

## The role of ZAP70 kinase in acute lymphoblastic leukemia infiltration into the central nervous system

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## Supplementary Figure and Table Legends

Supplementary Figure 1: *In vivo* effects of Zap-70 downregulation. A: Percentage of blasts in BM in 697, REH and JURKAT cells with and without knockdown of Zap-70 (unpaired t-test, two-sided p-value, n. s. = not significant). B: Survival of xenografts bearing 697-shGFP and -shZap-70 cells (Kaplan-Meier log rank test). C: Survival of xenografts bearing JURKAT-shGFP and -shZap-70 cells (Kaplan-Meier log rank test).

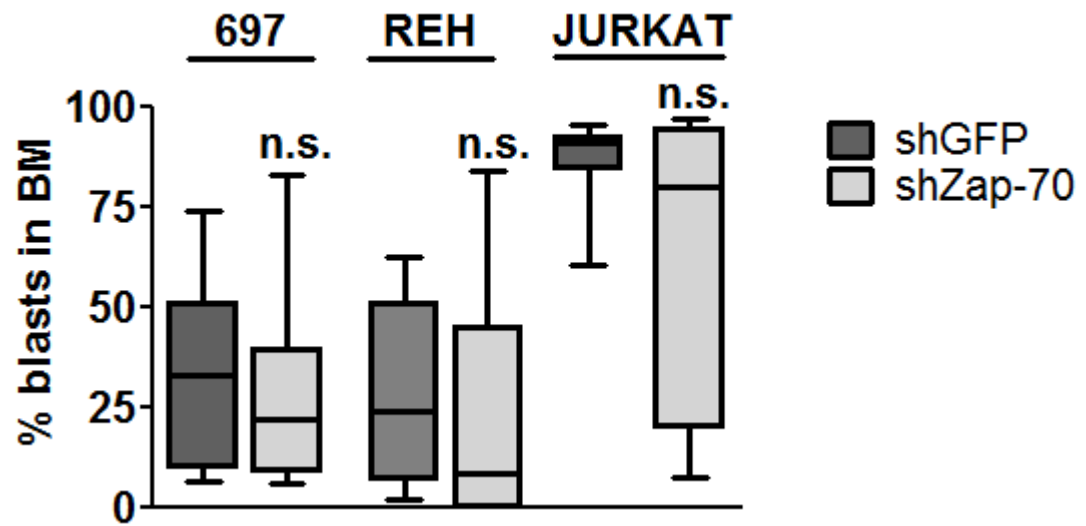
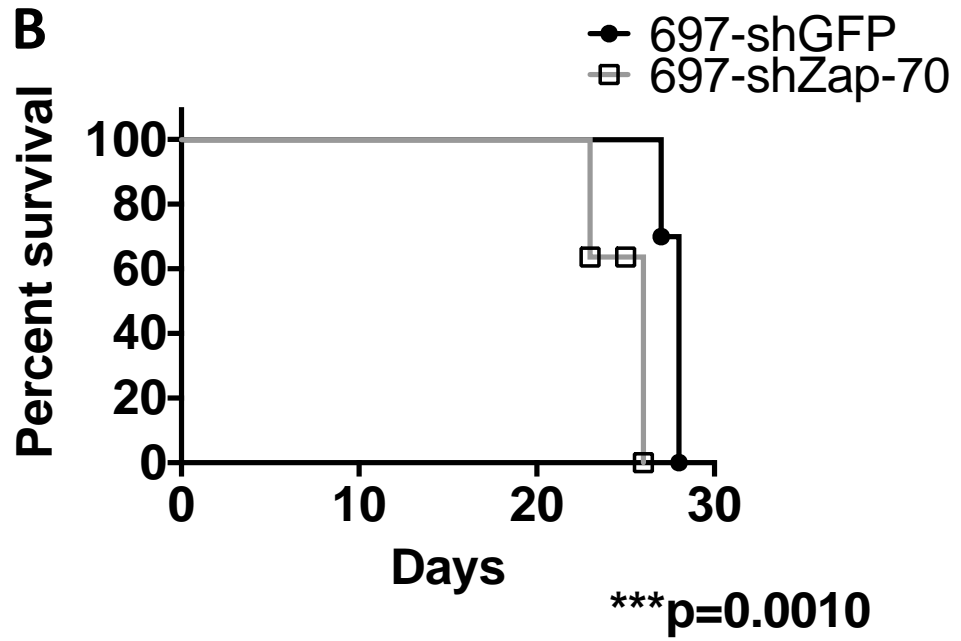
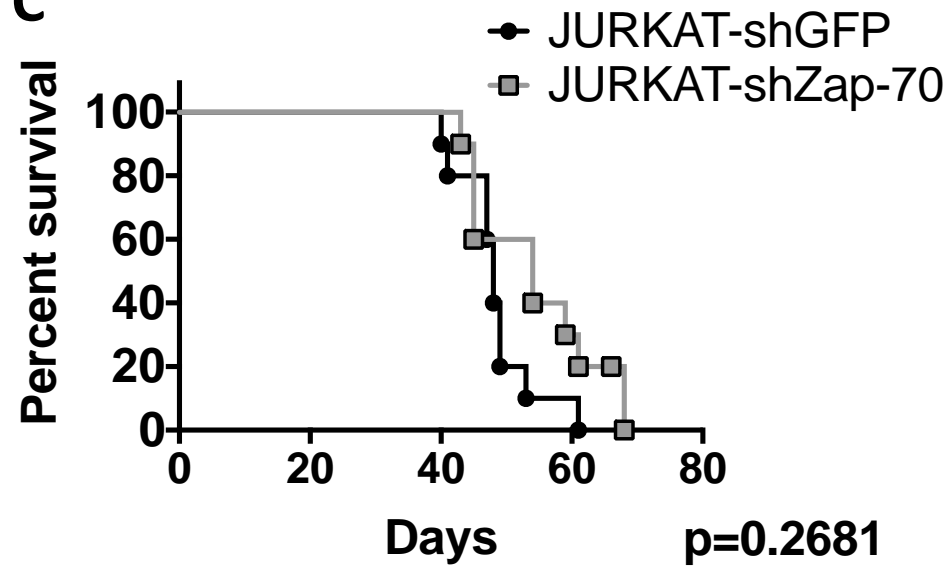
Supplementary Figure 2: CCL19 and CXCL12 induce Erk phosphorylation: A: Primary leukemic blasts from 4 BCP-ALL xenograft spleens were treated with anti-IgM and/or CCL19 as indicated and analyzed for Erk-phosphorylation. B: 697 and JURKAT cells were treated with CCL19 or CXCL12 as indicated and analyzed for Erk-phosphorylation. C: Primary leukemic blasts from 3 BCP-ALL xenograft spleens were treated with CXCL12 or left untreated and analyzed for Erk-phosphorylation. Blots were quantified using densitometry (Image J software). PV = Pervanadate, positive control.

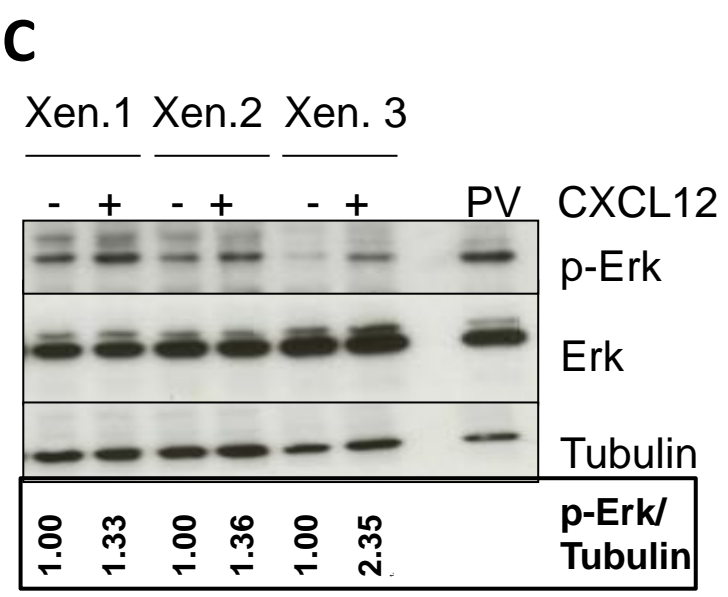
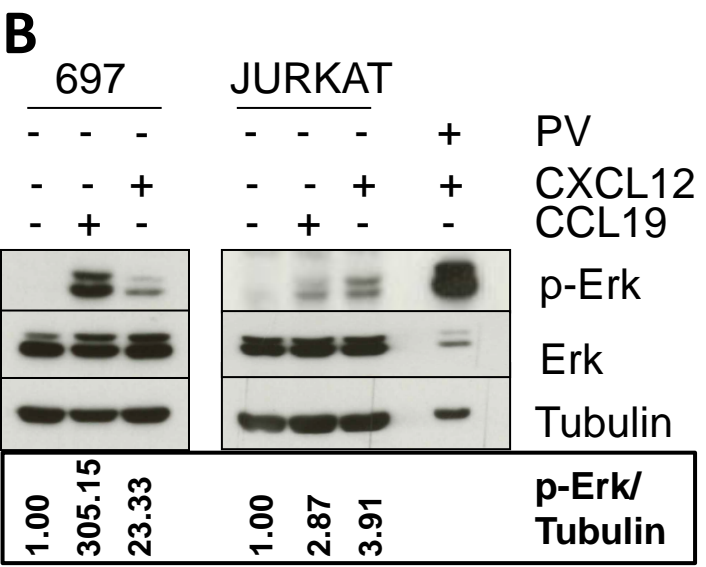
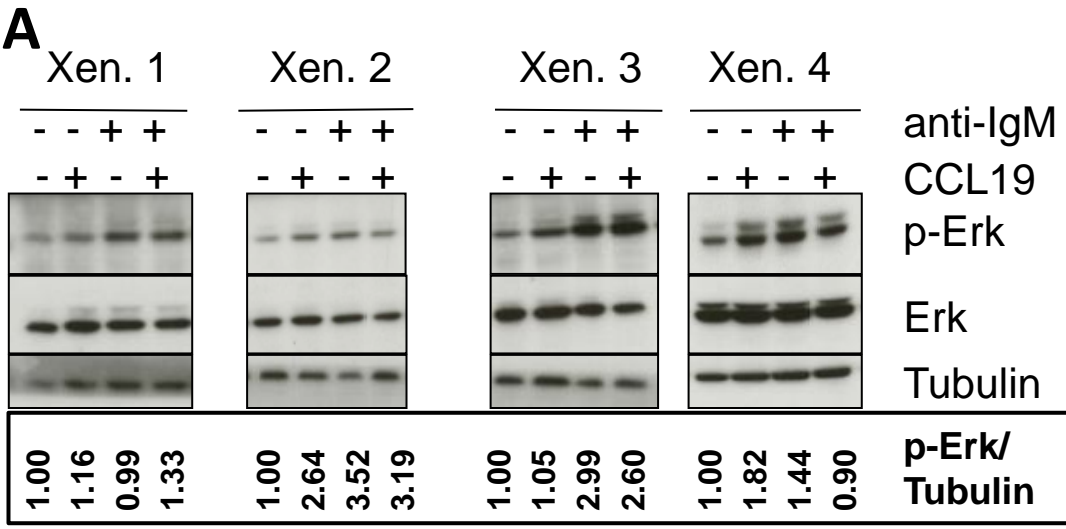
Supplementary Figure 3: Correlation of Zap-70 mRNA and protein expression in 9 xenograft samples (Spearman's rank correlation).

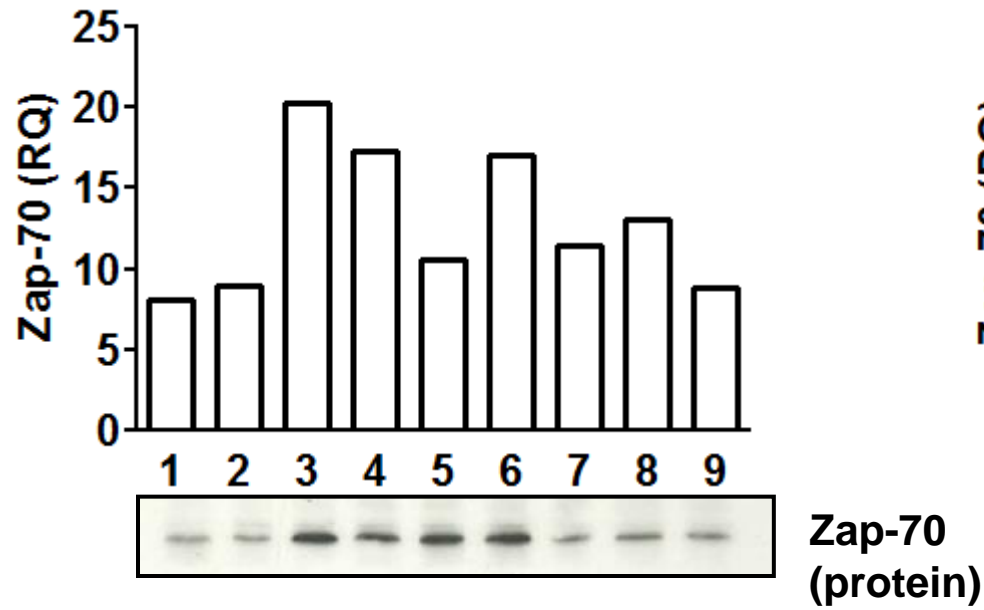
Supplementary Figure 4: Correlation between Zap-70 expression levels in T-ALL patients (A), CCR7 (B) and CXCR4 (C) expression levels in BCP-ALL patients and CNS group (CNS neg./no rel., CNS pos./no rel., CNS neg./rel., CNS pos./rel.). Further definitions are provided in Supplementary Table 2.

Supplementary Table 1: Basic characteristics of 10 t(1;19) positive BCP-ALL patients injected into NSG mice. Zap-70 mRNA levels are shown in reference to expression levels in 697 cells. Further definitions of patient CNS status are provided in Supplementary Table 2.

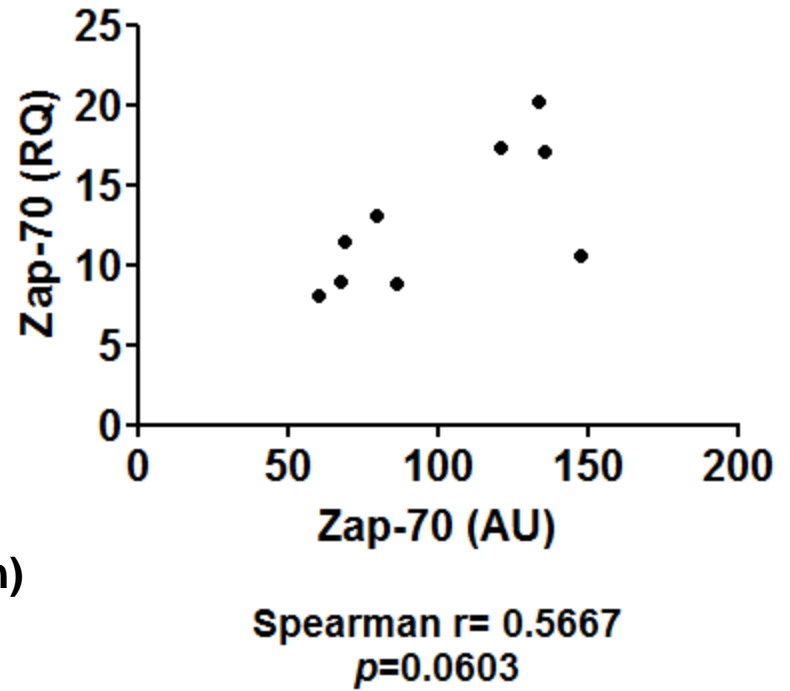
Supplementary Table 2: Characteristics of 130 BCP-ALL and 117 T-ALL pediatric patients at initial diagnosis.

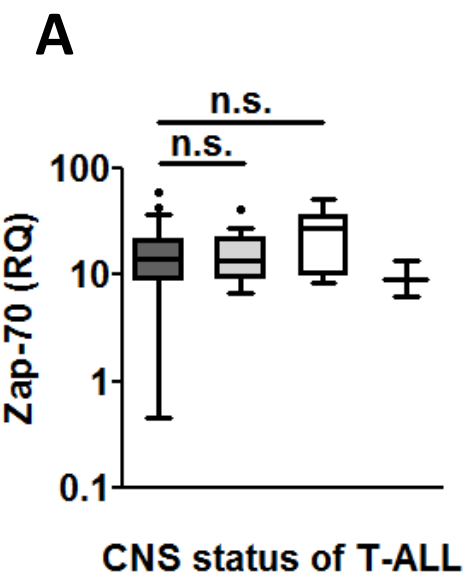
**A****B****C**



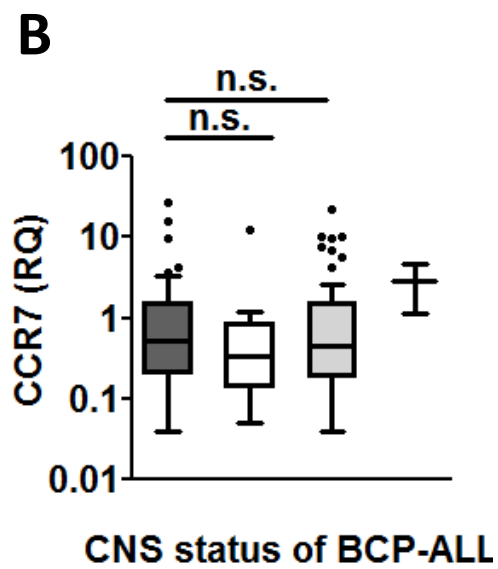


**Correlation Zap-70(protein)/Zap-70(mRNA)**

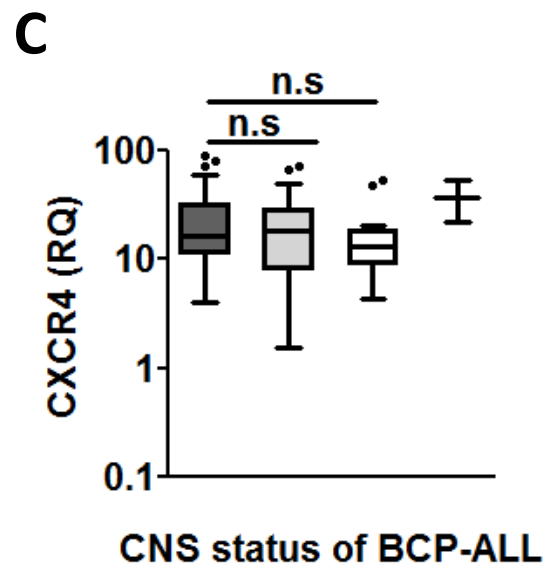




■ CNS neg.  
 ■ CNS pos.  
 □ CNS neg. rel.  
 ■ CNS pos. rel.



■ CNS neg.  
 ■ CNS pos.  
 □ CNS neg. rel.  
 ■ CNS pos. rel.



■ CNS neg.  
 ■ CNS pos.  
 □ CNS neg. rel.  
 ■ CNS pos. rel.

**Supplementary Table 1. Basic characteristics of the 10 t(1;19) positive patients injected into NSG mice to measure CNS infiltration.**

Patient	Age (y)	Sex	WBC	Patient CNS <sup>1</sup>	MRD Risk <sup>1</sup>	PR <sup>2</sup>	Zap-70 <sup>3</sup>	Zap-70 group	Engraftment <sup>4</sup>	% BM blasts	Survival (days)	Xenograft CNS <sup>5</sup>
1	4	F	≥100.000	3b	2	g	12.05	Hi	1, 1	80, N/A	71, 79	3,2
2	10.3	M	≥100.000	2a	1	g	10.56	Hi	3, 2	97, 94	103, 79	3,3
3	14.6	M	≥10.000 <50.000	1	1	g	10.03	Hi	1, 2	80, 95	94, 104	3,2
4	4	F	≥100.000	2b	2	g	7.26	Hi	2, 3	95, 86	71, 72	1,1
5	8.3	F	≥10.000 <50.000	2b	1	g	10.7	Hi	68, 46	100, 99	44, 55	1,3
6	12.4	F	≥100.000	3c	3	p	2.31	Lo	1, 1	96, N/A	76, 224	1,1
7	14.5	F	≥10.000 <50.000	1	2	g	2.84	Lo	20, 19	93, 85	49, 49	1,1
8	3.7	M	≥100.000	3b	2	g	6.48	Lo	1, 3	87, 99	71, 72	1,2
9	11.3	M	≥10.000 <50.000	3c	1	g	4.31	Lo	1, 3	91, 100	76, 76	1,1
10	12.8	F	≥10.000 <50.000	1	1	g	4.05	Lo	21, 23	99, 99	45, 44	1,1

<sup>1</sup>Definitions of patient CNS status and MRD risk groups are described in Supplementary Table 2.

<sup>2</sup>PR: Prednison response; g: good (less than 1000 leukemic blasts/ $\mu$ l blood on treatment day 8); p: poor (more than 1000/ $\mu$ l on day 8)

<sup>3</sup>Zap-70 mRNA expression levels were normalized to 697 cells

<sup>4</sup>Percentage of blasts in the peripheral blood 6 weeks after the injection

<sup>5</sup>CNS status in xenografted animals as shown in Figure 1F and defined in Materials and Methods

N/A Data not available

WBC: White blood cell count at initial diagnosis

Supplementary Table 2: Characteristics of 130 BCP-ALL and 117 T-ALL patients at initial diagnosis.

	<u>CNS neg./no rel. *</u>		<u>CNS pos./no rel. *</u>		<u>CNS neg./rel.*</u>		<u>CNS pos./rel.*</u>		<u>Statistics<sup>†</sup></u>	
	No.	%	No.	%	No.	%	No.	%	<i>P</i> <sup>1</sup>	<i>P</i> <sup>2</sup>
<b>BCP-ALL</b>	<b>64</b>	<b>100</b>	<b>46</b>	<b>100</b>	<b>18</b>	<b>100</b>	<b>2</b>	<b>100</b>		
<b>Sex</b>									0.5642	0.2823
Male	36	56.3	23	50	13	72.2	1	50.0		
Female	28	43.7	23	50	5	27.8	1	50.0		
<b>Age, years</b>									0.8263	0.9999
< 10 years	47	73.4	32	69.6	13	72.2	2	100.0		
≥ 10 years	17	26.4	14	30.4	5	27.8	0	0		
<b>WBC count</b>									0.9773	0.7683
< 10 000/μl	14	21.9	9	19.6	5	27.8	0	0		
≥10,000,<50,000/μl	26	40.6	18	39.1	5	27.8	0	0		
≥50,000,<100,000/μl	10	15.6	7	15.2	3	16.6	0	0		
≥ 100,000	14	21.9	12	26.1	5	27.8	2	100.0		
<b>Risk group<sup>‡</sup></b>									0.3913	0.0035
SR	25	39.1	13	29.5	2	11.1	0	0		
IR	18	28.1	18	41	13	72.2	0	0		
HR	21	32.8	13	29.5	3	16.7	1	100.0		
<b>Prednisone Response<sup>**</sup></b>									0.8108	0.4237
Good	51	81.0	36	78.3	16	94.1	0	0		
Poor	12	19.0	10	21.7	1	5.9	1	100.0		
<b>Cytogenetics</b>									0.8397	0.2200
BCR-ABL	1	1.5	2	4.3	2	11.1	0	0		
TEL-AML1	6	9.4	5	10.9	3	16.7	0	0		
MLL-rearranged	2	3.1	2	4.3	0	0	0	0		
E2A-PBX1	3	4.7	1	2.2	0	0	0	0		
Others/None	52	81.3	36	78.3	13	72.2	2	100		



<b>T-ALL</b>	<b>84</b>	<b>100</b>	<b>24</b>	<b>100</b>	<b>6</b>	<b>100</b>	<b>3</b>	<b>100</b>		
<b>Sex</b>									1.0000	1.0000
Male	61	72.6	17	70.8	5	83.3	2	33.3		
Female	23	27.4	7	29.2	1	16.7	1	66.7		
<b>Age, years</b>									0.1625	0.3935
< 10 years	50	59.5	10	41.7	2	33.3	1	33.3		
≥ 10 years	34	40.5	14	58.3	4	33.7	2	66.7		
<b>WBC count</b>									0.0179	0.4661
< 10 000/μl	5	6.0	3	12.5	1	16.7	0	0		
10,000<50,000/μl	20	23.8	3	12.5	1	16.7	0	0		
50,000< 100,000/μl	16	19.0	0	0	0	0	3	100.0		
≥ 100,000	43	51.2	18	75.0	4	66.6	0	0		
<b>Risk group<sup>‡</sup></b>									0.8408	0.2831
SR	9	10.7	2	8.3	0	0	0	0		
IR	37	44.0	8	33.3	1	16.7	1	33.3		
HR	38	45.2	14	58.3	5	83.3	2	66.7		
<b>Prednisone Response**</b>									0.3493	0.2127
Good	52	61.9	12	50.0	2	33.3	1	33.3		
Poor	32	38.1	12	50.0	4	66.7	2	66.7		

SR – standard risk, IR – intermediate risk, HR – high risk

WBC – white blood cell count in the peripheral blood at initial diagnosis

RBC – red blood cells

**\*CNS status is defined as follows:**

**CNS neg.:**

CNS1: neither clinical nor radiological signs of CNS involvement AND no blasts in the cerebrospinal fluid (CSF) cytopsin.

CNS2: neither clinical nor radiological signs of CNS involvement AND CNS2a: <10 per microliter RBC and no macroscopic blood; ≤ 5 per microliter WBC; positive blasts in cytopsin.

CNS2b: macroscopic blood and/or ≥ 10 per microliter RBC; ≤ 5 per microliter WBC; positive blasts in cytopsin.

CNS2c: macroscopic blood and/or  $\geq 10$  per microliter RBC;  $>5$  per microliter WBC; positive blasts in cytospin; negative according to algorithm  $(WBC_L/RBC_L)/(WBC_B/RBC_B) >2$ .

**CNS pos.:**

CNS3-CNS3a:  $<10$  per microliter RBC and no macroscopic blood;  $>5$  per microliter WBC; positive blasts in cytospin.

CNS3b: macroscopic blood and/or  $\geq 10$  per microliter RBC;  $>5$  per microliter WBC; positive according to algorithm  $(WBC_L/RBC_L)/(WBC_B/RBC_B) >2$ .

CNS3c: clinical signs of CNS involvement, radiologically detectable cerebral lesion, retinal infiltrations.

**CNS neg./rel.:** patients CNS neg. at initial diagnosis but with subsequent CNS relapse.

**CNS pos./rel.:** patients CNS pos. at initial diagnosis but with subsequent CNS relapse.

<sup>†</sup>Fisher's exact test, 2-sided p-value; For "Cytogenetics", a Chi-squared test was performed, 2-sided p-value; P<sup>1</sup>: Comparison between CNS pos./no rel. and CNS neg./no rel., P<sup>2</sup>: Comparison between CNS neg./rel. and CNS neg./no rel.

<sup>‡</sup>Risk stratification according to minimal residual disease (MRD) risk groups: MRD-SR: TP1+2 negative, MRD-IR: TP1 and/or TP2  $<10^{-3}$ , MRD-HR: TP2  $\geq 10^{-3}$ . In the BCP-ALL cohort, the MRD risk group was missing for 2 patients in the CNS pos./no rel. group and in 1 patient in the CNS pos./rel. group. Prednisone poor responders were stratified into the HR treatment group.

**\*\***Prednisone response was missing for 3 patients in the BCP-ALL cohort: 1 in the CNS neg./no rel., 1 in the CNS neg./rel. and 1 in the CNS pos./rel. groups.