminative value for relapse risk. MRD levels $>10^{-4}$ at more than 1 time-point after induction therapy were associated with a significantly higher relapse rate of 73% (11/15) compared to 13% (4/31) in patients with MRD $< 10^{-4}$ at all time-points [3]. According to these results in adult ALL a variety of potential clinical applications for MRD evaluation is already evident [17] [14]. This includes new definition of CR, MRD based risk stratification and efficacy monitoring of new treatment elements.

GMALL approach

According to these findings the GMALL group has started a pilot study (GMALL 06/99) with prospective monitoring of MRD [4] [17]. It has the following basic principles:

- Combination of "conventional" and "MRD based" risk factors in two sequential stratifications
- MRD based risk stratification in standard risk patients only
- Predefined technical pre-requisites
- Definition of MRD based risk groups based on MRD level and course during 1st year
- Prospective definition of treatment options for MRD based risk groups

Aim of the study was to develop a treatment model with stratification according to "conventional" risk factors (RF) and according to course of MRD (figure 1). MRD evaluation was based on TCR and Ig rearrangements. Initial treatment consisted of an intensified and shortened 7 drug induction therapy followed by early consolidation including HDMTX, HDAC and stem cell collection in all CR pts [17].

Risk stratification

Stratification I after 3 months (induction I and II and consolidation I) was performed according to "conventional" risk factors (figure 1). Patients were thereby allocated to a very high risk (VHR), a high risk (HR) and a standard risk (SR) group with approximately one third of the patients allocated to each group. VHR and HR patients received one more consolidation cycle and were candidates for SCT in CR1. SR patients received cyclic consolidation until month 12 and were followed for MRD.

A **2nd risk stratification** was introduced based on MRD results obtained at 9 time-points during the first year of chemotherapy in SR patients. The criteria for allocation to a MRD based low risk (MRD-LR) and high risk (MRD-HR) group are detailed in table 2. The remaining patients were allocated to a MRD intermediate risk (MRD-IMR) group. Scheduled treatment options were stop of therapy for MRD-LR, SCT or experimental therapy for MRD-HR. For the MRD-IMR group intensified maintenance therapy with 6 further consolidation cycles during the 2nd year of therapy was recommended (figure 2).

Results of the MRD based risk stratification in GMALL study 06/99

The study started 10/99 with more than 100 participating centers. An interim analysis of MRD risk stratification at month 12 was possible in 98 SR patients with at least 1 marker [15]. The risk groups according to MRD were distributed as follows: MRD-LR 36%, MRD-HR 9% and MRD-IMR 55%. The major reasons for allocation to MRD-IMR were lack of a 2nd marker (58%), insufficient sensitivity (51%) and inconclusive course of MRD (28%). Most patients in the MRD-IMR group had however combinations of several reasons e.g. only one marker and insufficient sensitivity which made an assessment of the relapse risk impossible.

Further treatment after MRD risk stratification was evaluable in 88 patients. In nearly all MRD-LR patients therapy was stopped. The relapse risk in this cohort is so far approximately 20%. In MRD-HR less than half of the patients could receive SCT. In several cases the relapses occurred shortly after the end of the first year of therapy and before the MRD results were available. Only patients with immediate SCT remain relapse free in the MRD-HR group. In MRD-IMR half of the the patients received intensified maintenance, in 25% treatment was stopped and 25% received conventional maintentance. In this group the relapse risk was overall also around 20% with lowest relapse risk for intensified maintenance and highest relapse risk for premature stop of therapy [15].

Summary and conclusions

MRD evaluation with several different methods is at least in specialised laboratories an established technical procedure in ALL. Altogether there is also sufficient evidence that level and course of MRD are strong independent prognostic factors in adult ALL. The published results indicate that the identification of high-risk patients by MRD analysis is relatively clear although the optimal time-point and necessary therapeutic consequences need to be defined. The definition of low-risk according to MRD is however still controversial and depends not only on time-point of evaluation but also on technical pre-requisites such as sensitivity, number of targets for MRD evaluation etc. and therapeutic consequences.

In the GMALL study 06/99 it was demonstrated for the first time in adult ALL that prospective MRD evaluation and risk stratification can be performed successfully in a large multicenter trial. Complete series of samples for one year and even more could be collected in the majority of patients.

In SR patients defined by conventional risk factors a group of **MRD-LR** patients could be identified in whom a considerable reduction of treatment duration from 2.5 years to 1 year may be justified. Only 10% **MRD-HR** patients were identified after 1 year mainly due to the fact that relapses occurred already during chemotherapy. It is concluded that MRD-HR patients must be identified earlier to initiate therapy intensification such as SCT or experimental therapy before clinical relapse. In the current study GMALL 07/2003 MRD interim analysis will be performed after induction and two consolidation cycles (month 4) with the major aim to identify MRD-HR patients defined as MRD > 10^{-4} at two time-points after induction as candidates for SCT in CR1.

Unexpectedly the **MRD-IMR** group was large (>50%) partly due to strict quality standards for MRD evaluation as mandatory for a prospective study. Similar observations have been made in childhood ALL [25]. Most importantly the therapeutic recommendation, which was intensified maintenance therapy, resulted in good outcome. Further characterisation of this subgroup also by

other means e.g. gene profiling is attempted [20]. Overall the interim results demonstrate that the treatment recommendations for the MRD risk groups are reasonable and should be pursued further. The MRD based risk stratification will therefore be continued in the ongoing GMALL study 07/2003.

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References

1. Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, Ferrara F, Peta A, Ciolli S, Deplano W, Fabbiano F, Sica S, Di Raimondo F, Cascavilla N, Tabilio A, Leoni P, Invernizzi R, Baccarani M, Rotoli B, Amadori S, Mandelli F. (2002) Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow- up of the GIMEMA ALL 0288 randomized study. Blood; 99:863-871.
2. Brisco MJ, Hughes E, Neoh SH, Sykes PJ, Bradstock K, Enno A, Szer J, McCaul K, Morley AA. (1996) Relationship between minimal residual disease and outcome in adult acute lymphoblastic leukemia. Blood; 87:5251-5256.
3. Brueggemann M, Droese J, Raff Th, Ritgen M, Scheuring U, Gökbuget N, Hoelzer D, Kneba M. (2001) The prognostic significance of minimal residual disease in adult standard risk patients with acute lymphoblastic leukemia. Blood 98: 314a.
4. Brueggemann M, Droese J, Scheuring U, Gökbuget N, Hoelzer D, Nakao M, Bartram CR, Kneba M. (2001) Minimal residual disease in adult patients with acute lymphoblastic leukemia during the first year of therapy predicts clinical outcome. The Hematology Journal; 1:700a.
5. Brueggemann M, Raff T, Gökbuget N, Hoelzer D, Kneba M. (2003) Early Tumor Kinetics in Adult Acute Lymphoblastic Leukemia (ALL) Has High Prognostic Impact. Blood; 102:Abstract #215.
6. Campana D, Pui C-H. (1995) Detection of minimal residual disease in acute leukemia: methodological advances and clinical significance. Blood; 85:1416-1434.
7. Cavé H, Van der Werff Ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E, for the European Organization for Research and Treatment of Cancer-Childhood Leukemia Cooperative Group. (1998) Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. N Engl J Med; 339:591-598.
8. Ciudad J, San Miguel JF, López-Berges MC, Vidriales B, Valverde B, Ocqueteau M, Mateos G, Caballero MD, Hernández J, Moro MJ, Mateos MV, Orfao A. (1998) Prognostic value of immunophenotypic detection of minimal residual disease in acute lyphoblastic leukemia. J Clin Oncol; 16:3774-3781.
9. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, Sandlund JT, Rivera GK, Rubnitz JE, Ribeiro RC, Pui CH, Campana D. (2000) Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood; 96:2691-2696.
10. Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, Behm FG, Pui C-H, Downing JR, Gilliland DG, Lander ES, Golub TR, Look AT. (2002) Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell; 1:75-87.
11. Foroni L, Harrison CJ, Hoffbrand AV, Potter MN. (1999) Investigation of minimal residial disease in childhood and adult acute lymphoblastic leukaemia by molecular analysis. Br J Haematol; 105:7-24.
12. Gökbuget N, Arnold R, Buechner Th, Ganser A, Ludwig WD, Maschmeyer G, Rieder H, Thiel E, Messerer D, Hoelzer D, for the GMALL Study Group. (2001) Intensification of induction and consolidation improves only subgroups of adult ALL: Analysis of 1200 patients in GMALL study 05/93. Blood 98: 802a.
13. Gökbuget N, Hoelzer D, Arnold R, Böhme A, Bartram CR, Freund M, Ganser A, Kneba M, Langer W, Lipp Th, Ludwig W-D, Maschmeyer G, Rieder H, Thiel E, Weiss A, Messerer D. (2000) Treatment of Adult ALL According to the Protocols of the German Multicenter Study Group for Adult ALL (GMALL). Hemat/Oncol Clin North Am; 14:1307-1325.
14. Gökbuget N, Kneba M, Raff T, Bruggemann M, Scheuring U, Reutzel R, Hoelzer D. (2002) Risk-adapted treatment according to minimal residual disease in adult ALL. Best Pract Res Clin Haematol; 15:639-652.
15. Gökbuget N, Raff T, Brueggemann M, Scheuring U, Reutzel R, Flohr T, Pfeifer H, Bartram CR, Kneba M, Hoelzer D. (2003) Prospective Risk Stratification Based on Minimal Residual Disease (MRD) Is Feasible in Adult ALL but Limited by a High Proportion of MRD-Intermediate Risk. Blood; 102:Abstract #1373.
16. Hoelzer D, Gökbuget N. (2000) New approaches in acute lymphoblastic leukemia in adults: Where do we go? Seminars in Oncology; 27:540-559.
17. Hoelzer D, Gökbuget N, Brüggemann M, Droese J, Scheuring U, Reutzel R, Thiel E, Kneba M. (2001) Clinical impact of minimal residual disease (MRD) in trial design for adult ALL. Blood 98: 584a.
18. Hoelzer D, Gökbuget N, Ottmann O, Pui CH, Relling MV, Appelbaum FR, van Dongen JJ, Szczepanski T. (2002) Acute lymphoblastic leukemia. Hematology (Am Soc Hematol Educ Program);162-192.
19. Hoelzer D, Thiel E, Löffler H, Büchner T, Ganser A, Heil G, Koch P, Freund M, Diedrich H. (1988) Prognostic factors in a multicenter study for treatment of acute lymphoblastic leukemia in adults. Blood; 71:123-131.
20. Hofmann W-K, Komor M, Huetter G, Schwartz S, Gökbuget N, Myloch S, de Vos S, Ottmann OG, Thiel E, Hoelzer D. (2003) Different Outcome of Adult Patients with Commonly Defined Standard Risk B-Precursor Acute Lymphoblastic Leukemia Can Be Predicted by Gene Expression Profiling. Blood; 102:Abstract #608.
21. Mortuza FY, Papaioannou M, Moreira IM, Coyle LA, Gameiro P, Gandini D, Prentice HG, Goldstone A, Hoffbrand AV, Foroni L. (2002) Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol; 20:1094-1104.
22. Panzer-Grümayer E, Schneider M, Panzer S, Faschin K, Gadner H. (2000) Rapid molecular response during early induction chemotherapy predicts good outcome in childhood acute lymphoblastic leukemia. Blood; 95:790-794.
23. Preudhomme C, Grardel N, Huyghe P, Roumier C, Lai J-L, Debotton S, Lepelley P, Cosson A, Bauters F, Fenaux P. (2001) Evaluation of Minimal Residual Disease (MRD) by Competitive Quantitative PCR Using IgH/TCR Rearrangements Has Prognostic Value in Adult Acute Lymphoblastic -Leukemia (ALL): A Single Center Study. Blood; 98:1331a.
24. Rowe JM, Richards S, Wiernik PH, Franklin IM, Burnett AK, Lazarus HM, Prentice HG, Harrison G, Neuberg D, Tallman MS, Goldstone AH. (1999) Allogeneic bone marrow transplantation (BMT) for adults with acute lymphoblastic leukemia (ALL) in first complete remission (CR): Early results from the international ALL trial (MRC UKALL/ECOG E2993). Blood; 94:732a.
25. Schrappe M, Flohr T, Beier R, Bartram CR. (2001) Risk stratification in childhood acute lymphoblastic leukemia based on clone-specific detection of minimal residual disease (MRD) with molecular genetics: performance in German Trial ALL-BFM 2000. The Hematology Journal; 1:695a.
26. Szczepanski T, Orfao A, van dV, V, San Miguel JF, van Dongen JJ. (2001) Minimal residual disease in leukaemia patients. Lancet Oncol; 2:409-417.
27. van Dongen JJ, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, Stolz F, Schrappe M, Masera G, Kamps WA, Gadner H, van Wering E, Ludwig WD, Basso G, de Bruijn MA, Cazzaniga G, Hettinger K, van der Does-van den Berg A, Hop WC, Riehm H, Bartram CR. (1998) Prognostic value of minimal residual disease in acute lymphoblastic leukemia in childhood. Lancet; 352:1731-1738.
28. Vidriales MB, Perez JJ, Lopez-Berges MC, Gutierrez N, Ciudad J, Lucio P, Vasquez L, Garcia-Sanz R, Consuelo del Canizo M, Fernandez-Calvo J, Ramos F, Rodriguez MJ, Calmuntia MJ, Porwith A, Orfao A, San-Miguel JF. (2003) Minimal residual disease (MRD) in adolescent (>14 years) and adult acute lymphoblastic leukaemias (ALL): Early immunophenotypical evaluation has high clinical value. Blood; First Edition Paper, prepublished online February 13, 2003.
29. Yeoh E-J, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, Behm FG, Raimondi SC, Relling MV, Patel A, Cheng C, Campana D, Wilkins D, Zhou X, Li J, Liu H, Pui C-H, Evans WE, Naeve C, Wong L, Downing JR. (2002) Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell; 1:133-143.

Results of the International LALA-94 Trial

H. DOMBRET, X. THOMAS, V. LHÉRITIER and D. FIÈRE
for the LALA Group, Paris, France

Study design

In this prospective multicenter study conducted by the French-Belgium-Swiss-Australian LALA Group, we analyzed the benefits of a risk-adapted post-remission strategy in adults (15–55 years) with acute lymphoblastic leukemia (ALL), re-evaluating stem cell transplantation (SCT) in the most aggressive cases. A total of 1,000 patients entered the trial between 1994 and 2002. The present analysis deals with 922 evaluable patients according to the following risk-groups: standard-risk ALL (group 1, #430); high-risk ALL (group 2, #238); Ph+ ALL defined by the presence of t(9;22) and/or *BCR-ABL* transcript (group 3, #198); central nervous system-positive (CNS+) ALL (group 4, #56). Criteria used to define the high-risk group 2 were to need a salvage course to reach CR and/or to have B-lineage ALL with at least one of the following bad factors: 1) WBC ≥ 30.10^9/L; 2) pro-B CD10-/CD20- phenotype; 3) presence of 11q23 abnormality and/or *MLL-AF4* transcripts or presence of t(1;19) and/or *E2A-PBX1* transcripts; 4) co-expression of myeloid antigens.

Treatments

All patients received a 4-drug/4-week induction course with an initial daunorubicin (DNR) *vs* idarubicin (IDA) randomization. Salvage therapy consisted of an intensive mitoxantrone and intermediate-dose cytarabine (MIDAC) course. Group 1 patients then received chemotherapy alone and were randomized between an intensive and a less intensive arm, except those needing salvage to reach CR who were then included in group 2. All group 2, 3, and 4 patients received intensive MIDAC as consolidation (or salvage) and those with an HLA-identical sibling were then assigned to allogeneic SCT in first CR. Autologous SCT was offered to all other CR patients in groups 3 and 4, while in group 2 they were randomized between chemotherapy and autologous SCT.

Results

The CR rate after one course of induction was 72%. Salvage rate was 53%. Overall, 771 patients achieved CR (84%). Resistance and mortality rates were 11% and 5%, respectively. With a median follow-up of 3.5 years, median disease-free survival (DFS) was 16 months with an estimated 3-year DFS at 33%. In patients receiving only chemotherapy, DFS was significantly improved in the IDA as compared to the DNR arm (p=0.04). In group 1, the 3-year DFS was 37% without difference between both post-CR treatment arms. In the 239 patients with T-ALL, most of them (**#x**) treated in the standard-risk group 1 (since only those who needed salvage were then included in group 2), results were disappointing with a 3-year DFS at 29%. In a subset of 91 T-ALL patients well-studied for immunophenotype and genotype, CR rate after induction was significantly lower in immature (cTCRβ negative) cases and HOX11L2 expression was associated with a poor outcome (Asnafi et al. ASH 2003). In group 2 and 4, the 3-year DFS was 36% and 42%, respectively. In these patients (group 2+4), DFS was significantly improved in those with an HLA-matched sibling (p=0.02). In group 2 patients, autologous SCT and chemotherapy resulted in comparable outcomes. In group 3 (Ph+ ALL), 3-year DFS was 21%. In a previous interim analysis of 154 group 3 patients, HLA-matched sibling availability and molecular response after induction and MIDAC were independent good-prognostic factors for outcome (Dombret et al. Blood 2002). In these patients, combined MIDAC and imatinib mesylate administration is under investigation (AFR03 study).

Conclusions

In high-risk ALL patients, allogeneic SCT was associated with longer DFS while no significant benefice of autologous SCT over chemotherapy was evidenced. Adapted treatments based on prognostic subsets characterization might be required for T-ALL patients. Combination of imatinib mesylate with intensive chemotherapy has to be evaluated and standardized in those with Ph+ ALL.

Main Session IX

Dose-Reduced Conditioning Therapy in High-Risk AML

H.-J. KOLB, C. SCHMID, M. SCHLEUNING, J. TISCHER, M. WEISSER, M. HUMANN, A. RANK, and G. LEDDEROSE
Hematopoietic Cell Transplantation, Dept. Medicine III,
University of Munich & GSF-National Research Center for Environment and Health, Munich, Germany

The aim of the conditioning treatment has changed from maximally tolerated doses of chemo-radiotherapy to dose-reduced conditioning, since the dominant role of the graft-versus-leukemia effect has been recognized. Dose-reduced conditioning has been studied in combination with cytoreductive therapy prior transplantation and transfusion of donor lymphocytes (DLT) after transplantation. Unfavorable karyotype, refractory or relapsed disease and an antecedent hematological disorder define high risk AML. MDS with blast excess are also included. We combined a 4 day course of chemotherapy with alloSCT after dose reduced conditioning. Fludarabin (30 mg/m^2), AraC (2×2 g/m^2) and Amsacrine (100 mg/m^2) were given on each of 4 consecutive days. After 3 days of rest, the patients were conditioned with 4Gy TBI, Cyclophosphamide and ATG. Most patients received mobilized blood stem cells (MBSC) from HLA.matched siblings and unrelated donors. DLT were scheduled to be given prophylactically on day 90i, if graft-versus-host disease (GVHD) was absent. 72 patients were treated (9 patients with unfavorable karyotype, delayed response to induction therapy or secondary AML in CR1, 7 patients in CR2, 27 patients in 1st or 2nd relapse, 23 patients in refractory disease, and 6 patients in untreated progressive MDS or secondary AML). 4 relapsed patients had received an autologous transplant before, and 22 patients had an unfavorable karyotype. Median age was 50.2 (18.5–65.8) years. 35 patients had a family and 37 an unrelated donor.

All patients surviving more than 30 days engrafted, and all but four patients were free of blasts in blood and bone marrow at day 30. Additionally, one patient achieved a CR after discontinuation of immune suppression. >95% donor chimerism was achieved in 85% of the patients at day 45 and in 92% at day 90. Overall survival and disease free survival was 50% after one and 42% after two years. Interestingly, survival was identical in patients with and without an unfavorable karyotype. Treatment related mortality was 18% before day 100. 58% of the patients developed acute and 40% developed chronic GvHD. 8 patients relapsed at a median of 102 days from transplant. Discontinuation of immune suppression and adoptive immunotherapy re-induced remissions in four cases. So far, 12 patients have received prophylactic DLT. Mixed chimerism was converted into complete chimerism in one patient, and 3 patients developed chronic GvHD. To date, none of the 12 patients has relapsed.

We conclude that the combination of cytoreductive chemotherapy and intensity reduced conditioning is well tolerated even in elderly patients and may induce long term remissions in patients with high risk AML and MDS. Only a minority of patients were given prophylactic DLT and the role of DLT for the maintenance of remissions remains to be defined.

Transplantation Strategies in the AML1996 Study

G. EHNINGER, U. PLATZBECKER, H. LINK, M. SCHAICH, T. ILLMER, and M. BORNHÄUSER
Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus, Fetscherstr. 74, 01307 Dresden, Germany

In the SHG AML 96 study for AML, patients younger than 60 years were treated according to their cytogenetic risk profile. In the intermediate risk group (including inv16) in patients below the age of 55 years an allogeneic transplantation was the first treatment option in patients with a related donor. In the high risk group, an unrelated donor search was initiated and a transplant was offered to all patients below the age of 45 years.

The results after allogeneic transplantation were worse compared to our previous studies out of the early '90 which might be explained by an increase of the median age of about 10 years. We therefore started to evaluate the use of reduced intensity conditioning protocols in AML. Sayer at al. recently published these data in BMT 2003. 113 patients received an allogeneic transplant after reduced intensity conditioning (predominantly busulfan/flud-arabine based) because of age, comorbitities, previous infections or relapse after autologous transplants. The results shown in figure 2 compare favourable with those after full dose conditioning in patients with a much better performance status.

Reduced intensity conditioning followed by an allogeneic transplant in AML resulted in engraftment and a disease-control was achieved even in poor-risk disease. Early TRM seems to be low, but the rate of GvHD and infectious complications is comparable to that after standard conditioning. The German cooperative transplant study group will follow a prospective randomized protocol in the context of the German Intergroup study within the competence network of acute and chronic leukemias. The standard conditioning therapy with 12 Gy TBI and 120 mg/kg cyclophosphamide will be compared in patients with AML in first complete remission with fludarabine and 8 Gy TBI.

The complete remission rate in AML patients with high risk cytogenetics is only around 50%. We therefore initiated a pilot study to transplant high risk patients very early after induction chemotherapy. The results looked very encouraging (Platzbecker et al, BMT 2001: 243-246) and this program will be evaluated in our current AML2003 program.

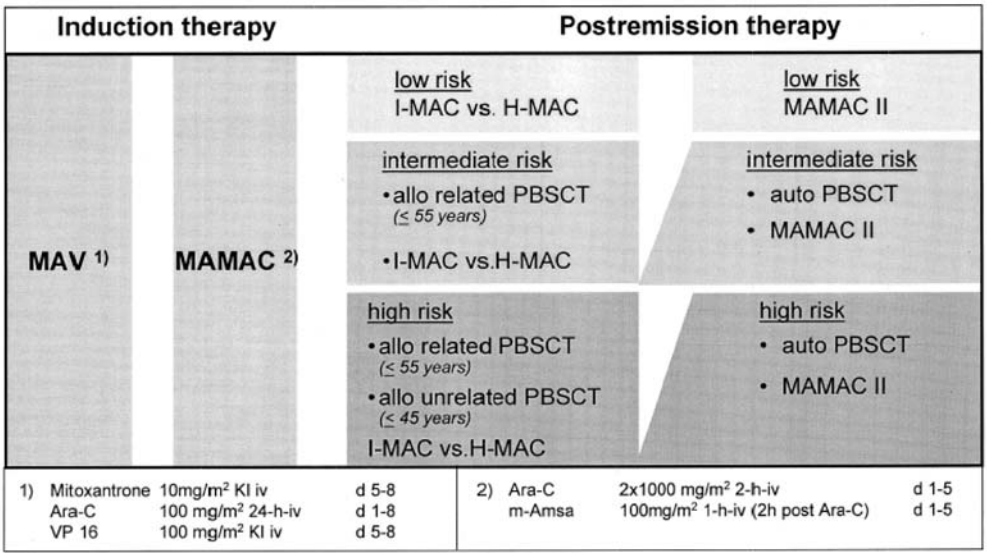

Figure 1: Design of the AML´96 study

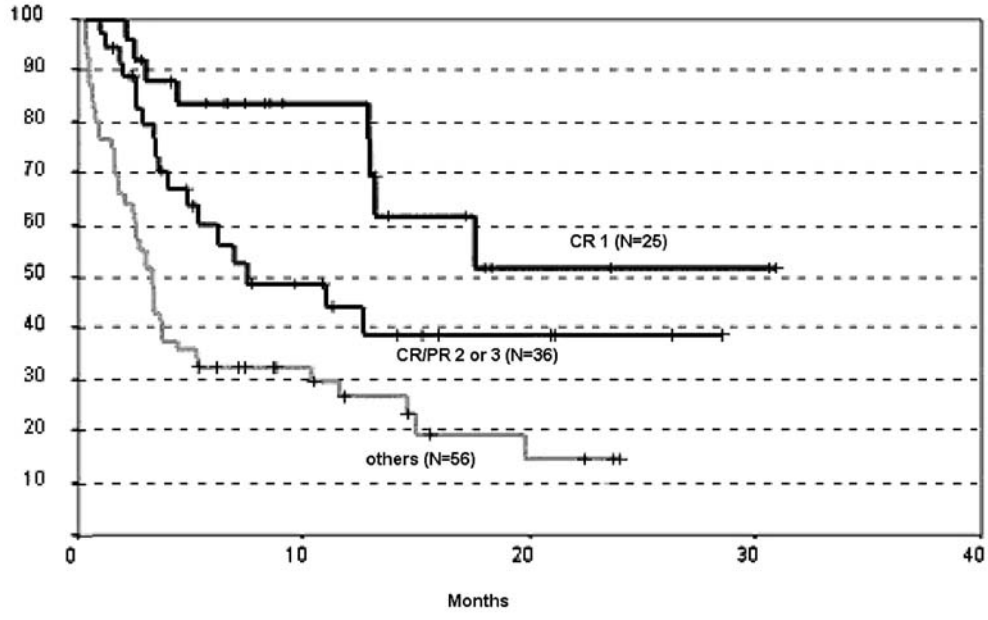

Figure 2: Probability of disease-free survival after reduced intensity conditioning in AML patients not qualifying for a full dose regimen (Sayer et al, BMT 2003:1089-1095)

Autologous versus Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia

R. WILLEMZE[1],S.SUCIU[2], F. MANDELLI[3], T. DE WITTE[4],
and S. AMADORI[5] on behalf of the EORTC and GIMEMA Leukemia Groups
[1] Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands; [2] EORTC Data Center, Brussels, Belgium;
[3] Department of Cellular Biotechnology and Hematology, University La Sapienza, Roma, Italy; [4] Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands;
[5] Department of Hematology, University Tor Vergata, Roma, Italy

Abstract

Using the data of the patients in complete remission (CR) up to the age of 45 years included in the EORTC-LG/GIMEMA AML-10 trial we investigated the value of the strategy to perform either an autologous (auto-SCT) or an allogeneic (allo-SCT) stem cell transplantation on an intention to treat basis. Between 1993 and 1999, out of 1198 pts, 822 achieved CR. 734 pts, constituting the study group, received an intensieve consolidation course: 293 had a sibling donor and 441 had not. Allo-SCT and auto-SCT was performed in 68.9% and 55.8%, respectively. Cytogenetics was successfully performed in 446 pts. Risk groups were: good (t(8;21), inv(16)), intermediate (NN or -Y only), bad/very bad (all others). Median follow-up was 4 years. The 4-year disease-free survival (DFS) rate of patients with a donor vs of those without a donor was 52.2% vs 42.2%, p=0.044; the relapse incidence was 30.4% vs 52.5%, death in first complete remission was 17.4% vs 5.3%, and the survival rate was 58.3% vs 50.8% (p=0.18). The DFS rates in pts with and without a sibling donor were similar in pts with good or intermediate risk cytogenetics, but 43.4% and 18.4%, respectively, in pts with bad or very bad risk cytogenetics. In younger patients (15–35 yrs), the difference was more pronounced. The strategy to perform an allo-SCT in patients where a family donor was available led to better overall results than to perform an auto-SCT, especially for younger patients or those with bad or very bad risk cytogenetics.

Introduction

The strategy to perform a transplantation with allogeneic or autologous bone marrow or blood derived stem cells after induction and consolidation courses for patients with acute myelogenous leukemia (AML) is a common practice in many large centers in the world. However, the value of such a transplantation strategy for individual prognostic subgroups of AML is unknown.

Previously, the AML-8A trial of the EORTC Leukemia Group and the Italian GIMEMA group showed that a strategy to perform an auto-SCT results in a significantly longer disease free survival (DFS) than to perform a second intensive consolidation course, but significant differences between auto-SCT and allo-SCT could not been detected due to the limited number of patients[1].

The EORTC and GIMEMA Leukemia Groups subsequently conducted the AML-10 study. All patients were randomized for one of three anthracyclines added to cytosine arabinoside and etoposide as remission induction treatment. After achievement of complete remissiom (CR) one intensive consolidation course was given. This course was to be followed by an allo-SCT in the patients with a HLA-identical family donor or by auto-SCT in those who lacked such a donor.

The AML-10 trial offered the possibility in patients up to the age of 45 years to assess the value of early allo-SCT vs auto-SCT on the basis of intention-to-treat[2] by comparing the outcome of the group of patients with an HLA identical sibling donor (“Donor” group) with the outcome of patients without such a donor (“No Donor” group).

Patients and Methods

The EORTC-LG/GIMEMA AML-10 study was conducted from November 1993 to December 1999 in 80 European centers. All patients, up to the age of 60, with previously untreated AML, except acute promyelocytic leukemia, with more than 30% blast cells in the bone marrow, were eligible.

Randomization for either daunorubicin, mitoxantrone or idarubicin was performed at the time of registration. Remission induction treatment consisted of 10 days of standard dose cytosine arabinoside, five days of etoposide and three days of either daunorubicin or mitoxantrone or idarubicin. In case of complete remission after one or two courses a single course of consolidation therapy is administered consisting of intermediate dose cytosine arabinoside and the anthracycline, randomized at registration. Patients with a sibling donor were assigned to undergo an allogeneic SCT. All patients without such a donor had to receive an autologous blood or bone marrow SCT.

2157 patients were randomized. Among 1198 patients between 15 and 46 years of age, 62 patients were considered as inevaluable for the treatment response, mainly due to the lack of clinical documentation. Out of the remaining 1136 pts, 822 (72.4%) entered complete remission after 1 or 2 courses of induction therapy. Among them, 50 patients were off–protocol treatment. Out of 772 patients who received consolidation, 38 have not been HLA–typed. Among the 734 patients, 55 had no sibling. Of the remaining 679 patients, 293 had an HLA identical sibling donor (“Donor” group) and 386 had no family donor. By adding to this latter group those 55 patients with no siblings, a 441 patient group has been formed, which was designated as “No Donor” group. The median follow-up was 4 years. The CALGB criteria for response to treatment and relapse were used. As cytogenetic classification the ISCN system has been applied[3]. Risk groups were: good (t(8;21), inv(16)), intermediate (NN or -Y only), bad/very bad (all others). The patients with unknown, not done or unsuccessful cytogenetics were grouped together as “unknown”. All analyses were based on the intent-to-treat principle.

Results

Allo-SCT was performed in 202 (68.9%) of 293 patients with a sibling donor and auto-SCT in 246 (55.8%) of 441 patients without such a donor.

The 4-year DFS rate (±SE) of the “Donor” group was superior to that of the “No Donor” group: 52.2% (±3.2%) vs 42.2% (±2.6%), p=0.044; The relapse incidence was 30.4% (±2.9%) vs 52.5% (±2.6%) (p<0.0001), and the incidence of death in CR was 17.4% (±2.4%) vs 5.3% (±1.1%) (p<0.0001), respectively. The survival from CR rate was 58.3% (±3.2%) vs 50.8% (±2.7%) (p=0.18).

Cytogenetics was successfully performed in 446 pts. Intention-to-treat analysis revealed that the 4-year disease-free survival (DFS) rates in patients with and without a sibling donor were 61% and 66%, respectively, for patients with good risk cytogenetics, and 45% and 49%, respectively, for patients with intermediate risk cytogenetics. In contrast the DFS in patients with bad or very bad risk cytogenetics was 43.4% for those in the donor group and only 18.4% for those in the no donor group. Patients with an “unknown” cytogenetic analysis (N=288) showed the same trends as the whole study group.

Overall, the results with respect to survival, DFS and relapse incidence were not different between the age groups (15–25, 26–35 and 36–45 years). TRM was slightly higher for the oldest age group. Within the “No Donor” group DFS, relapse incidence and TRM were similar in all age groups . In patients with a sibling donor, TRM showed a slight age effect; the older the patients the higher the TRM. In patients $\leq$35 years old, the DFS for the patients with a sibling donor was longer than for those without a donor, due to a lower incidence of relapse and a lower increase in the TRM incidence. In older patients (36–45) the lower incidence of relapse (29.6% vs 48.3%) for the “Donor” group was counterbalanced by a far higher incidence of death in CR (21.1% vs 5.5%).

Conclusion

In this study we show that using analysis by intention-to-treat of the EORTC-LG/GIMEMA AML-10 trial patients in complete remission under the age of 46 assigned to allo-SCT have a significantly better outcome than those planned to undergo an auto-SCT. This seems specifically true for patients with bad or very bad risk cytogenetics.

References

1. Zittoun RA, Mandelli F, Willemze R, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. N Engl J Med 1995; 332: 217-223.
2. Suciu S, Mandelli F, de Witte Th, Zittoun R, Gallo E, Labar B, de Rosa G, Belhabri A, Giustolisi R, Delarue R, Liso V, Mirto S, Leone G, Bourhis J-H, Fioritoni G, Jehn U, Amadori S, Fazi P, Hagemeijer A, Willemze R. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMA AML-10 trial. Blood, August 15, 2003;102 (4): 1232-1240
3. ISCN (International System for Human Cytogenetic Nomenclature). Guidelines for cancer cytogenetics. In: Mitelman F ed. Supplement to an International System for Human Cytogenetics Nomenclature. Basel, Switzerland: S. Karger; 1995.

The MRC Transplantation Strategies

A K BURNETT[1], R HILLS[2], A GOLDSTONE[3], D MILLIGAN[4], A G PRENTICE[5], B GIBSON[6], D WEBB[7], I HANN[7], and K WHEATLEY[2]
[1]University of Wales College of Medicine, Cardiff, [2]Birmingham Clinical Trial's Unit, Birmingham, [3]University College Hospital, London, [4]Heartland's NHS Trust ,Birmingham, [5] Derriford NHS Trust, Plymouth, [6]Royal Hospital for Sick Children, Glasgow, [7]Great Ormond Children's Hospital, London.

The Medical Research Council Leukaemia Working Parties have attempted to define the role of allogeneic and autologous stem cell transplantation in AML in two large prospective trials between 1998-2002. Nineteen hundred and sixty-six patients were recruited to AML10. In this trial the question being examined was what was the value of adding a transplant to 4 courses of intensive chemotherapy which itself resulted in an overall 40% survival. Allograft was assessed on a donor vs no donor analysis. Patients without a donor were randomised after course 3 to have 1 more course only or 1 course plus an autograft. Overall both types of SCT reduced the risk of relapse (allo 34% vs 50%/auto 37% vs 58%), improved the disease free survival (allo – 51% vs 44%/auto 53% vs 40%) but did not improve overall survival. In this trial a simple risk stratification was devised based on cytogenetics and morphological response to course 1. When the comparisons are done within the three risk groups there was a reduction in relapse risk in all groups, but no survival advantage was observed in patients of favourable or poor risk. Patients >45 years shown no survival benefit. Only standard risk patients had a survival advantage.

AML10 suggested that more treatment was better. The AML12 trial questioned whether the antileukaemic benefit of 'more' (ie transplant) could be achieved with an extra course of chemotherapy. A total of 4 vs 5 courses were then compared. Patients who were not good risk were randomised to stem cell transplant or not. Patients allocated to SCT who had a donor received an allograft, those who lacked a donor received an autograft.

Although further follow-up is required on the 1224 patients allocated to 4 vs 5 courses, there was no overall survival advantage for a 5th course but with a favourable trend in patients 15–35 years. There was no overall survival difference between those randomised to transplant (auto or allo) or chemotherapy, although there was a reduction in relapse risk. The decision to exclude favourable disease was vindicated since the outcome for these patients in AML12 (no SCT) was not different from AML10 (included SCT). When the comparisons were made within the risk and age subgroups no survival advantage was apparent in any subgroup.

Analysing the value of allograft using a donor vs no-donor comparison has been open to criticism, because as many as 40% of cases do not receive the transplant. A cohort of patients in these studies with donors who did or did not receive the allograft were compared, no survival difference was seen suggesting that the genetic analysis in this study was representative.

In designing the current study AML15 (opened June 2002), the aim as to continue to assess allogeneic transplant in standard and poor risk patents, as course 3 in the hope that more patients would receive the transplant so that more patients would access the greater antileukaemic mechanism. The question being posed is "does a transplant as course 3 improve survival compared with 4 or 5 courses of chemotherapy?"

Non-ablative transplants can be undertaken safely in older patients. The efficacy is encouraging but uncertain in AML. The AML15 trails provides that all standard risk patients with an available donor will, if under 35 years receive an early standard transplant. Patients >45 years will be eligible only for a non-ablative transplant. Patients 36–45 years may receive either approach. Because the efficacy of non-intensive allograft is not known, the transplant will be delivered after 3 courses of chemotherapy.

In summary, in neither of these major trials was there an overall survival advantage of adding transplant to 4 courses of chemotherapy although the risk of relapse could be reduced. Good risk patients derive no additional benefit. Patients over 35 years did not benefit in either trial in spite of the fact that the risk of relapse is increased in this age group. This is primarily due to an excess treatment related mortality which suggests that this may be the age threshold from which to evaluate the non-intensive approaches.

During this period 396 patients received SCT, of these 181 were autografts and 215 were allografts of which 117 were from volunteer unrelated donors.

References

Burnett AK, Goldstone AH, Stevens R, Hann IM, Rees JK, Gray RG, and Wheatley K (1998) Randomised comparison of addition of autologous bone-marrow transplantation in intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 Trial. Lancet 351:700-708.

Burnett AK., Wheatley K, Goldstone AH, Stevens RF, Hann IM, Rees JKH, and Harrison G (2002) The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: results of the UK MRC AML10 Trial. Brit.J.Haematol. 118:385-400.

Burnett A.K (2002) Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation? – An adult treater's view. Brit.J.Haematol. 118:357-364.

Grimwade D, Walker H, Oliver F, Wheatley K, Harrision C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, and Goldstone A (1998) The importance of diagnostic cytogenetics on outcome in AML: Analysis of 1,612 patients entered into the MRC AML: 10 Trial. Blood 92:2322-2333.

Wheatley K, Burnett AK, Goldstone AH, Gray RG, Hann IM, Harrison CJ, Rees JK, Stevens RF, and Walker H (1999) A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Brit.J.Haematol. 107 (1):69-79

Burnett AK, Wheatley K, Goldstone AH, Gibson B, Webb D, Prentice AG, Milligan D (2002) MRC AML12: A comparison of ADE vs MAE and S-DAT vs H-DAT±Retinoic Acid for induction and four vs five total courses using chemotherapy or stem cell transplant in consolidation in 3459 patients under 60 years with AML. Blood 100 (11):155a.

Stevens RF, Hann IM, Wheatley K, and Gray RG (1998) Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukaemia: results of the United Kingdom Medical Research Council's 10th AML trial. Brit.J.Haematol. 101:130-140.

Transplantation Strategies in AML: AMLCG Data

J. KIENAST[1], M. STELLJES[1], B. BERNING[1], M. KRÖGER[1], M. C. SAUERLAND[2], A. HEINECKE[2], C. SCHOCH[4], B. WÖRMANN[3], T. BÜCHNER[1], W. HIDDEMANN[4], and W. E. BERDEL[1] on behalf of the GERMAN AML COOPERATIVE GROUP (AMLCG)

[1]Dept. of Medicine / Hematology and Oncology and [2]Dept. of Medical Informatics and Biomathematics, University of Muenster, Muenster, Germany, [3]Dept. of Medicine, Städtisches Klinikum, Braunschweig, Germany, [4]Dept. of Medicine III, Ludwig-Maximilians-University, Grosshadern, Munich, Germany

Introduction

Allogeneic stem cell transplantation (allo-SCT) is considered the most potent postremission therapy for acute myeloid leukemia (AML) [1–3]. Its superior antileukemic activity is largely ascribed to the powerful graft-versus-leukemia (GvL) effects exerted by donor lymphocytes [4, 5]. However, due to considerable treatment-related lethality the gains in relapse prevention do not necessarily translate into survival advantages in the overall patient population. Therefore, allo-SCT for adult patients with AML in first complete remission (CR1) is currently recommended only for younger and medically fit patients who are at intermediate to high risk of relapse and have an HLA-identical sibling donor. Stem cell allografting from alternative donors in CR1 is considered an option for high risk patients as defined by cytogenetic abnormalities or incomplete response after one course of induction chemotherapy and should usually be performed in the context of a clinical protocol [6].

AMLCG Trials

Indications and Use of Allo-SCT in CR1. The protocol of the *AMLCG 1986* trial recommended allogeneic bone marrow transplantation (allo-BMT) for CR1 patients up to the age of 40 years with an HLA compatible sibling donor. The transplant was to be performed within 6 months of remission entry. Out of 495 evaluable patients in CR1 aged <60 years, 51 patients (10%) with a median age of 32 years (range: 16–49) underwent allo-BMT on this protocol. The transplants were performed at a median of 4 months after CR1 was documented.

The *AMLCG 1992* protocol recommended allo-BMT in CR1 for all patients up to the age of 50 years with an HLA-identical sibling donor. The transplant was to be performed within 3 months of remission entry. Out of 408 evaluable patients in CR1 aged <60 years, 42 patients (10%) with a median age of 36 years (range: 18–49) underwent allo-BMT. Deviant from the protocol recommendations, the transplants were performed after a median of 4 months since CR entry.

The ongoing *AMLCG 1999* trial follows a randomized factorial design comparing (i) high versus standard dose AraC during induction treatment, (ii) G-CSF priming versus no priming, and (iii) autologous SCT versus maintenance chemotherapy. All CR1 patients up to the age of 60 years are eligible for an allo-SCT from a matched sibling donor. A stem cell allograft from a related or unrelated donor is recommended for high risk patients with unfavorable complex karyotype abnormalities. The transplant is to be performed within 3 months of remission entry. As of 09/2003, 340 patients with de novo AML and below age 60 had entered CR after induction treatment. To date, 56 (16%) and 51 (15%) of those 340 patients underwent allo-SCT and auto-SCT, respectively.

Matched-Pair Analyses. The results of allo-BMT in CR1 were compared to postremission chemotherapy by means of a matched-pair analysis based on the combined data from the 1986 and 1992 trials. Patient pairs were matched for the following criteria: (i) time in CR1, (ii) age (± 2 years), (iii) type of induction therapy, (iv) gender, (v) time since enrolment into the protocol. For the overall patient population, the results shown in Figures 1–3 confirm the superior antileukemic activity of allo-BMT at the expense of a higher transplant-related lethality. Nonetheless, the significantly improved relapse free survival would favor allo-BMT. However, the trend suggesting improved overall survival fails to reach the level of statisti cal significance.

A matched-pair analysis for the 1999 trial is currently in progress and will be presented. This analysis will allow cross comparisons of allo-SCT, auto-SCT and conventional postremission treatment and will consider cytogenetic risk categories as the primary matching criterion.

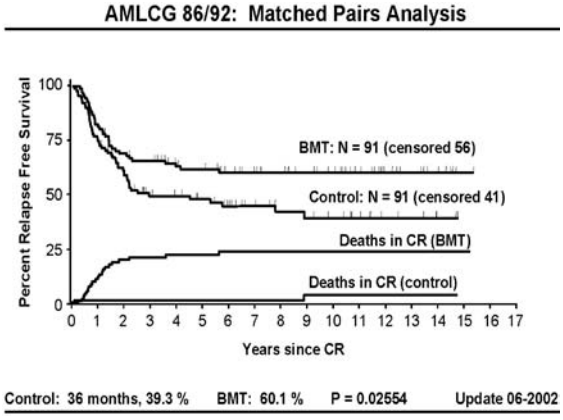

Figure 1

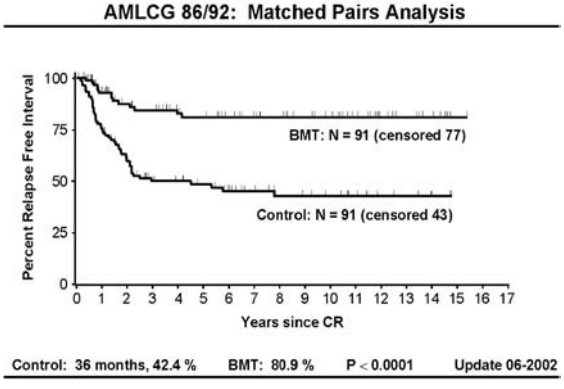

Figure 2

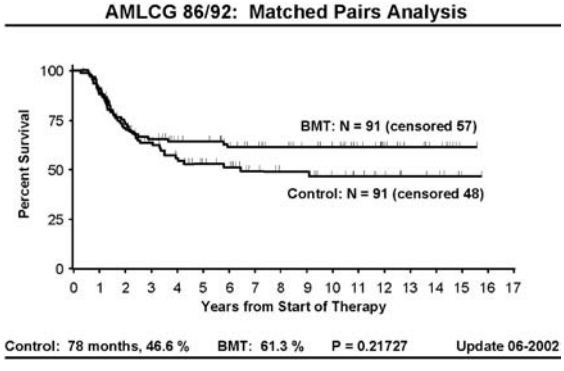

Figure 3

Reduced Intensity Ablative Conditioning. More recently, reduced intensity conditioning followed by allo-SCT has been explored in various hematological malignancies including AML [7, 8]. These novel transplant strategies aim at reducing transplant-related lethality and rely on harnessing GvL effects rather than the eradication of the malignant clone by high-dose chemoradiotherapy. In an intergroup pilot trial of the AMLCG and SHG Dresden as well as associated centers, a myeloablative conditioning regimen with reduced dose total body irradiation (TBI) followed by allo-SCT was evaluated. The rationale of the study was to lower transplant-related lethality while preserving the antileukemic effect of TBI. 50 patients with AML in CR1/CR2 (9/9), PR/untreated relapse (1/7) or with primary/secondary refractory disease (24) underwent allo-SCT from related (n=24) or unrelated (n=26) donors. Most of the patients were not considered eligible for conventional conditioning. Median age was 52 years (range: 20-65). The preparative regimen consisted of 8 Gy TBI, fludarabine (30 mg/m^2/d IV ×4), and optional ATG (rabbit, 20 mg/kg/d ×2) followed by immunosuppression with CsA/MTX. The median follow-up was 406 days (range: 9-1196).

Complete donor engraftment was documented within 6 weeks and sustained in all evaluable patients. Cumulative probabilities of grades II, III and IV acute GVHD were 12%, 4%, and 2%, respectively. Grade III-IV acute GVHD occurred in 0% of pts after related and in 12% after unrelated SCT and was fatal in 1 pt (2%). Estimated probabilities for treatment-related lethality at 2 years were 0% for CR1/CR2 (95% CI, 0% to 18%), 43% for PR/untreated relapse (95% CI, 18% to 59%), and 51% for refractory disease (95% CI, 8% to 75%). Probabilities for relapse lethality (2 years) were 5% for CR1/CR2 (95% CI, 0% to 27%), 38% for PR/untreated relapse (95% CI, 8% to 75%), and 42% for refractory disease (95% CI, 22% to 65%). 2-year estimates for relapse-free survival (RFS) were 89% for CR1/CR2 (95% CI, 62% to 97%) and 8% for refractory disease (95% CI, 1% to 27%). For PR/untreated relapse RFS at 1 year was 29% (95 CI, 4% to 61%).

Thus, for AML patients in CR conditioning with 8 Gy TBI/fludarabine±ATG prior to allo-SCT appears to result in markedly reduced transplant-related lethality and favorable survival data. Based on this phase II trial, a randomized German Intergroup Study comparing conventional conditioning (12 Gy TBI/cyclo-phosphamide) to the 8 Gy TBI/fludarabine regimen prior to allo-SCT in patients with AML in CR1 was initiated and integrated into the 2003 AMLCG protocol.

References

1. Zittoun RA, Mandelli F, Willemze R, et al. (1995) Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. N Engl J Med 332: 217-223
2. Harousseau JL, Cahn JY, Pignon B, et al. (1997) Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. Blood 90: 2978-2986
3. Cassileth PA, Harrington DP, Appelbaum FR, et al. (1998) Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N Engl J Med 339: 1649-1656
4. Appelbaum FR (2001) Haematopoietic cell transplantation as immunotherapy. Nature 411: 385-389
5. Riddell SR, Berger C, Murata M, et al (2003) The graft versus leukemia response after allogeneic hematopoietic stem cell transplantation.Blood Reviews 17: 153-162
6. Urbano-Ispizua A, Schmitz N, de Witte T, et al. (2002) Allogeneic and autologous transplantation for haematological diseases, solid tumors and immune disorders: definitions and current practice in Europe. Bone Marrow Transpl 29: 639-646
7. Giralt S, Estey E, Albitar M, et al. (1997) Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus- leukemia without myeloablative therapy.Blood 89: 4531-4536
8. Slavin S, Nagler A, Naparstek E, et al. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 91: 756-763