Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Schlenk RF, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1909-18.

Supplementary Table 1: Total number of patients and number of patients with normal karyotype per trial

Treatment trial	Total Number of Patients	Number of Patients With Normal Karyotype
AMLHD93*	223	101 (45%)
AML-2/95°	305	154 (50%)
AMLHD98A[#]	871	377 (43%)
AML-1/99[§]	520	240 (46%)
Total	1,919	872

In all trials written informed consent was obtained

*Schlenk et al. Leukemia 2003;17:1521-8.

°Heil et al. Ann Hematol 2004;83:336-44.

[#]Schlenk et al. Blood 2006;108:8a.

[§]Ganser et al. Blood 2006;108:183a.

Supplementary Table 2: Molecular cytogenetic and genetic diagnostic in AML

Method		Reference
Fluorescence *in situ* hybridization (FISH) analysis		Frohling S et al., J Clin Oncol 2002;20:2480-5 Krauter J et al., Br J Haematol 1998;103:72-8
Polymerase chain reaction (PCR)	**Fusion gene**	
t(15;17)(q22;q21)	*PML-RARA*	Slack JL et al., J Mol Diagn 2001;4:141-9
t(8;21)(q22;q22)	*RUNX1-CBFA2T1*	Mrozek K et al., J Clin Oncol 2001;19:2482-92
inv(16)(p13q22)/t(16;16)(p13;q22)	*CBFB-MYH11*	Mrozek K et al., J Clin Oncol 2001;19:2482-92
t(9;11)(p22;q23)	*MLL-MLLT3*	Scholl C et al., Genes Chromosomes Cancer 2003;38:274-80
Gene mutation screening		
FLT3-ITD/*FLT3*-TKD		Fröhling S et al., Blood 2002;100:4372-80
NPM1		Dohner K et al., Blood 2005;106:3740-6
CEBPA		Fröhling S et al., J Clin Oncol 2004;22:624-33
MLL-PTD		Döhner K et al., J Clin Oncol 2002;20:3254-61
NRAS[§]		Bowen DT et al., Blood 2003;101:2770-4

[§] For *NRAS* mutation screening reverse primer for the amplification of exon 1 and exon 2 were used according to Bowen et al.; forward primers were designed using the Primer3 program (http://frodo.wi.mit.edu/) [Primer exon 1: 5'-CTC CAG AAG TGT GAG GCC GAT-3'; exon 2: 5'-GGC AGA AAT GGG CTT GAA TA-3']. For PCR reaction the total reaction volume of 50 µL contained approximately 100 ng DNA, 10 pmol each primer,

deoxynucleotide triphosphate (dNTPs, 10 mmol/L each), 1.25 U HotStar Taq polymerase, and supplied buffer (Qiagen, Hilden, Germany). Samples were amplified using standard PCR conditions: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 55.5°C for 1 minute, 72°C for 1 minute; 72°C for 10 minutes. PCR products were sequenced in both directions with the same forward and reverse primers using the ABI Ready Reaction Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany).

1. Frohling S, Skelin S, Liebisch C, et al. Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia. J Clin Oncol 2002;20:2480-5.

2. Krauter J, Peter W, Pascheberg U, et al. Detection of karyotypic aberrations in acute myeloblastic leukaemia: a prospective comparison between PCR/FISH and standard cytogenetics in 140 patients with de novo AML. Br J Haematol 1998;103:72-78.

3. Slack JL, Bi W, Livak KJ, et al. Pre-clinical validation of a novel, highly sensitive assay to detect PML-RARalpha mRNA using real-time reverse-transcription polymerase chain reaction. J Mol Diagn 2001;4:141-9.

4. Mrozek K, Prior TW, Edwards C, et al. Comparison of cytogenetic and molecular genetic detection of t(8;21) and inv(16) in a prospective series of adults with de novo acute myeloid leukemia: a Cancer and Leukemia Group B Study. RA. J Clin Oncol 2001;9:2482-92.

5. Scholl C, Breitinger H, Schlenk RF, Döhner H, Fröhling S, Döhner K. Development of a real-time RT-PCR assay for the quantification of the most frequent MLL/AF9 fusion types resulting from translocation t(9;11)(p22;q23) in acute myeloid leukemia. Genes Chromosomes Cancer 2003;38:274-80.

6. Fröhling S, Schlenk RF, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood 2002;100:4372-80.

7. Dohner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 2005;106:3740-6.

8. Fröhling S, Schlenk RF, Stolze I, et al. *CEBPA* mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol 2004;22:624-33.

9. Döhner K, Tobis K, Ulrich R, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol 2002;20:3254-61.

10. Bowen DT, Frew ME, Rollinson S, et al. CYP1A1*2B (Val) allele is overrepresented in a subgroup of acute myeloid leukemia patients with poor-risk karyotype associated with NRAS mutation, but not associated with FLT3 internal tandem duplication. Blood 2003;101:2770-4.

Supplementary Table 3: Treatment after relapse

	No. of Relapses	
	Donor	**No Donor**
Therapy	**n=54**	**n=240**
Chemo followed by DLI/MRD-SCT	24	
DLI/MRD-SCT	11	
Chemo	8	86
Chemo followed by MUD-SCT	1	67
Auto-SCT followed by MUD-SCT		3
MUD-SCT		23
Chemo followed by Auto-SCT		7
Auto-SCT	1	
Chemo followed by Haplo-SCT		3

No intensive reinduction therapy	9	51

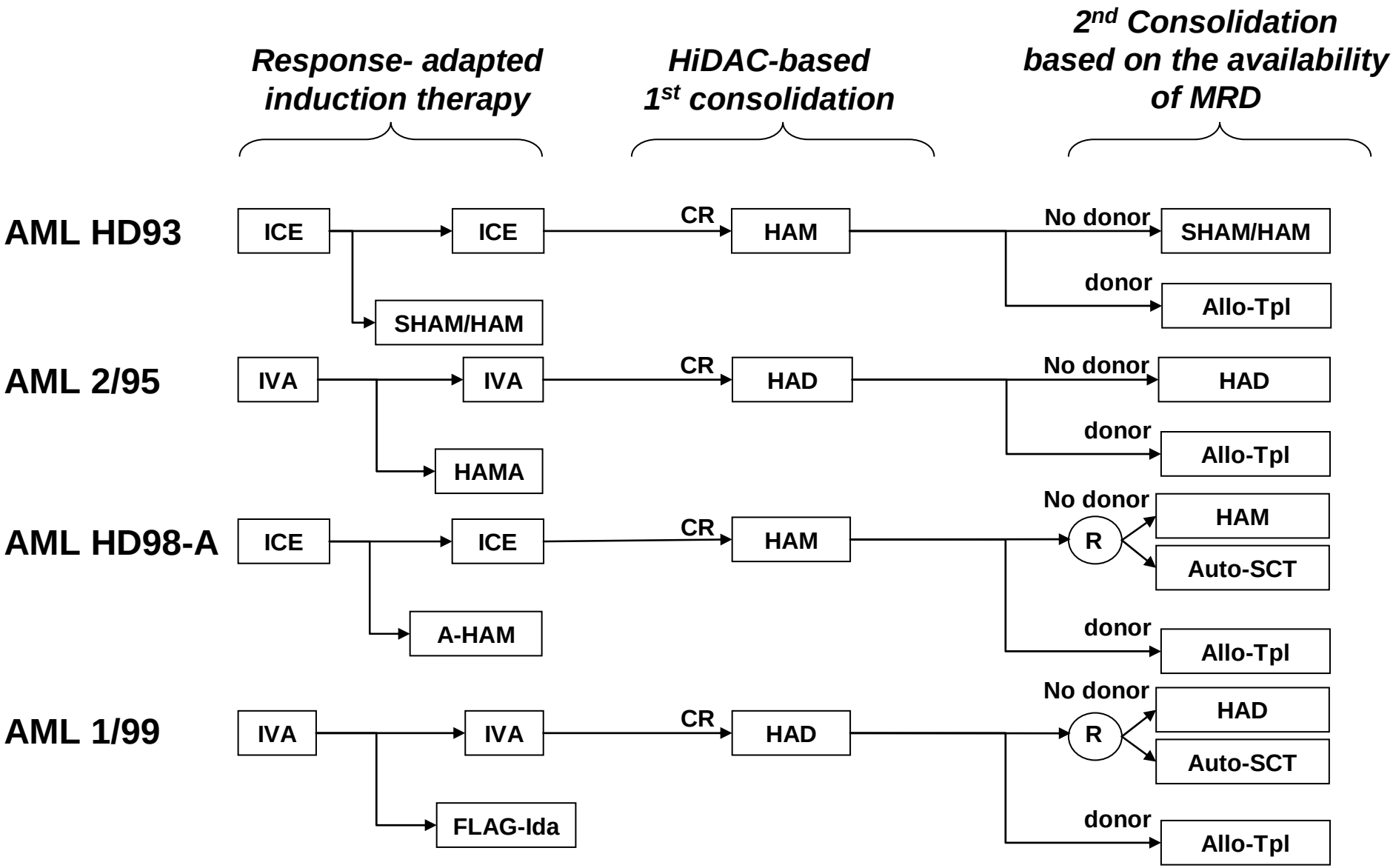
Chemo, Chemotherapy; DLI, donor lymphocyte infusion; MRD, matched related donor; SCT, stem cell transplantation; MUD, matched unrelated donor; Auto, autologous; Haplo, haploidentical related donor.

Supplementary Figure Legends:

Figure 1: Summary of the four prospective AMLSG treatment trials. HiDAC, high-dose cytarabine; MRD, matched related donor; IVA and ICE, idarubicin, etoposide, cytarabine; HAMA, high-dose cytarabine and amsacrin; HAD, high-dose cytarabine and daunorubicin; HAM, high-dose cytarabine, mitoxantrone; S-HAM, sequential HAM; A-HAM, all-*trans* retinoic acid and HAM; FLAG-Ida, fludarabine, cytarabine, granulocyte colony stimulating factor and idarubicin; Auto-SCT: autologous transplantation; Allo-SCT, allogeneic transplantation.

Figure 2: Summary of the individual-patient-data meta-analysis.

Supplementary Figure 1



Supplementary Figure 2