doi:10.4149/316_150910N482

Treatment and prognosis of childhood acute lymphoblastic leukemia on protocols ALL-BFM 90, 95 and ALL IC-BFM 2002: a retrospective single-center study from Olomouc, Czech Republic

J. VOLEJNIKOVA[1,2], M. JAROSOVA[3], D. POSPISILOVA[1,2], Z. NOVAK[1], J. VRBKOVA[2], M. HAJDUCH[2], V. MIHAL[1,2,*]

[1]Department of Pediatrics, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic; [2]Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic; [3]Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic

**Correspondence: vladimir.mihal@fnol.cz*

Received September 10, 2015 / Accepted December 4, 2015

Great progress has been made in the diagnostics and treatment of childhood acute lymphoblastic leukemia (ALL) over the past decades. The vast majority of children are cured, however, there is need for further improvement, especially in specific patient subgroups. Our aim was to retrospectively evaluate disease characteristics and treatment outcomes of children with ALL enrolled in a single center into consecutive treatment protocols (ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002) between years 1990 and 2007 and comprehensively summarize diagnostic and therapeutic advances between protocols. In total, 97 patients aged 0 to 18 years were treated for ALL at University Hospital Olomouc in the Czech Republic and steadily high relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) were observed during the evaluated time period without significant difference between the protocols (RFS 80-86%, EFS 75-83% and OS 84-92%). In conclusion, our center has demonstrated survival rates comparable to leading international study groups for childhood ALL over a substantial period of time. This has been achieved namely due to advances in diagnostics, excellent collaboration on regional, national and international level, quality assurance and high overall standard of care. The acquired experience has been crucial for current participation in the best performing Berlin-Frankfurt-Münster (BFM)-based international trials for childhood ALL.

Key words: acute lymphoblastic leukemia, BFM, childhood, Olomouc, prognosis

The therapy of acute lymphoblastic leukemia (ALL) in children is highly successful with survival rates approaching 90% in the best performing international trials. [1-3] This is achieved by administration of intensive multi-agent chemotherapy, dosed with respect to exactly defined prognostic features in order to optimally balance the risk of relapse and the toxicity of the treatment. Benefit of new diagnostic methods and improvements in chemotherapy and supportive care has been enhanced by centralization and intense cooperation on both national and international levels. The International Berlin-Frankfurt-Münster Study Group (I-BFM SG), whose core introduced the basic principles of childhood ALL treatment, nowadays comprises national study groups from more than 30 countries worldwide. This group regularly launches treatment protocols adjusted to the newest knowledge and based on the randomization results obtained on large cohorts of patients (http://www.bfm-international.org). [4]

Our aim was to review the development of Berlin-Frankfurt-Münster (BFM)-based childhood ALL therapy in the Czech Republic between 1990 and 2007 and evaluate its impact on patient prognosis in a single-center setting.

Patients and methods

Patients. In total, 97 children (age 0 to 18 years, 50 girls and 47 boys) newly diagnosed with ALL between October 1990 and October 2007 were included in the study. Patients were treated at the Department of Pediatrics, University Hospital Olomouc, which is one of 8 centers (including 5 university hospitals) providing care for pediatric hematooncology patients in the Czech Republic. Characteristics of children treated with the successive protocols ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002 are depicted in Table I. Distribution of patients according to the major clinical features did not differ

significantly between the respective regimens. The research was approved by the relevant institutional ethics committee. All patients or their parents/guardians gave an informed consent to participate in the study. Clinical data of the nationwide cohort have been published as a part of previous studies. [5-7] The median follow-up period of the study group was 16.3, 12.6 and 7.7 years for ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002, respectively.

Diagnostics. The main goal of ALL diagnostics is to estimate the individual disease risk and optimize the treatment burden by stratification of patients into the standard risk (SR), intermediate risk (IR) and high risk (HR) group. This classification is based on precisely defined and internationally accepted criteria such as age, leukocyte count, presence of particular fusion genes and treatment response (for details see below). [4, 7, 8] The summary of diagnostic methods is given in Supplementary Table I.

Since 1995, molecular diagnostics and immunophenotyping of childhood ALL in the Czech Republic has been centralized in Laboratory of Molecular Genetics and Laboratory of Flow Cytometry, Childhood Leukemia Investigation Prague (CLIP), adjacent to the Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague.

During the ALL-BFM 90 trial, screening for *BCR/ABL1* and *MLL/AF4* translocations by reverse transcriptase-polymerase chain reaction (RT-PCR) was introduced (in June 1993 and March 1995, respectively). *TEL/AML1* status has been examined since 1996, but has not been implemented in the risk stratification.

Flow cytometry immunophenotyping of bone marrow aspirates has been performed centrally (see above) and since 1995 it has undergone multiple advances in software and laser technology and has been improved by the implementation of new antibodies and fluorochromes. [9] Already in 1990, cytogenetic examination was performed in larger centers with sufficient equipment (including Olomouc) and today, 6 out of 8 Czech centers are accredited for this examination. New techniques such as chromosome G-banding analysis and fluorescence in situ hybridization (FISH) have been gradually introduced by individual centers and performed according to international standards. [10, 11] In order to obtain analyzable metaphase cells, at least 1-3 ml of bone marrow aspirates were cultured in vitro for 24 or 48 hours in an in-house medium containing 1μg of interleukin 7 and subsequently treated with colcemid.

Monitoring of intraerythrocytic methotrexate levels and tetrazolium (MTT) assay, although not required by the protocols, were performed in all patients treated in our center. [12]

All data were recorded centrally. Frozen bone marrow aspirates and trephine biopsy samples from all patients are stored and thus available for possible further analyses.

Treatment protocols. Children were enrolled in following treatment protocols: ALL-BFM 90 (Jun 1990-May 1996), ALL-BFM 95 (Sep 1995-Oct 2002) and ALL IC-BFM 2002 (Nov 2002-Nov 2007). Risk group stratification and therapy elements of these protocols have been described in detail elsewhere (Supplementary Figure I). [4, 7, 8] Diagnostic and therapeutic approaches have been similar over time, however, following changes were introduced:

a) between ALL-BFM 90 and ALL-BFM 95:
   1. Centralization of diagnostics (see above) and implementation of flow cytometry;
   2. Replacement of the BFM risk factor (BFM-RF), reflecting the initial leukemic mass, by a new stratification strategy using age, white blood cell count (WBC) at diagnosis and immunophenotype in addition to the assessment of response to prednisone and induction treatment plus presence of unfavorable translocations t(9;22) and t(4;11);
   3. Reduction of anthracyclines from 4 to 2 doses of 30 mg/m^2 daunorubicin during Induction I_A in SR patients;

Table I. Characteristics of children with ALL treated in Olomouc, Czech Republic.

	ALL-BFM 90	ALL-BFM 95	ALL IC-BFM 2002	p
Patients total	37	36	24*	-
Gender				
Male	18	14	15	0.20
Female	19	22	9	
Age at diagnosis				
<6 years	20	17	12	0.84
≥6 years	17	19	12	
WBC at diagnosis (μ1^{-1})				
<20 000	25	26	15	0.73
≥20 000	12	10	9	
Immunophenotype				
BCP-ALL	35	31	19	0.31
T-ALL	2	3	4	
Other	0	2 MPAL	1 MPAL	
Molecular genetics				
BCR/ABL1	0	2	1	ND
MLL translocations	2	0	0	
TEL/AML1	ND	10	7	
None of the above	35	24	15	
Risk group				
SR	16	12	7	0.55
IR	17	18	14	
HR	4	1	3	
Relapse				
No	30	31	20	0.94
Yes	7	5	4	
Other event				
No	35	34	24	0.67
Yes	2	2	0	

*ALL IC-BFM 2002: 1 SR patient excluded (steroid-pretreated); WBC – white blood cell count; BCP-ALL – B-cell precursor ALL; T-ALL – T-lineage ALL; MPAL – mixed phenotype acute leukemia; ND – not performed; SR – standard risk; IR – intermediate risk; HR – high risk.

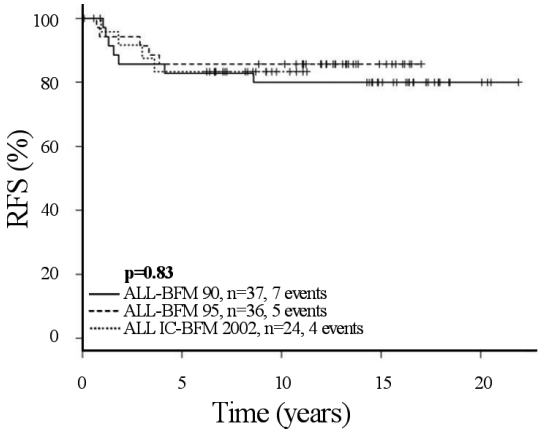


Figure 1. Relapse-free survival (RFS) for children with ALL treated in Olomouc between 1990-2007. Summary graph for protocols ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002. N, number of patients.

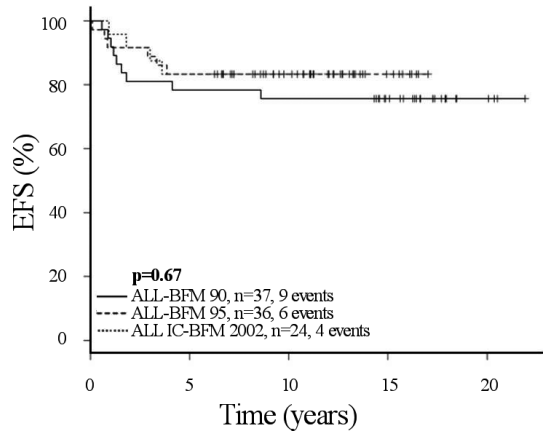


Figure 2. Event-free survival (EFS) for children with ALL treated in Olomouc between 1990-2007. Summary graph for protocols ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002. N, number of patients.

4. Successive introduction of high dose (5 g/m^2) instead of intermediate dose (1 g/m^2) of methotrexate in Protocol M for SR and IR patients by individual centers during the ALL-BFM 90 trial;
5. Omission of preventive cranial radiotherapy (12 Gy) in patients with IR B-cell precursor ALL (BCP-ALL) and its restriction to T-lineage ALL (T-ALL) and/or HR patients;
6. Reduction of cranial irradiation for children with central nervous system (CNS) involvement between 1 and 2 years of age from 18 to 12 Gy and for children older than 2 years of age from 24 to 18 Gy. Cranial irradiation for infants under 1 year of age is contraindicated;
7. Intensification of consolidation/reinduction for the HR group – minor changes in composition of the HR-1, HR-2 and HR-3 block elements and substitution of three final HR blocks by Protocol II; [4]
8. Prolongation of maintenance therapy for boys in the SR group until 36 months instead of 24 months from initial diagnosis;
9. Intensification of maintenance therapy of ALL-BFM 95 by pulses of dexamethasone and vincristine in the IR group - no benefit for survival was demonstrated; [13]
10. Advances in the field of hematopoietic stem cell transplant (HSCT), which is indicated in HR, particularly very-high-risk (VHR), patients; [14, 15]

b) between ALL-BFM 95 and ALL IC-BFM 2002:

1. Reduction of intravenous methotrexate from 5 g/m^2 to 2 g/m^2 in Protocol M for patients with BCP-ALL and its replacement by 1 intrathecal dose of methotrexate;
2. Withdrawal of 4 doses of 200 mg/m^2 cytosine-arabinoside in Protocol M for patients with BCP-ALL;
3. Randomization before treatment reinduction in all risk groups and introduction of shortened Protocol III with reduced intensity chemotherapy for SR and IR group;
4. Re-shortening maintenance therapy for SR boys: cessation at 24 months from initial diagnosis;
5. Evolution of distinct treatment for infants under 1 year of age (protocols POG 9407, Interfant-99 [16] and Interfant-06 [17]) and *BCR/ABL1*-positive ALL (protocol EsPhALL since 2003, introduction of imatinib mesylate) and subsequent outflow of these patients from the "mainstream" protocol.

For overview of changes between protocols and their explanation see Supplementary Table II.

Statistical methods. Statistical analyses were performed using software R version 3.1.0 (http://www.r-project.org) and GraphPad Prism, version 4.0 (San Diego, CA, USA). Differences between distributions of major clinical features in trials were tested using the Fisher's exact test or Chi-square test (for categorical variables) and the Mann Whitney test (for continuous variables). Relapse-free survival (RFS) was calculated from the date of diagnosis to the last follow-up or to relapse, event-free survival (EFS) was calculated from the date of diagnosis to the last follow-up or to event (relapse, death, or secondary malignancy) and overall survival (OS) was calculated from the date of diagnosis to the last follow-up or to death. Survival rates were calculated according to the Kaplan-Meier method and evaluated at the following time points: 15 years after initial diagnosis for ALL-BFM 90, 10 years after initial diagnosis for ALL-BFM 95 and 5 years after initial diagnosis for ALL IC-BFM 2002. Log-rank test was used to reveal differences in survival between trials. The level of significance for all tests was set at 5%.

Results

Prognosis. Figures 1, 2 and 3 show Kaplan-Meier survival plots for RFS, EFS and OS of children with ALL treated in Olomouc between years 1990 and 2007 (RFS: 80.0±6.8%,

85.7±5.9% and 83.3±7.6%; EFS: 75.7±7.1%, 83.3±6.2% and 83.3±7.6%; OS: 83.8±6.1%, 91.7±4.6% and 91.7±5.6% for ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002 protocols, respectively). The observed results were rather steady and did not differ significantly between consecutive protocols (p=0.83, p=0.67 and p=0.52, respectively).

Other events included 2 deaths in remission in the ALL-BFM 90 protocol (1 from graft-versus-host disease after HSCT and 1 infectious death) and 1 death in induction due to hemorrhagic pancreatitis and 1 secondary malignancy (malignant schwannoma of the mediastinum metastasized to the cervical spine) in the ALL-BFM 95 protocol (Table I). These events occurred 10 months, 7 months, 1 month and 15 years after diagnosis, respectively.

Adverse late effects of treatment were present in 3 children: two boys suffered from hypergonadotropic hypogonadism after testicular relapse of ALL and 1 girl who underwent HSCT due to relapse developed transient hypogonadism, growth hormone deficiency, chronic heart failure and chronic renal disease stage 3.

Cytogenetics. Results of cytogenetic examination in 97 patients treated at University Hospital Olomouc are categorized in Table II. Cytogenetics was successful in 82/86 (95%) examined patients and the yield of cytogenetic examination increased gradually. Chromosomal changes were detected (by conventional cytogenetics and FISH) in 77/82 (94%) examined patients. There were no statistically significant differences in distribution of chromosomal aberrations between protocols (p=0.23).

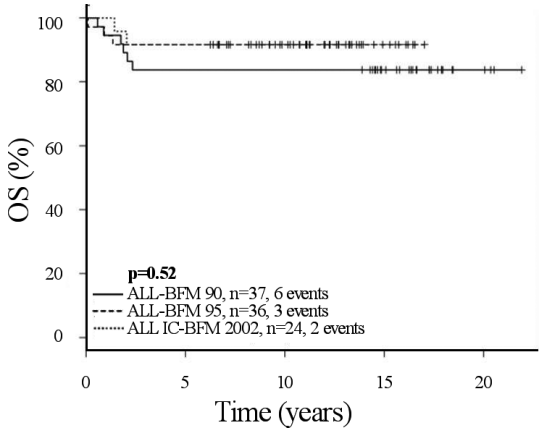


Figure 3. Overall survival (OS) for children with ALL treated in Olomouc between 1990-2007. Summary graph for protocols ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002. N, number of patients.

Discussion

In this paper, we comprehensively summarize diagnostics and treatment of childhood ALL in the Czech Republic during the period of 1990 to 2007 from a perspective of a single center, Department of Pediatrics at University Hospital Olomouc. Single center studies of childhood ALL have been published and can not only provide interesting insight into history but also help elucidate impact of a wide variety of diagnostic and therapeutic measures on outcome by comparison of different centers worldwide. [18] Consecutive trials ALL-BFM 90, ALL-BFM 95 and ALL IC-BFM 2002 were optimized to decrease treatment burden where possible (e.g. by reduction of cranial radiotherapy and cardiotoxic anthracyclines) while retaining optimal leukemia control. This strategy was successful and led to a significant improvement in prognosis, in the Czech Republic mainly between ALL-BFM 95 and ALL IC-BFM 2002, with EFS approaching 85% and survival exceeding 90% on the latter protocol. Here we demonstrate that our single-center results were steadily comparable to the nationwide data. [6, 19]

The mentioned outcomes surpassed international results of the ALL IC-BFM 2002 trial (5-year EFS 74%, OS 82%) [7] and were comparable with world's leading childhood leukemia trials – e.g. a 7-year EFS 80.4% and OS 91.8% for AIEOP-BFM ALL 2000; 5-year OS 90.4% for protocol of the Children's Oncology Group (COG). [2, 3] This achievement can be attributed to multiple improvements in both diagnostics and treatment of childhood ALL and would not be possible without centralization of diagnostics and excellent collabora-

Table II. Results of cytogenetic examination in 97 children with ALL treated in Olomouc, Czech Republic.

	ALL-BFM 90	ALL-BFM 95	ALL IC-BFM 2002
T-ALL (n=9)			
TCR rearrangement	2	1	2
SIL/TAL	0	0	1
SIL deletion	0	0	1
Complex changes	0	2 (6%)	0
BCP-ALL (n=88)			
Normal karyotype	2	3	0
High hyperdiploidy (≥50 chromosomes)	8	9	6
Hyperdiploidy (47-49 chromosomes)	7	3	2
Pseudodiplody	5	11	7
Hypodiploidy (35-45 chromosomes)	2	4	3
Near haploidy (23-34 chromosomes)	0	0	1
Unsuccessful cytogenetics	3	1	0
ND	8*	2*	1*
Total	37	36	24
TEL/AML1 (*RUNX1/ETV6*)	1	10	7
BCR/ABL1	0	2	0
MLL rearrangement	2	0	0
E2A/PBX1	2	3	0
Complex karyotype	2	12	5

*Diagnostic examination was done in a different center in 7, 2 and 1 of these patients, respectively. n – number of patients; ND – not performed; BCP-ALL, B-cell precursor ALL; T-ALL – T-lineage ALL; TCR – T-cell receptor.

tion between centers (including regular meetings of the Czech Pediatric Hematology Working Group with demonstrations of all newly diagnosed and relapsed patients).

The success rate of cytogenetics in our center was even superior compared to the published data of childhood ALL where rates exceeding 90% are considered to be remarkable. [9, 20] This confirms a good quality of examination with guaranteed detection of rarer chromosomal aberrations even in small patient cohorts. We also report high abnormality detection rate. [21, 22] We assume that the success in the examination is largely determined by the amount of biological material available and could have been further enhanced by use of the in-house culture medium, especially in hyperdiploidy, whose detection may frequently fail due to unsuccessful cell cultivation. Nevertheless, only *BCR/ABL1* and *MLL* translocations (detected by RT-PCR) were considered for the risk stratification in all mentioned protocols. Interestingly, *BCR/ABL1* fusion was found by molecular genetics but not by cytogenetics in 1 patient from the ALL IC-BFM 2002 protocol.

We summarize multiple changes between respective treatment protocols; these were applied equally by all centers. Between 1997 and 2003, changes regarding the management of *BCR/ABL1*-positive patients and infants under 1 year of age were made (see above). This led to a redistribution of these patients with poor prognosis to different treatment protocols and might have ameliorated the results of survival analyses. Whereas the introduction of tyrosine kinase inhibitors improved the prognosis of *BCR/ABL1*-positive ALL, [23] the prognosis of infant ALL remains poor and represents diagnostic and therapeutic challenge. [16, 24, 25] Quantification of minimal residual disease (MRD) was not used for guiding the therapy in any given protocol, although it has been already performed during the ALL IC-BFM 2002 trial. [5]

In conclusion, we have demonstrated a steady good quality of childhood ALL diagnostics and treatment in the Czech Republic during a considerable time span (1990-2007) in both regional and nationwide setting. The future of childhood ALL management lies in the individualization of care in order to further reduce the incidence of relapse and treatment-related morbidity. This can be achieved through the refinement of diagnostics, prognostication and therapy; e.g. by MRD quantification, introduction of next generation sequencing, search for new prognostic markers, progress in pharmacogenomics and pharmacokinetics, [26] development of new chemotherapeutics and targeted treatment, possible omission of cranial irradiation, [27] etc. As a result of our joint effort, the Czech Republic has since 2010 allied to the international AIEOP-BFM ALL 2009 protocol of the BFM consortium, which is based on MRD quantification and belongs to the world's leading leukemia trials.

Acknowledgments: This work was supported by a grant from the Czech Ministry of Education, Youth and Sports (NPU LO 1304) and Czech Ministry of Health (IGA UP 2015-001).

The authors would like to thank Iveta Janotova for data management and acknowledge all collaborating members of the Czech Pediatric Hematology Working Group.

Supplementary information is available in the online version of the paper.

References

[1] CONTER V, ARICO M, BASSO G, BIONDI A, BARISONE E et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 2010; 24: 255–264. http://dx.doi.org/10.1038/leu.2009.250

[2] CONTER V, BARTRAM CR, VALSECCHI MG, SCHRAUDER A, PANZER-GRUMAYER R et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood 2010; 115: 3206–3214. http://dx.doi.org/10.1182/blood-2009-10-248146

[3] HUNGER SP, LU X, DEVIDAS M, CAMITTA BM, GAYNON PS et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol 2012; 30: 1663–1669. http://dx.doi.org/10.1200/JCO.2011.37.8018

[4] MORICKE A, ZIMMERMANN M, REITER A, HENZE G, SCHRAUDER A et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 2010; 24: 265–284. http://dx.doi.org/10.1038/leu.2009.257

[5] FRONKOVA E, MEJSTRIKOVA E, AVIGAD S, CHIK KW, CASTILLO L et al. Minimal residual disease (MRD) analysis in the non-MRD-based ALL IC-BFM 2002 protocol for childhood ALL: is it possible to avoid MRD testing? Leukemia 2008; 22: 989–997. http://dx.doi.org/10.1038/leu.2008.22

[6] STARY J, JABALI Y, TRKA J, HRUSAK O, GAJDOS P et al. Long-term results of treatment of childhood acute lymphoblastic leukemia in the Czech Republic. Leukemia 2010; 24: 425–428. http://dx.doi.org/10.1038/leu.2009.255

[7] STARY J, ZIMMERMANN M, CAMPBELL M, CASTILLO L, DIBAR E et al. Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. J Clin Oncol 2014; 32: 174–184. http://dx.doi.org/10.1200/JCO.2013.48.6522

[8] SCHRAPPE M, REITER A, LUDWIG WD, HARBOTT J, ZIMMERMANN M et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 2000; 95: 3310–3322.

[9] KALINA T, FLORES-MONTERO J, VAN DER VELDEN VH, MARTIN-AYUSO M, BOTTCHER S et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012; 26: 1986–2010. http://dx.doi.org/10.1038/leu.2012.122

[10] HARRISON CJ, MOORMAN AV, BARBER KE, BROADFIELD ZJ, CHEUNG KL et al. Interphase molecular cytogenetic screening for chromosomal abnormalities of prognostic significance in childhood acute lymphoblastic leukaemia: a UK Cancer Cytogenetics Group Study. Br J Haematol 2005; 129: 520–30. http://dx.doi.org/10.1111/j.1365-2141.2005.05497.x

[11] SIMONS A, SHAFFER LG, HASTINGS RJ Cytogenetic Nomenclature: Changes in the ISCN 2013 Compared to the 2009 Edition. Cytogenet Genome Res 2013; 141: 1–6. http://dx.doi.org/10.1159/000353118

[12] MIHAL V, HAJDUCH M, NOSKOVA V, FEKETOVA G, JESS K Differential antileukemic activity of prednisolone and dexamethasone in freshly isolated leukemic cells. Adv Exp Med Biol 1999; 457: 461–471. http://dx.doi.org/10.1007/978-1-4615-4811-9_50

[13] CONTER V, VALSECCHI MG, SILVESTRI D, CAMPBELL M, DIBAR E et al. Pulses of vincristine and dexamethasone in addition to intensive chemotherapy for children with intermediate-risk acute lymphoblastic leukaemia: a multicentre randomised trial. Lancet 2007; 369: 123–131. http://dx.doi.org/10.1016/S0140-6736(07)60073-7

[14] SCHRAUDER A, REITER A, GADNER H, NIETHAMMER D, KLINGEBIEL T et al. Superiority of allogeneic hematopoietic stem-cell transplantation compared with chemotherapy alone in high-risk childhood T-cell acute lymphoblastic leukemia: results from ALL-BFM 90 and 95. J Clin Oncol 2006; 24: 5742–5749. http://dx.doi.org/10.1200/JCO.2006.06.2679

[15] BALDUZZI A, VALSECCHI MG, UDERZO C, DE LORENZO P, KLINGEBIEL T et al. Chemotherapy versus allogeneic transplantation for very-high-risk childhood acute lymphoblastic leukaemia in first complete remission: comparison by genetic randomisation in an international prospective study. Lancet 2005; 366: 635–642. http://dx.doi.org/10.1016/S0140-6736(05)66998-X

[16] PIETERS R, SCHRAPPE M, DE LORENZO P, HANN I, DE ROSSI G et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet 2007; 370: 240–250. http://dx.doi.org/10.1016/S0140-6736(07)61126-X

[17] VAN DER SLUIS I, MORICKE A, ESCHERICH G, VON STACKELBERG A, HOLTER W et al. Pediatric acute lymphoblastic leukemia: efficacy and safety of recombinant E. coli-asparaginase in infants (less than one year of age) with acute lymphoblastic leukemia. Haematologica 2013; 98: 1697–1701. http://dx.doi.org/10.3324/haematol.2013.090563

[18] JATCZAK-GACA A, STYCZYNSKI J, KOLTAN A, DEBSKI R, POGORZALA M et al. Results of therapy in children with acute lymphoblastic leukemia in over 50 years of experience in a single center in Poland. Leuk Lymphoma 2015; 56: 2212–2214. http://dx.doi.org/10.3109/10428194.2014.999325

[19] ZDRAHALOVA K, STERBA J, DOMANSKY J, BLAZEK B, PTOSZKOVA H et al. The prospects for children with acute lymphoblastic leukemia of being cured has increased in the Czech Republic in the 21st century to 90% - outcome of the ALL-IC BFM 2002 trial. Cas Lek Cesk 2015; 154: 79–89.

[20] CHESSELS JM, SWANSBURY GJ, REEVES B, BAILEY CC, RICHARDS SM Cytogenetics and prognosis in childhood lymphoblastic leukaemia: results of MRC UKALL X. Medical Research Council Working Party in Childhood Leukaemia. Br J Haematol 1997; 99: 93–100. http://dx.doi.org/10.1046/j.1365-2141.1997.3493163.x

[21] JAROSOVA M, HOLZEROVA M, JEDLICKOVA K, MIHAL V, ZUNA J et al. Importance of using comparative genomic hybridization to improve detection of chromosomal changes in childhood acute lymphoblastic leukemia. Cancer Genet Cytogenet 2000; 123: 114–122. http://dx.doi.org/10.1016/S0165-4608(00)00310-1

[22] STRUSKI S, DOCO-FENZY M, CORNILLET-LEFEBVRE P Compilation of published comparative genomic hybridization studies. Cancer Genet Cytogenet 2002; 135: 63–90. http://dx.doi.org/10.1016/S0165-4608(01)00624-0

[23] BIONDI A, SCHRAPPE M, DE LORENZO P, CASTOR A, LUCCHINI G et al. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol 2012; 13: 936–945. http://dx.doi.org/10.1016/S1470-2045-(12)70377-7

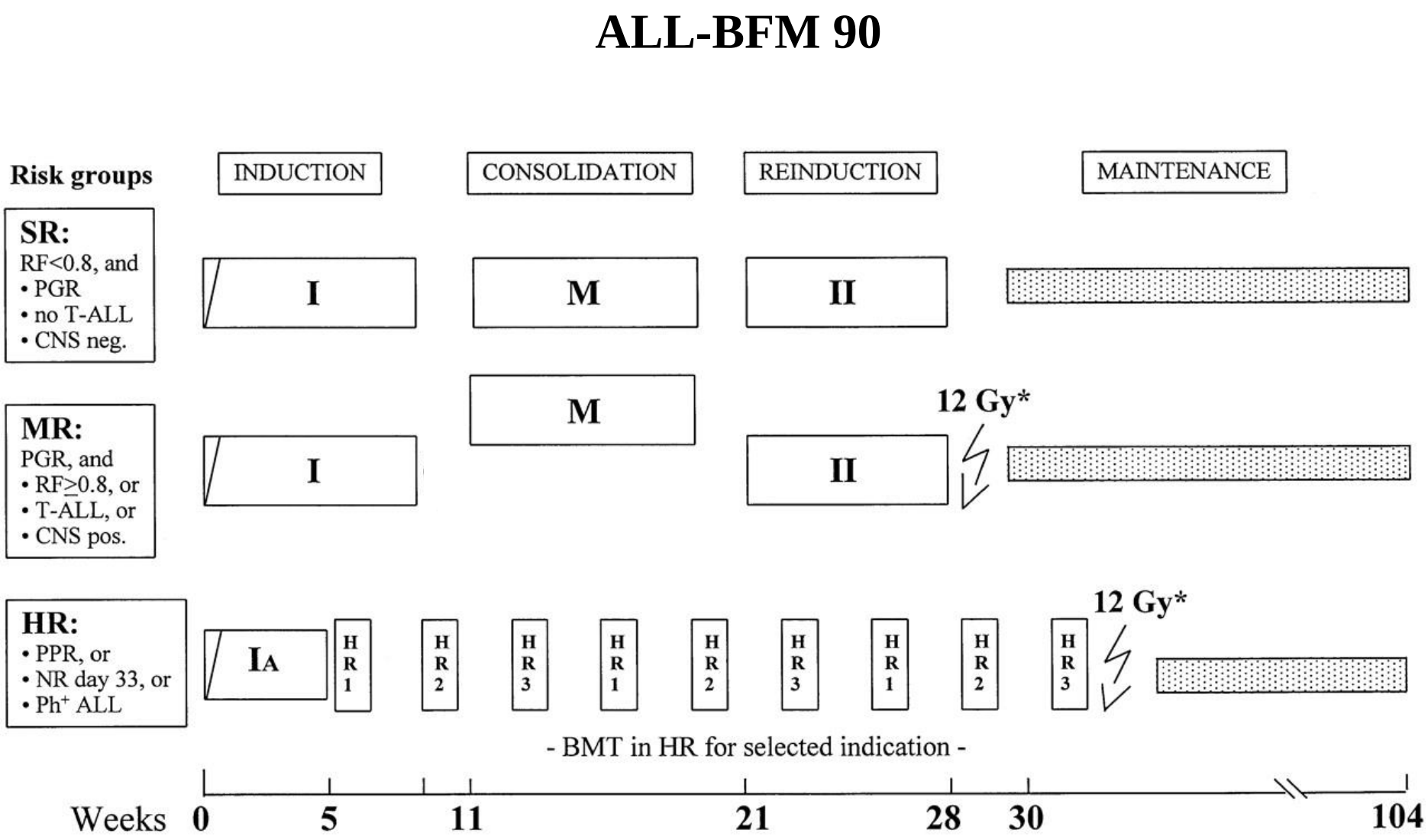
[24] JABALI Y, STARY J, HAK J, BLAZEK B, HRSTKOVA H et al. Acute lymphoblastic leukemia in infants: a decade of experience in the Czech Republic. Med Pediatr Oncol 2000; 35: 493–495. http://dx.doi.org/10.1002/1096-911X(20001101)35:5<493::AID-MPO9>3.0.CO;2-P

[25] SALZER WL, JONES TL, DEVIDAS M, DREYER ZE, GORE L et al. Decreased induction morbidity and mortality following modification to induction therapy in infants with acute lymphoblastic leukemia enrolled on AALL0631: A report from the children's oncology group. Pediatr Blood Cancer 2015; 62: 414–418. http://dx.doi.org/10.1002/pbc.25311

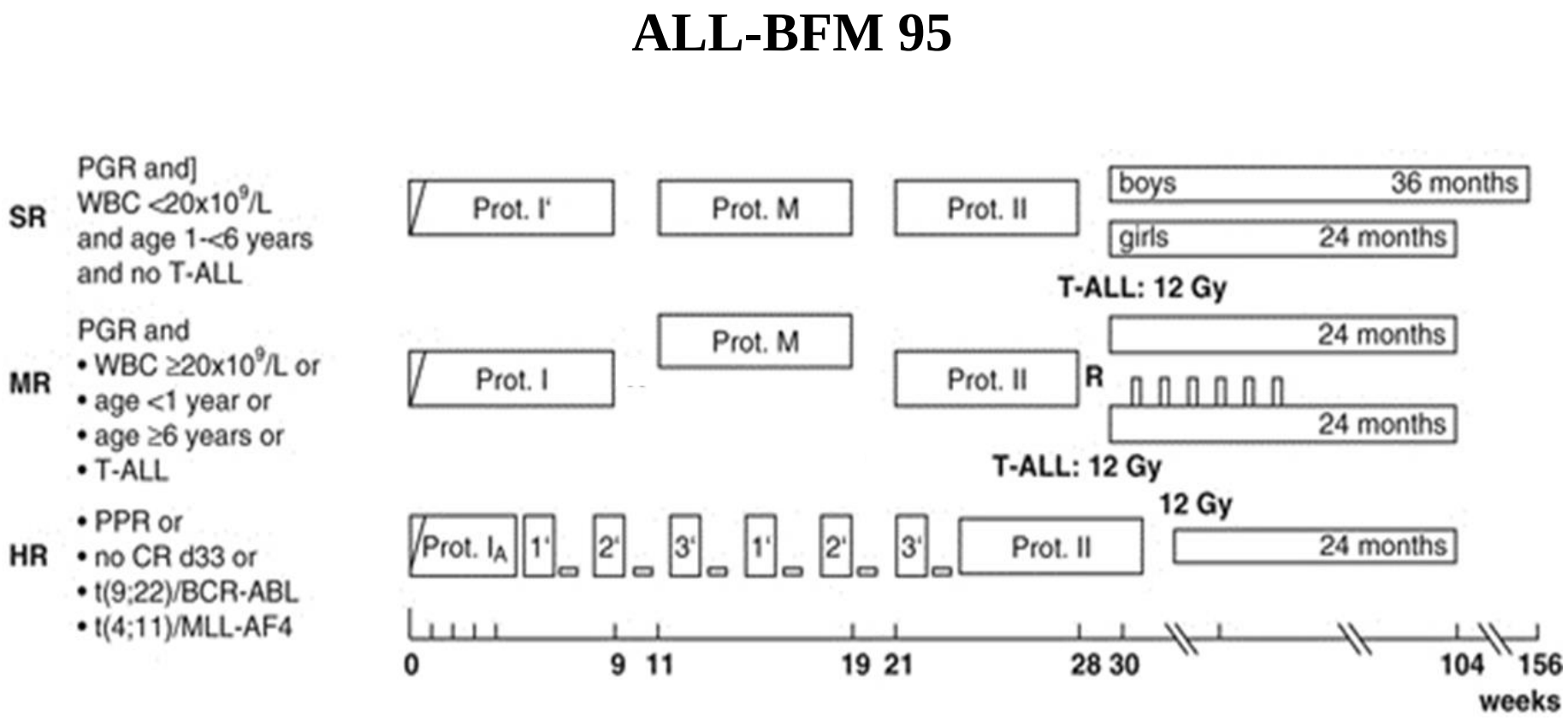
[26] SCHMIEGELOW K, NIELSEN SN, FRANDSEN TL, NERSTING J Mercaptopurine/Methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. J Pediatr Hematol Oncol 2014; 36: 503–517. http://dx.doi.org/10.1097/MPH.0000000000000206

[27] PUI CH, CAMPANA D, PEI D, BOWMAN WP, SANDLUND JT et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med 2009; 360: 2730–2741. http://dx.doi.org/10.1056/NEJMoa0900386

Supplementary Figure 1A. Scheme of the ALL-BFM 90 protocol.

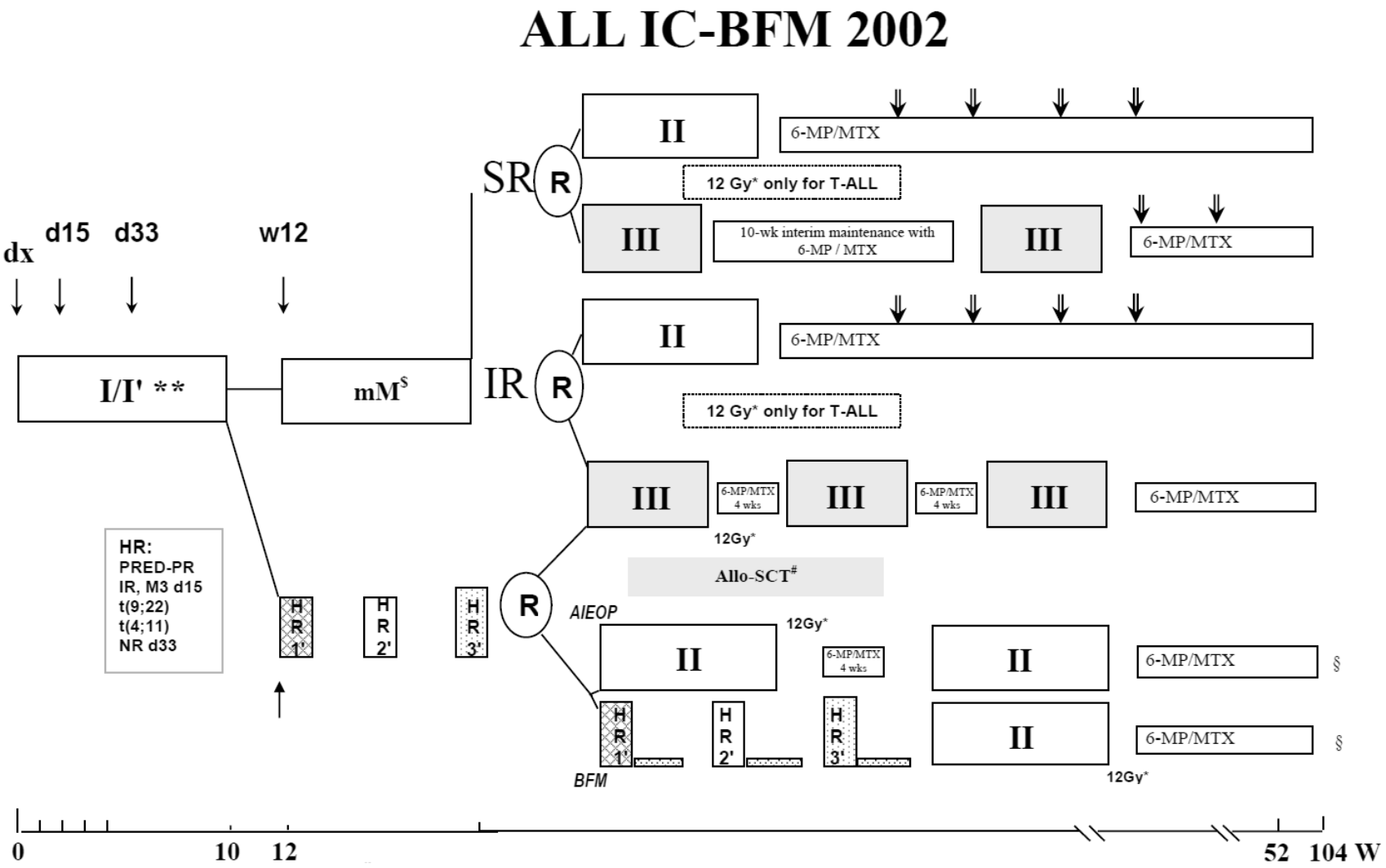


Supplementary Figure 1B. Scheme of the ALL-BFM 95 protocol.



Schrappe et al, Blood 2000

Supplementary Figure 1C. Scheme of the ALL IC-BFM 2002 protocol.



SR, standard risk; MR, medium risk; IR, intermediate risk; HR, high risk; I, I′, I_A, M, II, III, HR 1, 2, 3, 1′, 2′, 3′, chemotherapy blocks; 6-MP, 6-mercaptopurine; BMT, bone marrow transplant; dx, diagnosis; d15, day 15; d33, day 33; MTX, methotrexate; NR, non remission; PGR, prednisone good response; PPR, PRED-PR, prednisone poor response; R – randomization; RF, risk factor; SCT, stem cell transplant; w12, week 12; W, weeks; WBC, white blood cell count;

Supplementary Table I. Summary of diagnostic methods in childhood ALL.

Diagnostic method	Output	Time point	Purpose, advantages and disadvantages
Differential blood count, blood smear	Total WBC	Diagnosis	Basic examination; leukemia detection; WBC≥20 000/μl indicates worse prognosis
	Prednisone response	Day 8	Simple, traditional and strong predictor of outcome; patients with prednisone poor response (≥1000 blasts/μl) stratified into HR group
Bone marrow morphology	Percentage of leukemic blasts in the bone marrow	Diagnosis	Leukemia diagnosis (>25% blasts in the bone marrow)
		Day 15	Simple but invasive; patients with M3 marrow (>25% blasts) stratified into HR group
		Day 33	Definition of complete remission: non-responders with >5% blasts stratified into HR group
Cytogenetics	Karyotype, translocations	Diagnosis	Including t(12;21), t(9;22), t(11q23); hypodiploidy and complex karyotype indicate poor prognosis
Flow cytometry	Immunophenotype	Diagnosis	Comprehensive characteristics of the leukemic clone (lineage, developmental stage, special conditions[+])
	MRD[*]	Day 15	Fast, sensitive, used for risk stratification in the Czech Republic since 2010 (AIEOP-BFM ALL 2009 protocol)
Molecular genetics	Fusion genes	Diagnosis	*TEL/AML1*, *BCR/ABL1*, *MLL* translocations; crucial for leukemia characteristics and targeted treatment; patients with *BCR/ABL1* and *MLL* translocations stratified into HR group
	MRD[*]	Day 33, week 12	Sensitive but laborious, costly, time demanding; increasingly important predictor of outcome – used for risk stratification in the Czech Republic since 2007 (Interim AIEOP-BFM ALL 2000 and AIEOP-BFM ALL 2009 protocols)

[*]Not used for risk stratification in the Czech Republic during the study period; [+]e.g. mixed phenotype acute leukemia (MPAL) and lineage switch; WBC, white blood cell count; HR, high risk; MRD, minimal residual disease.

Supplementary Table II. Changes between treatment protocols for childhood ALL between 1990-2007.

	ALL-BFM 90	ALL-BFM 95	ALL IC-BFM 2002	Indication	Reason	Result
Diagnostics	Immunophenotyping and cytogenetics at selected centers with better equipment and experience; PCR screening for *BCR/ABL1* and *MLL/AF4* centrally	Immunophenotyping at selected centers; flow cytometry centrally; *BCR/ABL1*, *MLL/AF4* and *TEL/AML1* centrally	Immunophenotyping at 6 centers; flow cytometry centrally; *BCR/ABL1*, *MLL/AF4* and *TEL/AML1* centrally; MRD examined experimentally	All patients	Technical developments, new requirements (data reporting)	Fast, effective, comprehensive, centralized diagnostics
Risk stratification	BFM-RF, BCP-ALL vs. T-ALL, CNS status, prednisone response, complete remission, t(9;22)	WBC and age at diagnosis, immunophenotype, prednisone response, complete remission, t(9;22) and t(4;11)	WBC and age at diagnosis, immunophenotype, prednisone response, bone marrow response, complete remission, t(9;22) and t(4;11)	All patients	BFM-RF (reflecting initial leukemic cell mass) insufficient to separate different risk groups within PGR patients	More precise criteria for risk stratification; decreasing treatment burden and risk of relapse
Induction treatment	4 doses of daunorubicin	2 doses of daunorubicin	2 doses of daunorubicin	SR patients	Reducing cardiotoxicity	Treatment burden decreased while retaining excellent survival
Protocol M	Methotrexate increased from 1 g/m^2 to 5 g/m2 gradually during the study	5 g/m2 methotrexate		SR and IR patients	Preventing extramedullary (CNS) relapse	No increase in CNS relapse
			Reduction of methotrexate to 2 g/m^2 but addition of 1 intrathecal dose of methotrexate; withdrawal of 4 doses of cytosine-arabinoside	Patients with BCP-ALL		
Cranial irradiation	Preventive cranial irradiation 12Gy	Omission of preventive irradiation	Omission of preventive irradiation	Patients with IR BCP-ALL	Reducing neurotoxicity	Treatment burden decreased

						without deteriorating survival
	Children 1-2 years: 18 Gy Children >2 years: 24 Gy	Children 1-2 years: 12 Gy Children >2 years: 18 Gy	Children 1-2 years: 12 Gy Children >2 years: 18 Gy	Patients with CNS involvement	Reducing neurotoxicity	Treatment burden decreased without deteriorating survival
Consolidation/ reinduction	Protocol II once	Protocol II once	Introduction of shortened Protocol III	SR and IR patients	Reducing intensity of Protocol III ; randomization	Treatment burden decreased without deteriorating survival
	9 HR blocks	6 HR blocks and Protocol II; increase in alkylating agents and anthracyclines	6 HR blocks and Protocol II	HR patients	Intensification of therapy for HR patients	Markedly improved outcome of HR patients
Maintenance therapy	Until 24 months from diagnosis	Until 36 months from diagnosis	Until 24 months from diagnosis	SR boys	Preventing late relapses	No benefit for survival demonstrated
	Methotrexate and 6-mercaptopurine	Methotrexate and 6-mercaptopurine plus pulses of dexamethasone and vincristine	Methotrexate and 6-merc aptopurine	IR patients	Reducing relapse rate	No benefit for survival demonstrated
Treatment of infant ALL	Within the frontline treatment protocol	Protocol POG 9407 until 1999; Protocol Interfant-99 since 2000	Protocol Interfant-06 since 2006	Children under 1 year of age	Need for distinct treatment approach	Improved outcome
Treatment of *BCR/ABL1*-positive ALL	Within the frontline treatment protocol	Within the frontline treatment protocol	Protocol EsPhALL with imatinib mesylate since 2003	Children with *BCR/ABL1*-positive ALL	Need for distinct treatment approach	Improved outcome

MRD, minimal residual disease; BFM-RF, BFM risk factor; WBC, white blood cell count; BCP-ALL, B-cell precursor ALL; T-ALL, T-lineage ALL; CNS, central nervous system; SR, standard risk; IR, intermediate risk; HR, high risk; HSCT, hematopoietic stem cell transplant.