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Improved outcome for children and adolescent with acute lymphoblastic leukemia in the first decade of the 21st century: a report from the Slovak Republic

A. KOLENOVA[1,*], E. KAISEROVA[1], M. MAKOHUSOVA[1,4], Z. SUBOVA[1], E. BUBANSKA[2], I. ORAVKINOVA[3], P. SVEC[1], O. FABRI[1], S. P. HUNGER[5], S. SUFLIARSKA[1], S. HORAKOVA[1], V. MRAZOVA[4], J. STARY[6]

[1]Department of Pediatric Hematology and Oncology , Comenius University – Medical School and University Children's Hospital, Bratislava, Slovak republic; [2]Department of Pediatric Oncology and Hematology, Children's University Hospital, Banská Bystrica, Slovak republic; [3]Department of Pediatric Hematology and Oncology, University Children´s Hospital Kosice, Slovak Republic; [4]Department of Chemistry, University of SS. Cyril and Methodius, J. Herdu 2, Trnava,Slovak Republic; [5]University of Pennsylvania Perelman School of Medicine and The Children's Hospital of Philadelphia, Philadelphia PA USA; [6]Department of Pediatric Oncology and Hematology, Charles University and University Hospital Motol, Czech Republic

**Correspondence: sasa.kolenova@gmail.com*

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Our aim was to analyze event-free (EFS) and overall survival (OS) among children and adolescents with acute lymphoblastic leukemia (ALL) treated with International BFM Intercontinental trial (ALL IC 2002) therapy in the Slovak Republic. In total, 280 children and adolescent age 1 to 18 years were treated with ALL IC BFM 2002 based therapy from 2002 to 2012, which was divided into two periods. During 2002-2007, when patients were actively enrolled in the ALL IC-BFM 2002 trial, and during 2008-2012 when the trial was closed and patients were treated with the same therapy without randomization. Five-year EFS and OS rates were 79% (+/- 2.6%) and 86% (+/- 2.1%), respectively, similar to results obtained in the ALL-BFM 95 trial, which was the basis for ALL IC BFM 2002 therapy. The EFS ($p<0.012$) and OS ($p<0.003$) were significantly better than the prior Slovak experience in 1997-2001. Survival is improved in standard and intermediate risk groups, including those age 1 to 6 years, and older; with B-cell or T-cell immunophenotype, and is also excellent for those with good early response. The rate of death in induction, cumulative incidence of death in complete remission and of relapse decreased. However, outcome was suboptimal for patients in the high risk group. Current EFS and OS rates for children and adolescents with ALL in the Slovak Republic resembled those obtained in Western Europe as a result of clinical trial participation, and clinical experience acquired with intensive BFM type treatment.

Key words: acute lymphoblastic leukemia, children, clinical trial

The treatment of childhood acute lymphoblastic leukemia (ALL) is one of the success stories of clinical oncology [1, 2, 3]. Pediatric ALL 5-year overal survival (OS) rates have improved to approximately 90% in trials conducted in North America and Western Europe with risk stratification by biological features of leukaemic cells and response to treatment and improved supportive care [4, 5, 6, 7, 8, 9, 10]. The outstanding outcome has been obtained in countries with highly developed health care systems and has been possible because of high enrollment in clinical trials by cooperative groups and large institutions, with the two largest being the Children's Oncology Group (COG) based in North America and the International Berlin-Frankfurt-Munster (BFM) group based in Western Europe [11].

Stary et al have recently reported the results of more than 5,000 children with ALL enrolled in the International BFM Intercontinental trial (ALL IC-BFM 2002) between 2002 and 2007. Fifteen countries participated in the ALL IC-BFM 2002 trial, contributing from 36 to 1,270 patients each [12]. Children with ALL in Slovakia were included in the ALL IC-BFM 2002 trial, which was designed for countries inside the I-BFM study group who had achieved event-free survival (EFS) rates of at least 65%, but were not able to utilize the minimal residual disease (MRD) testing employed in recent BFM trials [11, 12].

In this report, we analyze the outcome of 280 children diagnosed with ALL in Slovakia from 2002 to 2012. During the first part of this period (2002 – 2007) patients were enrolled in the ALL IC-BFM 2002 trial. After study enrollment was closed, the same therapy was used without randomization (2008-12). Since 2013 Slovakian children with ALL have been enrolled in the new ALL IC BFM 2009 trial.

The ALL IC BFM 2002 prospective randomized international trial provided the first experience for Slovakia to participate in an international pediatric ALL treatment study. Prior to 1992, Slovakian children diagnosed with ALL and other malignancies were treated in several different children's departments using a variety of therapies. Starting in 1992, pediatric cancer therapy in Slovakia was centralized in three children's oncology centers that used the same treatment strategy. The treatment with protocols for ALL initiated in the early 70´s when Čáp, Koza and Černý worked out the first protocols according to American authors Holland and al. [13, 14, 15]. In 1995 the ALL BFM 90 protocol was gradually introduced and since 1997 all three departments of paediatric oncology treated children and adolescents with ALL following the ALL BFM 95 protocol, which formed the basis of the ALL IC-BFM 2002 trial [16].

The objective of this report is to analyze EFS and OS among children and adolescents with ALL treated with ALL IC 2002 therapy in the Slovak Republic between 2002 and 2012, and compare these outcomes to those obtained in 1997-2001 using the same basic therapy without clinical trial participation.

Patients and methods

Patients. From December 9, 2002 until December 31, 2012, a total of 280 children (age 1-18 years, median 5.7 years) were diagnosed with ALL and treated with ALL IC BFM 2002 therapy at one of 3 Slovak centers in Bratislava (n =130; 46.4%), Košice (n =90; 32.1%), and Banská Bystrica (n = 60; 21.4%). Children or their parents gave informed consent to enroll in ALL IC-BFM 2002 (2002-07) or be treated with the same backbone therapy after the trial was closed to enrollment (2008-12). The median follow up period for the analyzed patients was 5.35 years. Because the EFS and OS rates for those enrolled on the trial in 2002-07 (n=140) and those treating with the same regimens in 2008-12 (n=140) were very similar, the whole group was pooled to provide more power to examine outcome in patient subgroups (Table I).

Diagnosis. The diagnosis of ALL was based on the French-American-British classification and flow cytometric immunophenotyping using a standard set of monoclonal antibodies according to the European Group for Immunological Characterization of Leukemia [17]. Conventional cytogenetics, fluorescence *in situ* hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR) screening for the *ETV6*/*RUNX1*, *BCR*/*ABL1* and *MLL*/*AF4* fusion genes were routinely performed on samples from each patient [18].

Treatment response and relapse criteria. Treatment response was evaluated using cytomorphology, including day 8 prednisone response in peripheral blood, and day 15 and 33 bone marrow (BM) response. Peripheral blood prednisone poor response (PPR) was defined as $\geq 1x10^9$/L blasts and prednisone good response (PGR) as $<1x10^9$/L blasts after 7 days of treatment with prednisone and a single dose of intrathecal methotrexate. Bone marrow response was classified as M1 (<5% blasts), M2 (≥5 to < 25% blasts), or M3 (≥25% blasts) using standard morphological criteria (12). Complete remission (CR) was defined as less than 5% blasts in regenerating BM, the absence of leukemic blasts in blood and CSF, and no evidence of extramedullary leukemia. Relapse was defined as recurrence of ≥ 25% lymphoblasts in the BM or localized leukemic infiltrates in any site after achieving CR.

Risk stratification and therapy. Risk stratification was performed as described by Stary [12]. Patients with favorable age (1 to 6 years), initial white blood cell count (WBC) < 20 $x10^9$/L, and good response on day 8 (PGR), on day 15 (bone marrow M1 or M2) and on day 33 (bone marrow M1) were defined as standard risk (SR). Patients with any one of the following characteristics were defined as high risk (HR): PPR, M3 marrow on day 15, M2 or M3 on day 33, or *BCR-ABL1* or *MLL-AF4* detected by cytogenetics and/or FISH/RT-PCR. Patients that did not meet SR or HR criteria were defined as intermediate risk (IR).

From 2002-2007, patients were enrolled in the ALL IC-BFM 2002 trial and received therapy including randomized interventions as recently published [12, 19]. From 2008-2012, the ALL IC-BFM 2002 trial was closed to enrollment and patients were treated with the same backbone according to the BFM recommendations. The SR and IR patients recieved only a single protocol II block. Patients in the HR group received 6 HR blocks with a single Protocol II reinduction block. Cranial radiotherapy was applied as described by Stary et al. Maintenance therapy continued for all patients until a total treatment duration of 24 months, and consisted of daily oral mercaptopurine and weekly oral methotrexate. Allogeneic SCT was indicated for patients in very HR group who had a matched sibling donor available [7, 17].

Statistical analyses. The OS rate was calculated from the date of diagnosis to the date of last follow-up or the date of death. Event-free survival and survival times were calculated from the date of diagnosis to the date of event. Free survival or death events were resistance to therapy (nonresponse), relapse, secondary malignant neoplasm (SMN) or death from any cause. The Kaplan-Meier method was used to estimate survival rates [20]. The Log-Rank test (Mantel-Cox test) was used to compare differences of survival curves [21]. Cumulative incidence curves for relapse and deaths in complete remission were compared with the Gray test [22]. Tests with 0.05 significance level indicated a statistical difference in the survival and cumulative incidence curves between different groups. Analyses were carried out using *X*LSTAT 2014 and IBM SPSS Statistics 19.

Table 1. Patient characteristics and treatment results and by risk group for all patients

		All				SR, % (n= 82)		MR, % (n= 161)		HR, % (n=37)	
Variable		**n**	**%**	**Events, n**	**5-y EFS (SE)**	**n**	**%**	**n**	**%**	**n**	**%**
	All	280	100.0	58	0.788 (0.026)	82	100.0	161	100.0	37	100.0
Sex	Male	185	66.1	39	0.784 (0.032)	57	69.5	99	61.5	29	78.4
	Female	95	33.9	19	0.794 (0.044)	25	30.5	62	38.5	8	21.6
Age[b]	1 to less than 6 y	145	51.8	21	0.857 (0.031)	82	100.0	55	34.2	8	21.6
	6 to less than 10 y	52	18.6	8	0.822 (0.058)	0	0.0	44	27.3	8	21.6
	10 y and older	83	29.6	29	0.647 (0.054)	0	0.0	62	38.5	21	56.8
Initial WBC (/μL)[a]	Less than 20 000	172	61.4	33	0.808 (0.032)	82	100.0	77	47.8	13	35.1
	20 000 to less than 100 x10^9/L	72	25.7	13	0.845 (0.043)	0	0.0	66	41.0	6	16.2
	100 000 and over	36	12.9	12	0.663 (0.080)	0	0.0	18	11.2	18	48.6
CNS status[b]	CNS1	203	72.5	38	0.810 (0.029)	61	74.4	123	76.4	19	51.4
	CNS2	57	20.4	11	0.805 (0.053)	20	24.4	28	17.4	9	24.3
	CNS3	20	7.1	9	0.495 (0.124)	1	1.2	10	6.2	9	24.3
Precursor	Precursor T	42	15.0	10	0.760 (0.066)	1	1.2	24	14.9	17	45.9
	Precursor B	238	85.0	48	0.793 (0.028)	81	98.8	137	85.1	20	54.1
ETV6/RUNX 1	Negative	223	79.6	46	0.792 (0.029)	65	79.3	123	76.4	35	94.6
	Positive	46	16.4	8	0.811 (0.061)	16	19.5	29	18.0	1	2.7
	No data	11	3.9	4	0.582 (0.169)	1	1.2	9	5.6	1	2.7
BCR/ABL	Negative	262	93.6	49	0.808 (0.026)	79	96.3	152	94.4	31	83.8
	Positive	5	1.8	5	0.000 (0.000)	0	0.0	0	0.0	5	13.5
	No data	13	4.6	4	0.692 (0.128)	3	3.7	9	5.6	1	2.7
MLL/AF4	Negative	266	95.0	54	0.792 (0.026)	79	96.3	153	95.0	34	91.9
	Positive	4	1.4	0	1.000 (0.000)	1	1.2	1	0.6	2	5.4
	No data	10	3.6	4	0.600 (0.155)	2	2.4	7	4.3	1	2.7
Hyperdiploidy[a]	Negative	250	89.3	53	0.785 (0.027)	70	85.4	145	90.1	35	94.6
	Positive	26	9.3	4	0.826 (0.080)	11	13.4	14	8.7	1	2.7
	No data	3	1.1	0	0.000 (0.000)	1	1.2	2	1.2	0	0.0
T lineage NCI risk criteria	Standard risk	8	2.9	1	0.875 (0.117)	1	1.2	6	3.7	1	2.7
	High risk	34	12.1	9	0.733 (0.076)	0	0.0	18	11.2	16	43.2
Non-T lineage NCI risk criteria	Standard risk	149	53.2	20	0.865 (0.030)	81	98.8	63	39.1	5	13.5
	High risk	89	31.8	28	0.678 (0.051)	0	0.0	74	46.0	15	40.5
Prednisone response[b]	Good	257	91.8	49	0.806 (0.026)	82	100.0	161	100.0	14	37.8
	Poor	23	8.2	9	0.598 (0.105)	0	0.0	0	0.0	23	62.2
BM day 15[b]	M1	193	68.9	25	0.868 (0.026)	69	84.1	121	75.2	3	8.1
	M2	66	23.6	19	0.709 (0.059)	13	15.9	40	24.8	13	35.1
	M3	21	7.5	14	0.291 (0.108)	0	0.0	0	0.0	21	56.8
Comlete remission day 33[b]	Yes	266	95.0	47	0.819 (0.025)	82	100.0	161	100.0	23	62.2
	No	14	5.0	11	0.214 (0.110)	0	0.0	0	0.0	14	37.8

Results

Patient characteristics. The demographic and clinical characteristics of the 280 children with ALL treated in the Slovak Republic between 2002 and 2012 with patients' basic characteristics (sex, age, WBC, immunophenotype, specific cytogenetic aberrations, CNS status) and treatment response on day 8, 15 and 33 of the total 280 patients and according to risk groups are summarized in Table I. Patient characteristics in the two time periods are provided in Table SI.

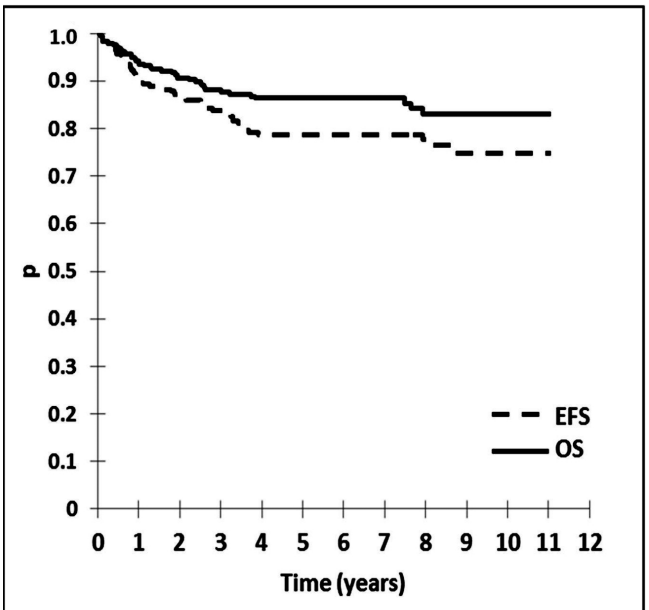
Event-free and overall survival. For the whole group of 280 patients, the 5-year EFS (+/- standard error (SE)) and OS rates were 79% (SE 2.6%) and 86% (SE 2.1%), respectively. The corresponding estimates were 85% (SE 4.1%) and 95% (SE 2.5%) for the SR group (n=82, 29% of patients), 84% (SE 3.1%) and 90% (SE 2.4%) for the IR group (n=161, 58% of patients), and 42% (SE 8.9%) and 48% (SE 9.6%) for the HR group (n=37, 13% of patients). (Table II, Figure 1, 2, 3).

Study vs non study patients. The overall outcome for patients enrolled in the ALL IC-BFM 2002 trial during 2002-2007 was not significantly different from that of patients treated in the 2nd period, (p=0.491). The 5-year EFS/OS rates were 77% (SE 3.5%)/81% (SE 3.8%) for study patients and 85% (SE 3%)/88% (SE 3%). There were significant differences in the outcome of patients age 6 to less than 10 years (p=0.044), and for patients with M3 bone marrow on day 15 (p=0.047), with better outcome for patients treated in the 2nd period (Figure S1).

Clinical characteristics and outcome (Table I). The EFS rates were similar for males and females (p=0.957), Figure S2. Age was highly predictive of outcome (p=0.001). As expected patients age 1 to less than 6 years had the best outcome with 5-year EFS and OS of 86% (SE 3.1%) and 93% (SE 2.2%), while patients 10 years and older had the worst outcome with 5-year EFS and OS of 65% (SE 5.4%) and 75% (SE 4.9%). Patients age 6 to <10 years had intermediate outcomes with 5-year EFS and OS of 82% (SE 5.8%) and 86% (SE 5.3%), Figure S3. Initial WBC was also highly predictive of outcome with 5-year EFS and OS rates 81% (SE 3.2%) and 91% (SE 2.3%) for those with an initial WBC <20 x10^9/L, as compared to 66% (SE 8.0%) and 64% (SE 8.4%) for those with an initial WBC ≥100 x10^9/L Figure S4. There was no difference in outcome based on precursor B-cell versus T-cell immunophenotype (p value for EFS 0.447 Figure S6). Patients with CNS 1 and 2 had a favorable outcome compared to those classified as CNS 3 (p=0.039 for OS and p=0.009 for EFS; Figure S5).

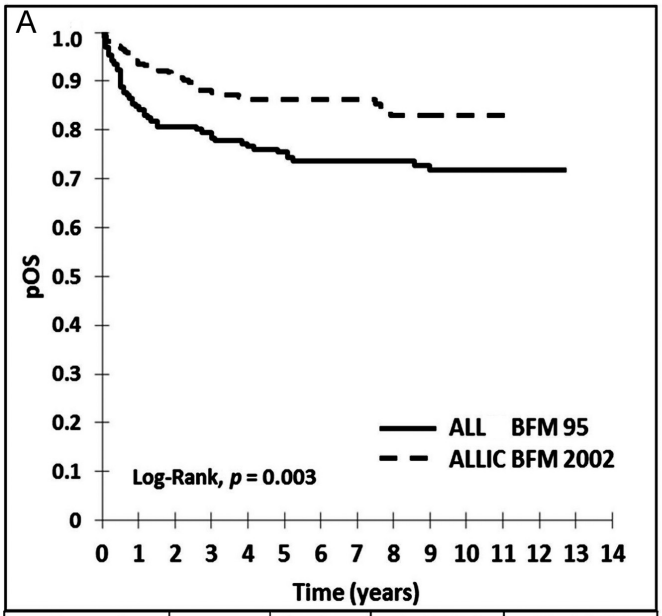
Cytogenetic subgroups. ETV6/RUNX 1 was detected in 16% of patients, and hyperdiploidy in 9% . The 5-year EFS for these subgroups was similar to that of the overall group at 81% (SE 6.1%) and EFS 83% (SE 8%), respectively. Only a small percentage of patients were identified to have *BCR/ABL1* (1.8%) or *MLL/AF4* fusion (1.4%), Table I.

Treatment response. Prednisone good response was achieved in 92% of patients (5-year EFS 81%, SE 2.6%) and PPR in 8% (5-year EFS 59.8%, SE 10.5%; p<0.003), Figure S7. Day 15 BM response was also highly predictive of outcome (p<0.0001). Patients with an M3 marrow had a 5-year EFS of only 29% (SE 11%) compared to patients with an M1 (87%, SE 2.6%) or M2 marrow (71% SE 5.9%), Figure S8. Seven patients with BM M3 on day 15 were shifted

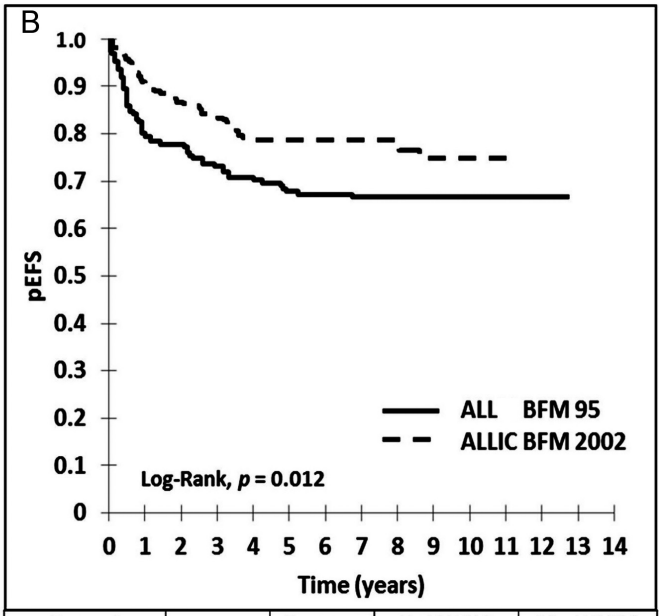


n	Deaths/Events, n	5-y Survival (SE)	10-y Survival (SE)
OS	39	0.863 (0.021)	0.831 (0.028)
EFS	58	0.788 (0.026)	0.749 (0.033)

Figure 1. Survival and event free survival of all patients treated in Slovakia during period 2002-2012

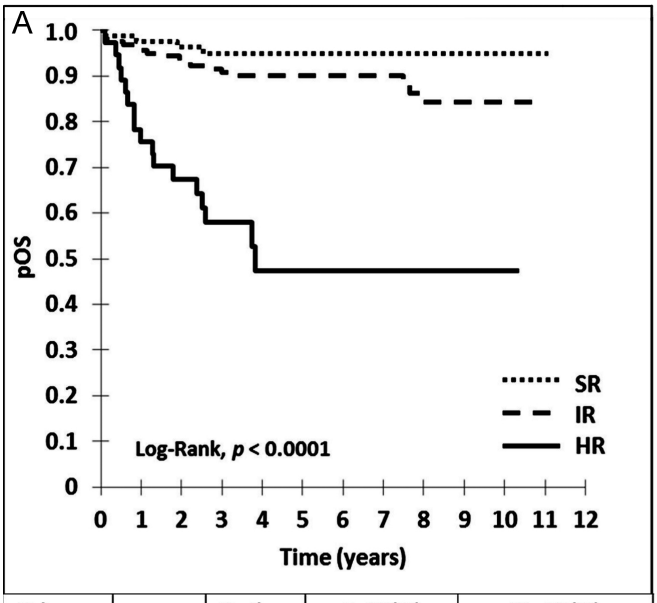


Protocol	n	Deaths, n	5-y OS (SE)	10-y OS (SE)
ALL BFM 95	171	47	0.754 (0.033)	0.718 (0.035)
ALLIC BFM 2002	280	39	0.863 (0.021)	0.831 (0.028)

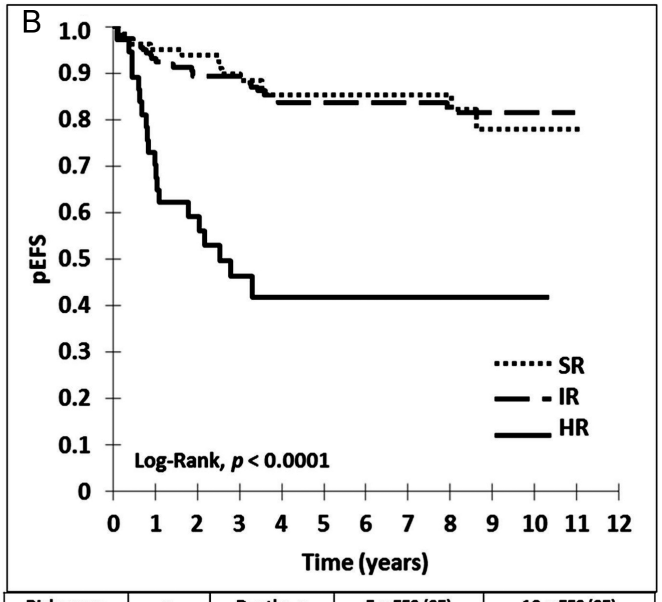


Protocol	n	Events, n	5-y EFS (SE)	10-y EFS (SE)
ALL BFM 95	171	57	0.673 (0.036)	0.667 (0.036)
ALLIC BFM 2002	280	58	0.788 (0.026)	0.749 (0.033)

Figure 2. (A) Comparison of survival in BFM 95 and in ALLIC BFM 2002. (B) Comparison of event free survival in BFM 95 and in ALLIC BFM 2002



Risk group	n	Deaths, n	5-yOS (SE)	10-y OS (SE)
SR	82	4	0.949 (0.025)	0.949 (0.025)
IR	161	18	0.901 (0.024)	0.842 (0.040)
HR	37	17	0.475 (0.096)	0.475 (0.096)



Risk group	n	Deaths, n	5-y EFS (SE)	10-y EFS (SE)
SR	82	13	0.852 (0.041)	0.779 (0.063)
IR	161	25	0.838 (0.031)	0.816 (0.037)
HR	37	20	0.417 (0.089)	0.417 (0.089)

Figure 3. (A) Survival by risk groups in ALLIC BFM 2002. (B) Event free survival by risk groups in ALLIC BFM 2002.

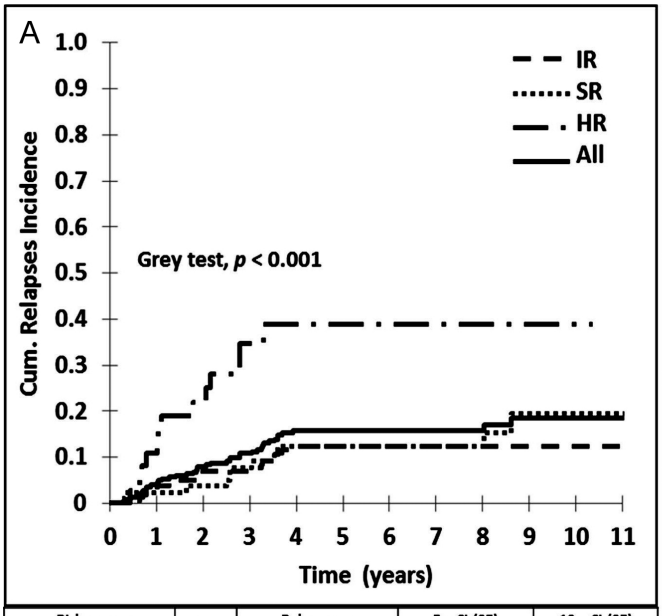
from IR to HR. Patients who did not achieve remission on day 33 (n=14), had a very poor outcome (p<0.0001) with 5-year EFS and OS only 21% (SE 11%) and 34% (SE 13%), Figure S9.

Events (Table II)

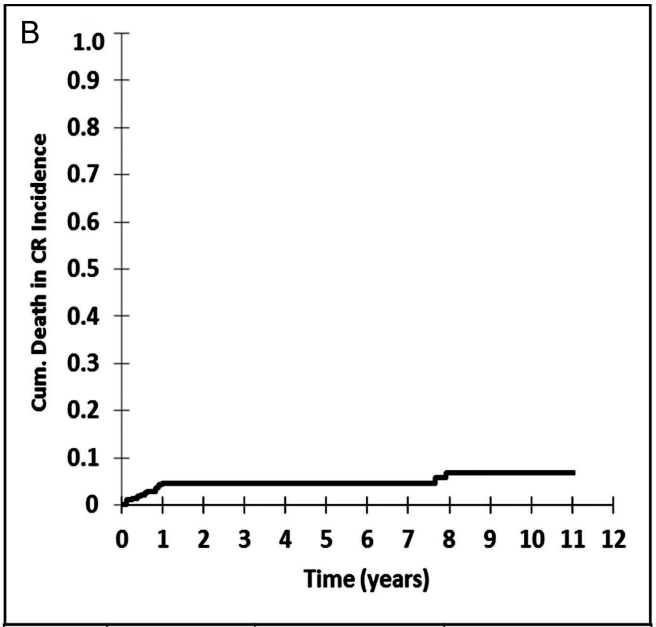
Remission failure and induction deaths. A total of 266/280 (95%) patients achieved CR and 14 failed to achieve CR on day 33. Two patients died during induction (0.7%). Of the 12

Table 2. Treatment results

	All		SR		IR		HR	
	n	%	n	%	n	%	n	%
Overall	280	100.0	82	100.0	161	100.0	37	100.0
Events (relapses + deaths)	57	20.4	13	15.9	25	15.5	20	54.1
Deaths	39	13.9	4	4.9	18	11.2	17	45.9
Death before CR	2	0.7	-	-	-	-	2	5.4
Resistant disease	1	0.4	-	-	-	-	1	2.7
Death in first CR	15	5.4	1	1.2	8	5.0	5	13.5
During/after chemotherapy	13	4.6	1	1.2	7	4.3	5	13.5
After stem cell transplantation	2	0.7	-	-	1	0.6	1	2.7
Relapses	42	15.0	11	13.4	18	11.2	13.0	35.1
Isolated BM	29	10.4	7	8.5	13	8.1	9	24.3
Isolated CNS	1	0.4	0	0.0	1	0.6	0	0.0
Isolated testes	2	0.7	1	1.2	1	0.6	0	0.0
Combined CNS/BM involved	5	1.8	1	1.2	2	1.2	2	5.4
Combined BM/other (without CNS)	2	0.7	1	1.2	0	0.0	1	2.7
Secondary neoplasms	2	0.7	1	1.2	1	0.6	0	0.0
Other relapses	1	0.4	0	0.0	0	0.0	1	2.7
Stem cells transplantation	23	8.2	4	4.9	7	4.3	12	32.4
SCT in 1. CR	10	3.6	0	0.0	0	0.0	10	27.0
SCT in 2. CR	11	3.9	3	3.7	6	3.7	2	5.4
SCT in 3. CR	2	0.7	1	1.2	1	0.6	0	0.0



Risk group	n	Relapses, n	5-y CI (SE)	10-y CI (SE)
SR	82	11	0.123 (0.039)	0.196 (0.061)
IR	161	18	0.125 (0.028)	0.125 (0.028)
HR	37	13	0.388 (0.086)	0.388 (0.086)
All	280	42	0.158 (0.023)	0.184 (0.029)



n	Deaths	5-y CI (SE)	10-y CI (SE)
280	15	0.046 (0.013)	0.069 (0.020)

Figure 4. (A) Cumulative incidence of relapse, and by risk groups in ALLIC BFM 2002. (B) Cumulative incidence of death in complete remission in ALLIC BFM 2002.

patients who did not enter remission at day 33, 11 achieved remission at later time points, and 1 patient had resistant disease.

Death in CR. As a result of treatment – related events a total of 15 patients died in CR, with cumulative incidence rate (CIR) of 4.6% (SE 1.3) at 5 years (Figure 4). Of these deaths, 13 occurred in patients receiving chemotherapy and 2 occurred among the ten patients that underwent SCT in first remission.

Relapse. The most common cause of treatment failure was relapse with a 5-year CIR of 15.8% (SE 2.3%), including 10.4% for isolated BM relapse, 0.4% for isolated CNS relapse, 1.8% for combined BM/CNS relapse, 0.7% for isolated testicular relapse, 0.7% for combined BM/other (without CNS), 0.4% of other relapses. The 10-year CIR of relapse was slightly higher at 18.0% (SE 2.9%). The 5-year CIR for relapse differed among the risk groups: 12.3% (SE 3.9%) for SR, 12.5 % (SE 2.8%) for IR, and 38.8 % (SE 8.6%) for HR ($p<0.001$; Figure 4).

Second malignant neoplasms (SMN). Two SMN occurred, one patient developed an aggressive histiocytic sarcoma during maintenance therapy and died within 6 months and another patient developed myelodysplasia with monosomy 7, 3.5 years after ALL was diagnosed and underwent allogenic HSCT and is alive in 2nd CR.

Hematopoietic stem cell transplantation (HSCT). Ten patients (0.4%) underwent HSCT from a matched sibling or matched unrelated donor in first remission. Four have died (one due to post-transplant lymphoproliferative disorder, one due to graft-versus-host disease and 2 from relapse) and six remain in first remission. Nine patients were transplanted in 2nd CR, 5 of whom are alive in remission, and 4 who died (1 due to aspergillosis, 1 due to graft-versus-host disease and 2 after another relapse). Two patients were transplanted in CR3, both of whom subsequently died.

Discussion

Each year in the Slovak Republic, which has about 1.1 million children, approximately 40 pediatric leukemia cases are diagnosed, including 30 cases of acute lymphoblastic leukemia, 6–8 cases of acute myeloid leukemia, and 1–2 cases of chronic myeloid leukemia [16, 23, 24]. Children with leukemia are treated in 3 centers, which are well distributed across Slovakia. In Bratislava there are about 50% of patients and in Banska Bystrica and Kosice equally about 25% of patients.

Treatment and results in historical perspective. From 1971 until 1991, a variety of different treatment regimens derived from those used in the United States were used to treat children with ALL [13, 14, 15]. The 10-year EFS and OS rates in this period were 46% and 50%, respectively [16]. Starting in 1997, BFM-based ALL treatment regimens have been used. Initially from 1997-2001, the ALL BFM 95 regimen was adopted and children were treated with this regimen in all

of the three centers in the Slovak Republic, but not as part of a formal clinical trial. The 10-year EFS and OS rates from this period were 67% and 72%, with 2.9% induction deaths and 10.8% deaths in 1st CR, and a 20% relapse rate [16]. In order to participate in the formal ALL IC-BFM 2002 trial, several important steps were taken including standardized methods for diagnosis and treatment response, development of a common database to facilitate country-wide randomization and data capture, and institution of standardized supportive care guidelines across the three centers.

Current results. In this report, we present the results of 280 patients, treated in the Slovak Republic from 2002 – 2012. The main result of our analyses is that the 5-year EFS and OS rates were 79% and 86%, which are significantly better than those obtained using the same BFM-95 based regimen in 1997-2001 (p=0.012 for EFS and p=0.003 for OS; Figure 2). Regarding comparison with larger groups; our results compare favorably to those of the total ALL IC BFM 2002 trial (5-year EFS 74%, OS 82%) [12] and are very similar to those achieved in Western Europe in the ALL BFM 95 trial that utilized the same treatment regimen. Moricke et al report results for 2283 pts with 6-years EFS of 79.6% and OS of 86.3% [25, 26] and almost as good as those obtained in the most recent reports of AEIOP/BFM ALL 2000 trial (7-year EFS and OS of 80.4% and 91.8%, respectively [27]).

One important potential cause for lower survival rates in countries with lower incomes and/or less familiarity with intensive therapy are higher rates of treatment-related mortality. The rate of induction death (0.7%) in this study is essentially the same as that in the ALL BFM 95 study (25). However, we encountered higher rates of death in 1st CR. Prior to 1997, the rate of death in 1st CR decreased gradually in Slovak protocols from 10.4% to 1.8%, but then significantly increased (10.8%) with introduction of the ALL BFM 95 protocol in Slovakia in 1997 due to insufficient experience with intensive BFM-type therapy (16). Since 2002, the cumulative incidence of death in 1st CR decreased to 4.6%, which is still about two times higher than the results of ALL BFM 95 (2.1%) and three times higher than current achievements of leading international collaborative ALL treatment groups (1.4%) (27). Thus, decreasing the rate of death in remission remains a important challenge in Slovakia.

Risk groups stratification. Patient in the SR group (n=82) achieved excellent results with 5-year EFS and OS of 85% and 95%, respectively. Fifteen events occurred in the SR group (4 deaths and 11 relapses). In the IR group, EFS and OS were 84% and 90% (n =161 patients, 24 events, 18 relapses). Our results in the SR and IR groups are better than overall outcomes for these groups in the whole ALL IC BFM 2002 trial. While the number of HR patients was small (n = 37, with 20 events and 13 relapses), our results in this group are quite unsatisfactory and need improvement due to high toxicity.

Relapse rate. The relapse rate for children with ALL in Slovakia decreased from 42% to 35% before the introduction of BFM based therapy, with a 9% rate of CNS relapse. These rates have decreased significantly since the introduction of BFM-based therapy in 1997, with cumulative incidence of relapse of 20.5% (CNS 1.8%) in 1997-2001, and 15.4% with only 0.4% CNS in 2002-12. The biggest challenge is for HR patients who had a very high CIR rate of 39.5%. Although patient numbers are small in this group in Slovakia (n= 37), this appears to be worse than the overall results of international ALL IC BFM 2002 (25.2%), but about the same as achieved in ALL BFM 95 (38%) [25].

Study vs non study patients. The patients enrolled in ALL IC BFM 2002 in 2002 – 2007 (n= 140) achieved 5-year EFS and OS of 77% and 85%. The cohort (n=140) enrolled in 2008-2012 after the study was closed had a trend toward improved outcome, with 5-year EFS and OS of 81% and 88%. While it is not statistically different in overall results (significant statistical difference was achieved in age group 6 to less than 10 years old and in M3 marrow on day 15), there is a trend toward better EFS/OS in the latter period. The results suggest that outcome may be improving over time with increased familiarity with the treatment regimen.

Response to treatment. Response in BM on day 15 had significant impact on outcome, patients with good response on day 15 (67%) achieved 5 years EFS 87% and those with bone marrow M3 response has significantly worse outcome. On day 33, 95% of patients achieved complete remission. These results are consistent with those published by Stary.

Cytogenetic. We observed lower rates of specific sentinel chromosome translocations and numerical abnormalities than those reported by larger groups. This is likely due to technical factors, and efforts are ongoing to improve cytogenetic methodology in Slovakia.

Conclusion

Slovak Republic participation in the ALL IC BFM 2002 trial resulted in statistically significant improvement in 5-year EFS and OS, due to decreases in CIR of relapse, and in induction and treatment related deaths over past decade. Our results are almost as good as those achieved by the larger BFM group in Western Europe in the late 1990s using the same treatment approach. This improvement was achieved not by changing treatment strategies, but by several other factors: development of central diagnostic infrastructure, improvements in supportive care, familiarities with BFM-based treatment and participation in the intercontinental clinical trial. However, our results for patients in the HR group remain quite unsatisfactory and need improvement. The next challenge in treatment of childhood ALL in the Slovak Republic is to adopt contemporary approaches to MRD-based risk stratification to facilitate more precise patient allocation to risk groups [27, 28, 29, 30, 31].

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

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doi:10.4149/neo_2015_098

Supplementary Information

Improved outcome for children and adolescent with acute lymphoblastic leukemia in the first decade of the 21st century: a report from the Slovak Republic

A. KOLENOVA[1,*], E. KAISEROVA[1], M. MAKOHUSOVA[1,4], Z. SUBOVA[1], E. BUBANSKA[2], I. ORAVKINOVA[3], P. SVEC[1], O. FABRI[1], S. P. HUNGER[5], S. SUFLIARSKA[1], S. HORAKOVA[1], V. MRAZOVA[4], J. STARY[6]

[1]Department of Pediatric Hematology and Oncology , Comenius University – Medical School and University Children's Hospital, Bratislava, Slovak republic; [2]Department of Pediatric Oncology and Hematology, Children's University Hospital, Banská Bystrica, Slovak republic; [3]Department of Pediatric Hematology and Oncology, University Children´s Hospital Kosice, Slovak Republic; [4]Department of Chemistry, University of SS. Cyril and Methodius, J. Herdu 2, Trnava,Slovak Republic; [5]University of Pennsylvania Perelman School of Medicine and The Children's Hospital of Philadelphia, Philadelphia PA USA; [6]Department of Pediatric Oncology and Hematology, Charles University and University Hospital Motol, Czech Republic

**Correspondence: sasa.kolenova@gmail.com*

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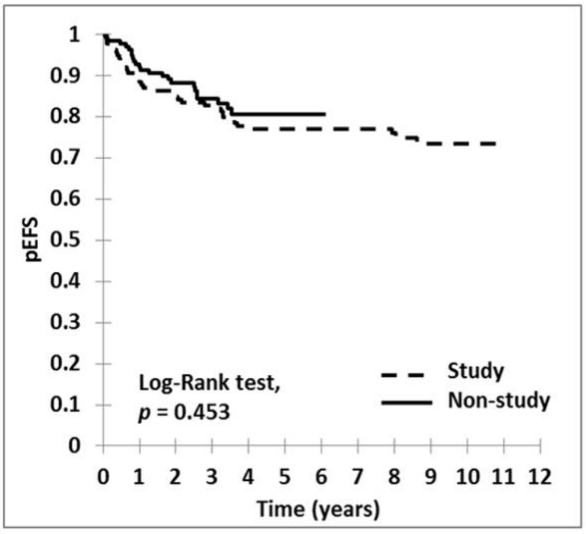

Treatment group	n	Events, n	5-yEFS
Study	140	35	0.771 (0.035)
Non-study	140	23	0.807 (0.038)

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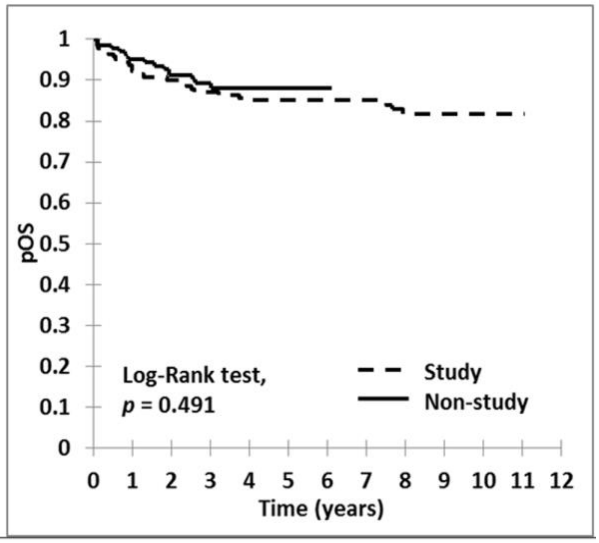

Treatment group	n	Deaths, n	5-yOS
Study	140	24	0.850 (0.030)
Non-study	140	15	0.880 (0.030)

Figure S1. (A) Comparison of survival in period 2002-2007 (study) and in 2008-2012 (non study). (B) Comparison of event free survival in period 2002-2007 (study) and in 2008-2012 (non study)

Table S1. Study and non-study patients' characteristics

Variable		Study group (total n=140)				Non study group (total n=140)				
		n	%	Events, n	5-y EFS (SE)	n	%	Events, n	5-y EFS (SE)	*p*
	All	140	100.0	35	0.771 (0.035)	140	100.0	23	0.815 (0.041)	0.453
Sex	Male	88	62.9	22	0.773 (0.045)	97	69.3	17	0.789 (0.047)	0.655
	Female	52	37.1	13	0.769 (0.058)	43	30.7	6	0.848 (0.059)	0.464
Age	1 to less than 6 y	71	50.7	11	0.873 (0.03.9)	74	52.9	10	0.834 (0.050)	0.584
	6 to less than 10 y	25	17.9	7	0.720 (0.09.0)	27	19.3	1	0.963 (0.036)	0.044[a]
	10 y and older	44	31.4	17	0.636 (0.073)	39	27.9	12	0.661 (0.081)	0.768
Initial WBC (/µ L)	Less than 20 000	88	62.9	19	0.818 (0.041)	84	60.0	14	0.794 (0.051)	0.736
	20 000 to less than 100 x10^9/L	38	27.1	9	0.763 (0.069)	34	24.3	4	0.882 (0.055)	0.314
	100 000 and over	14	10.0	7	0.500 (0.134)	22	15.7	5	0.773 (0.089)	0.093
CNS status	CNS1	108	77.1	24	0.806 (0.038)	95	67.9	14	0.812 (0.048)	0.826
	CNS2	24	17.1	7	0.708 (0.093)	33	23.6	4	0.874 (0.059)	0.099
	CNS3	8	5.7	4	0.500 (0.177)	12	8.6	5	0.533 (0.161)	0.832
Precursor	Precursor T	20	14.3	5	0.750 (0.097)	22	15.7	5	0.767 (0.092)	0.739
	Precursor B	120	85.7	30	0.775 (0.038)	118	84.3	18	0.815 (0.041)	0.444
ETV6/ RUNX 1	Negative	118	84.3	28	0.788 (0.038)	105	75.0	18	0.800 (0.044)	0.830
	Positive	16	11.4	3	0.813 (0.098)	30	21.4	5	0.807 (0.079)	0.985
	No data	6	4.3	4	0.333 (0.192)	5	3.6	0	1.000 (0.000)	-
Hyperdiploidy	Negative	126	90.0	32	0.770 (0.037)	124	88.6	21	0.807 (0.039)	0.529
	Positive	13	9.3	2	0.846 (0.100)	13	9.3	2	0.750 (0.153)	0.894
	No data	-	-	-	-	3	2.1	0	1.000 (0.000)	-
T lineage NCI risk criteria	Standard risk	3	2.1	0	1.000 (0.000)	5	3.6	1	0.800 (0.179)	-
	High risk	17	12.1	5	0.706 (0.111)	17	12.1	4	0.755 (0.107)	0.574
Non-T lineage NCI risk criteria	Standard risk	69	49.3	12	0.855 (0.042)	80	57.1	8	0.874 (0.043)	0,727
	High risk	51	36.4	18	0.667 (0.066)	38	27.1	10	0.697 (0.083)	0.804
Prednisone response	Good	134	95.7	31	0.791 (0.035)	123	87.9	18	0.825 (0.039)	0.491
	Poor	6	4.3	4	0.333 (0.192)	17	12.1	5	0.688 (0.118)	0.074
BM day 15	M1	98	70.0	13	0.888 (0.032)	95	67.9	12	0.837 (0.045)	0.412
	M2	31	22.1	12	0.645 (0.086)	35	25.0	7	0.786 (0.073)	0.322
	M3	11	7.9	10	0.091 (0.087)	10	7.1	4	0.583 (0.161)	0.047[a]
Completed remission on day 33	Yes	130	92.9	27	0.815 (0.034)	136	97.1	20	0.824 (0.037)	0.838
	No	10	7.1	8	0.200 (0.126)	4	2.9	3	0.250 (0.217)	0.981
BFM risk criteria	SR	45	32.1	7	0.889 (0.047)	37	26.4	7	0.786 (0.081)	0.288
	IR	80	57.1	16	0.813 (0.044)	81	57.9	9	0.871 (0.041)	0.372
	HR	15	10.7	12	0.200 (0.103)	22	15.7	8	0.614 (0.109)	0.022[a]

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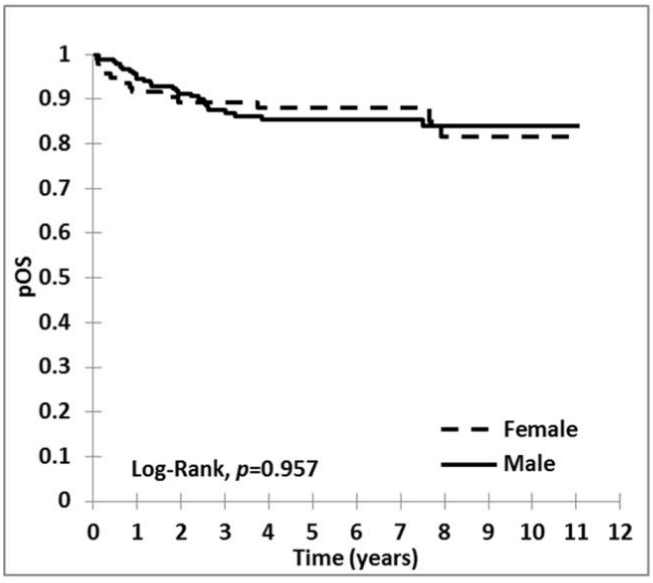
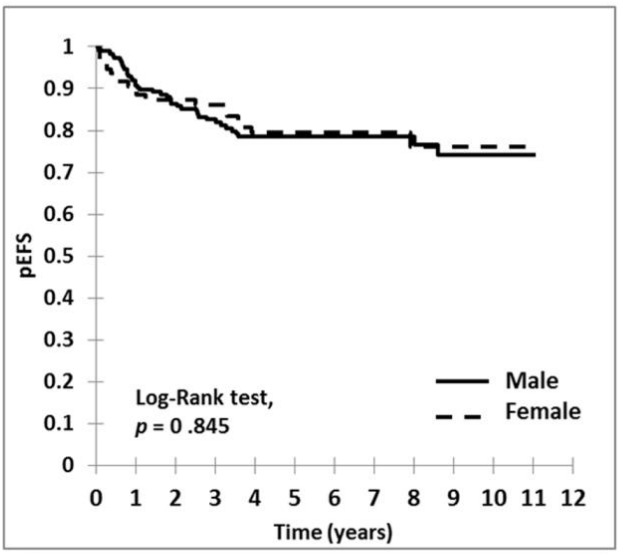


Gender	n	Deaths, n	5y-pOS (SE)
Male	185	26	0.855 (0.027)
Female	95	13	0.880 (0.034)

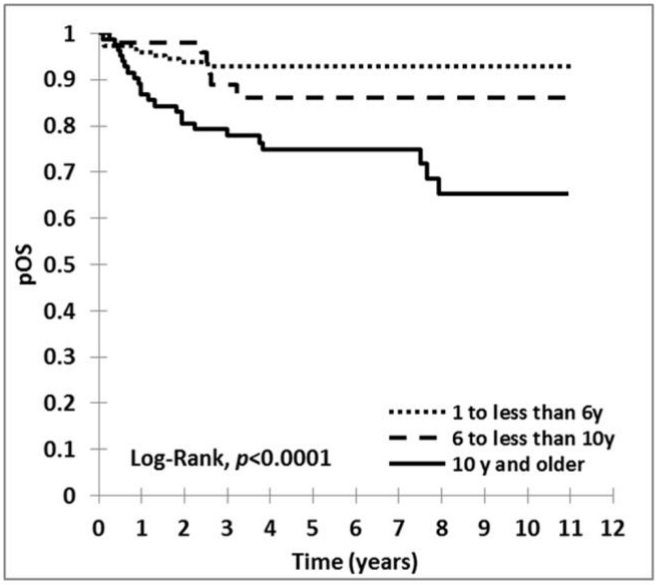
B



Sex	n	Events, n	5-y EFS (SE)
Male	185	39	0.784 (0.032)
Female	95	19	0.794 (0.044)

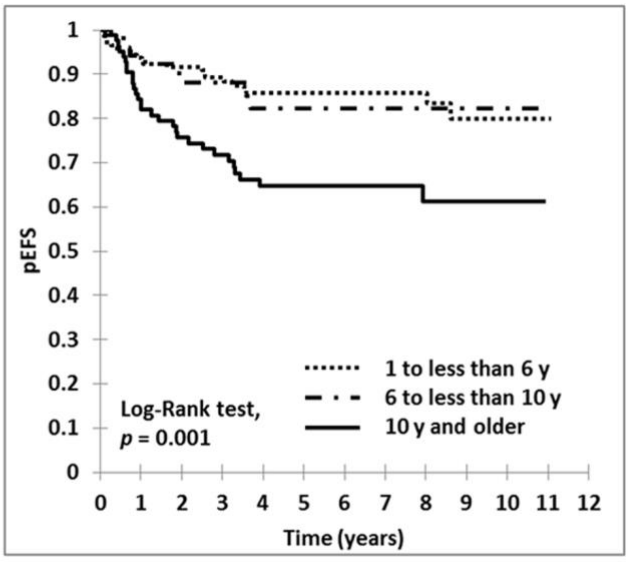
Figure S2. (A) Survival by sex. (B) Event free survival by sex.

A



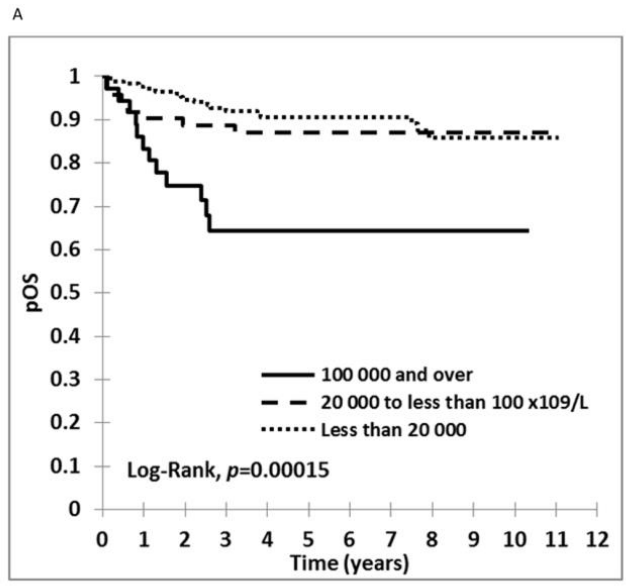
Age	n	Deaths, n	5y-EFS (SE)
1 to less than 6y	145	10	0.929 (0.022)
6 to less than 10y	52	6	0.862 (0.053)
10 y and older	83	23	0.748 (0.049)

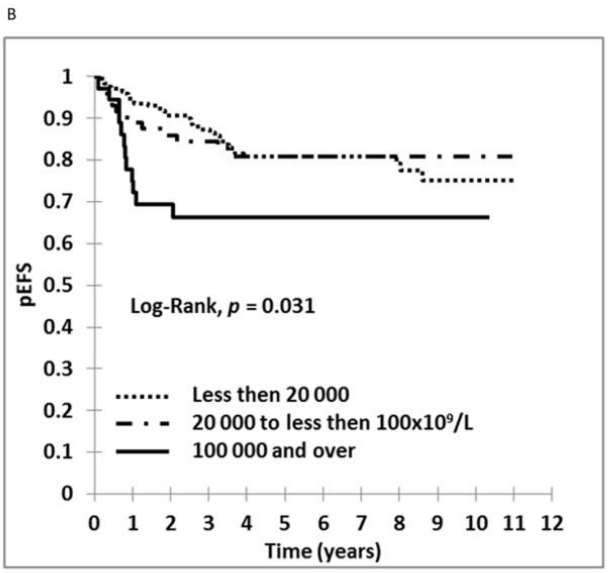
B



Age	n	Events, n	5-y EFS (SE)
1 to less than 6 y	145	21	0.857 (0.031)
6 to less than 10 y	52	8	0.822 (0.058)
10 y and older	83	29	0.647 (0.054)

Figure S3. (A) Survival by age. (B) Event free survival by age

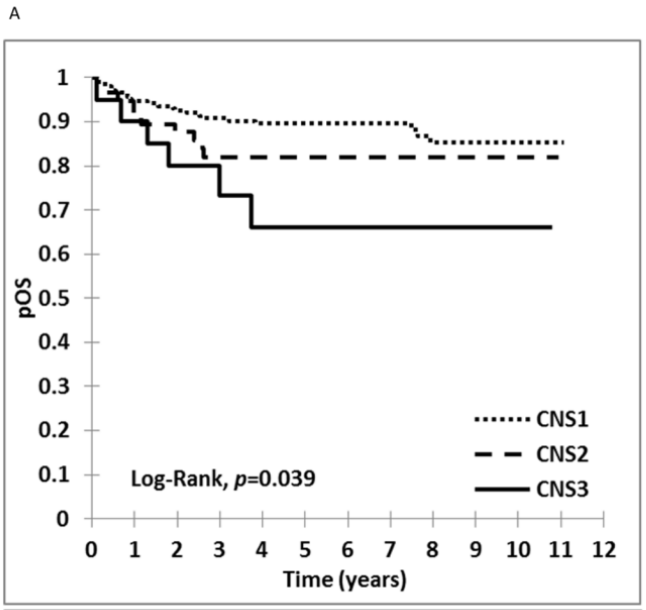


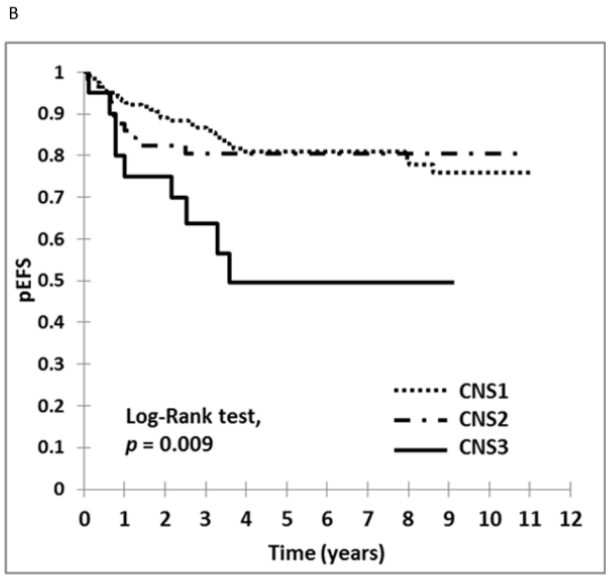


Initial WBC	n	Deaths, n	5y-EFS (SE)
Less than 20 000	172	18	0.905 (0.023)
20 000 to less than 100 x109/L	72	9	0.870 (0.041)
100 000 and over	36	12	0.644 (0.084)

Initial WBC	n	Events, n	5-y EFS (SE)
Less than 20 000	172	33	0.808 (0.032)
20 000 to less than 100 x10⁹/L	72	13	0.845 (0.043)
100 000 and over	36	12	0.663 (0.080)

Figure S4. (A) Survival by initial white blood cell count. (B) Event free survival by initial white blood cell count.



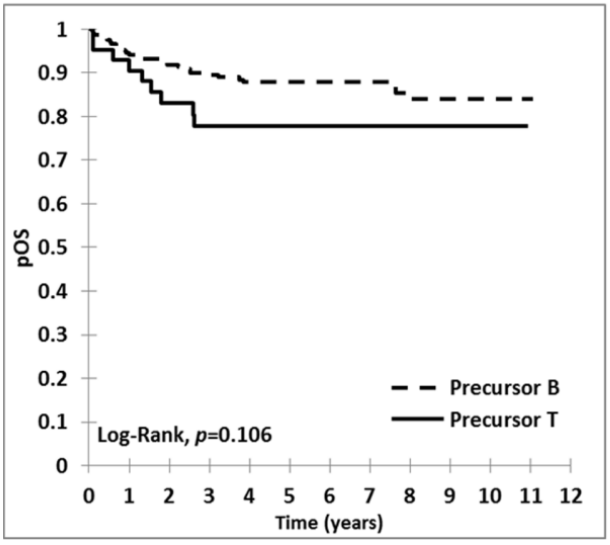


CNS status	n	Deaths, n	5y-EFS (SE)
CNS 1	203	23	0.895 (0.022)
CNS 2	57	10	0.819 (0.052)
CNS 3	20	6	0.660 (0.117)

CNS status	n	Events, n	5-y EFS (SE)
CNS1	203	38	0.810 (0.029)
CNS2	57	11	0.805 (0.053)
CNS3	20	9	0.495 (0.124)

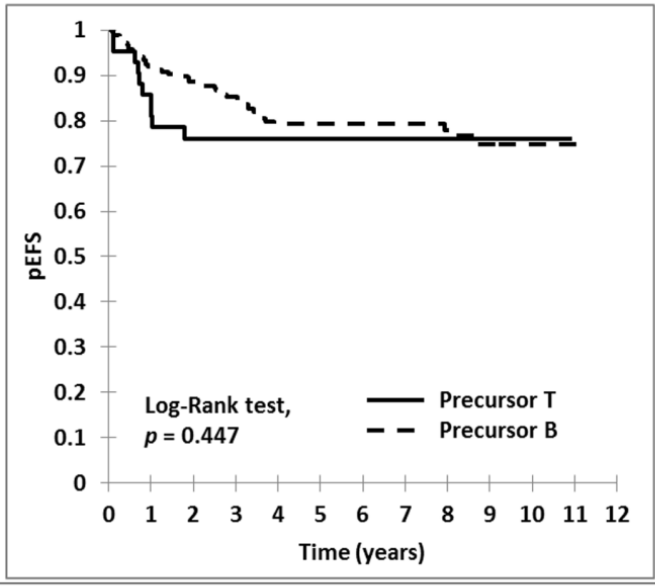
Figure S5. (A) Survival by CNS status. (B) Event free survival by CNS status.

A



Precursor	n	Deaths,n	5y-pOS (SE)
Precursor B	238	30	0.879 (0.022)
Precursor T	42	9	0.777 (0.066)

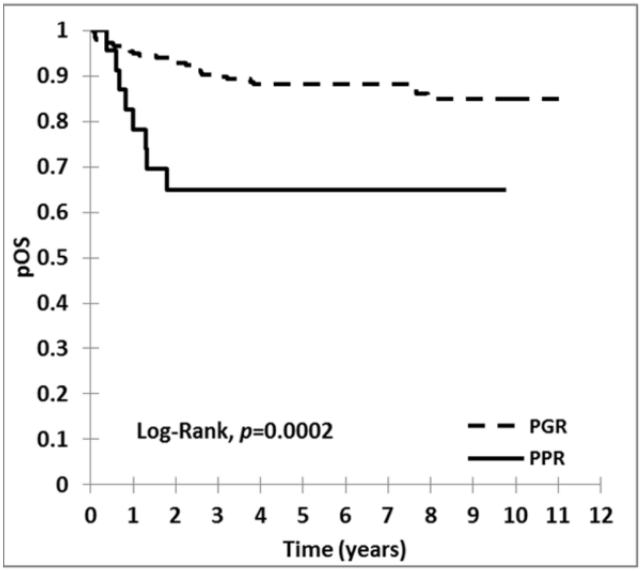
B



Precursor	n	Events, n	5-y EFS (SE)
Precursor T	42	10	0.760 (0.066)
Precursor B	238	48	0.793 (0.028)

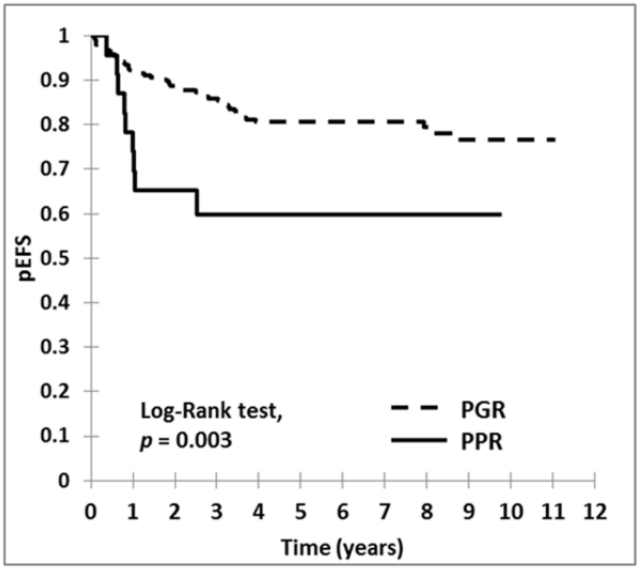
Figure S6. (A) Survival by imunophenotype. (B) Event free survival by immunophenotype.

A



Prednisone response	n	Deaths, n	5y-OS (SE)
Good	257	31	0.883 (0.021)
Poor	23	8	0.649 (0.100)

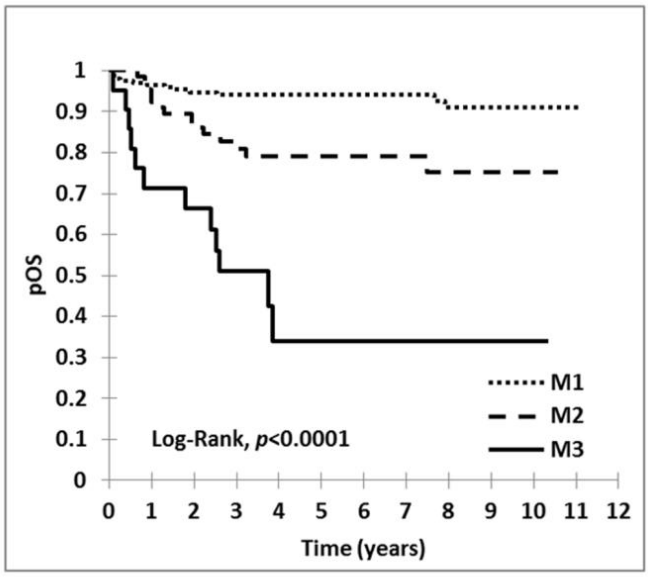
B



Prednisone response	n	Events, n	5-y EFS (SE)
Good	257	49	0.806 (0.026)
Poor	23	9	0.598 (0.105)

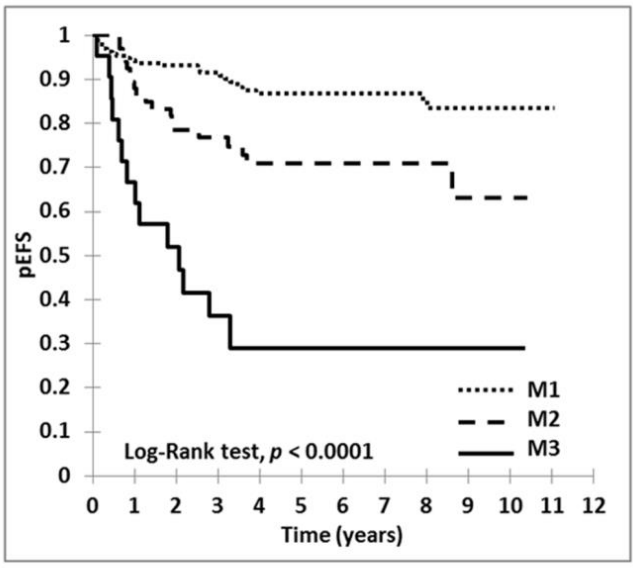
Figure S7. (A) Survival by prednisone response on day 8. (B) Event free survival by prednisone response on day 8.

A



Bone marrow day 15	n	Deaths, n	5y-EFS (SE)
M1	193	13	0.942 (0.017)
M2	66	14	0.791 (0.052)
M3	21	12	0.340 (0.123)

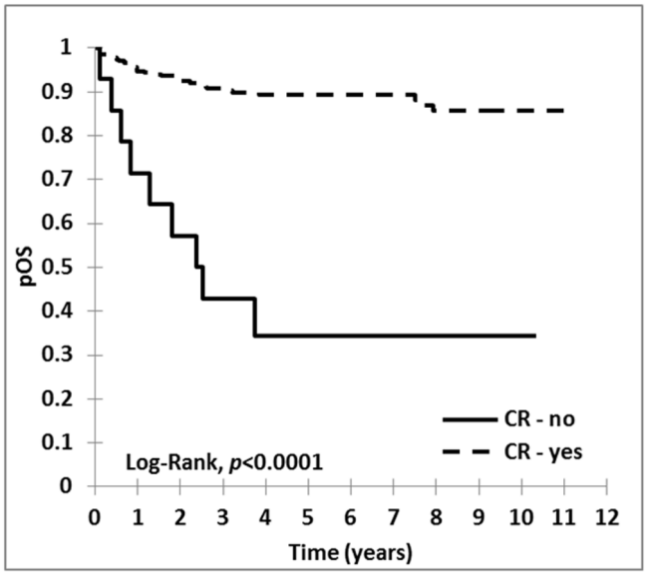
B



Bone marrow day 15	n	Events, n	5y-EFS (SE)
M1	193	25	0.868 (0.026)
M2	66	19	0.709 (0.059)
M3	21	14	0.291 (0.108)

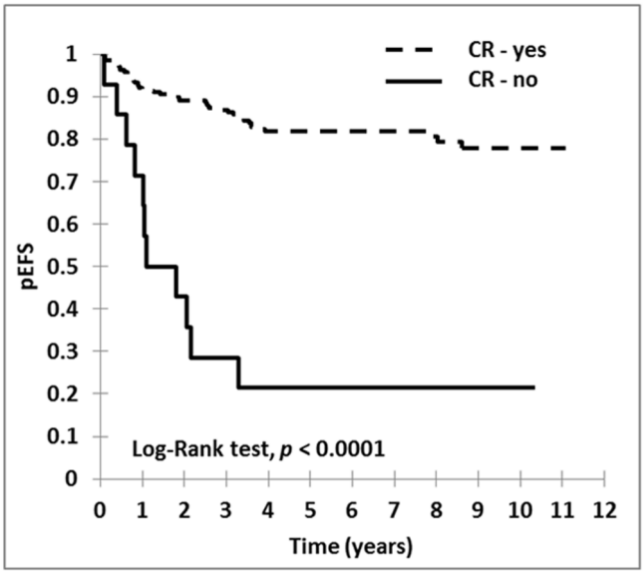
Figure S8. (A) Survival by response in bone marrow on day 15. (B) Event free survival by response in bone marrow on day 15.

A



Completed remission	n	Deaths, n	5y-EFS (SE)
No	14	9	0.343 (0.131)
Yes	266	30	0.892 (0.020)

B



Completed remission	n	Events, n	5y-EFS (SE)
Yes	266	47	0.819 (0.025)
No	14	11	0.214 (0.110)

Figure S9. (A) Survival by response in bone marrow on day 33. (B) Event free survival by response in bone marrow. on day 33.