Supplementary Data

ALL06 Study Protocol version 5.0 (09 June 2017)

To be included in supplementary data

Table S1. Grade 3 and 4 Adverse Events in Protocol I

Achieved Protocol M/HR by Day 94 (n=34)				Did not Achieve Protocol M/HR by Day 94 (n=48)			
	Grade				Grade		
Toxicity	**3**	**4**	**Total (n=)**	**Toxicity**	**3**	**4**	**Total (n=)**
Acute kidney injury	1		1	*Acute kidney injury*			
Alanine aminotransferase increased	2		2	*Alanine aminotransferase increased*	5		5
Alkaline phosphatase increased	2		2	*Alkaline phosphatase increased*			
Anemia	31	1	32	*Anemia*	13	1	14
Anorexia				*Anorexia*	1		1
Arachnoiditis	1		1	*Arachnoiditis*			
Aspartate aminotransferase increased	2		2	*Aspartate aminotransferase increased*	1		1
Atrial fibrillation	1		1	*Atrial fibrillation*			
Blood bilirubin increased	1		1	*Blood bilirubin increased*	4	1	5
Colitis	2		2	*Colitis*			
Constipation				*Constipation*	1		1
Depression				*Depression*	1		1
Dystonia				*Dystonia*	1		1
Fatigue	2		2	*Fatigue*	1		1
Febrile neutropenia	18		18	*Febrile neutropenia*	29	1	30
Fever	4		4	*Fever*	1		1
Fibrinogen decreased	3		3	*Fibrinogen decreased*	2		2
Gastric perforation		1	1	*Gastric perforation*			
GGT increased	2		2	*GGT increased*	4		4
Headache	5		5	*Headache*	2		2
Hematoma				*Hematoma*	1		1
Hepatic failure				*Hepatic failure*		1	1

Hepatobiliary disorders - Other	2		2	*Hepatobiliary disorders - Other*	4		4
Hyperammonemia				*Hyperammonemia*		1	1
Hyperglycemia	2		2	*Hyperglycemia*	3		3
Hypertension	1		1	*Hypertension*	1		1
Hypertriglyceridemia				*Hypertriglyceridemia*	1		1
Hypoalbuminemia	1		1	*Hypoalbuminemia*			
Hypokalemia				*Hypokalemia*	1		1
Hyponatremia	2		2	*Hyponatremia*	2		2
Hypotension	1		1	*Hypotension*			
Infections and infestations	8		8	*Infections and infestations*	19	2	21
Lower gastrointestinal hemorrhage				*Lower gastrointestinal hemorrhage*		1	1
Mucositis oral	1		1	*Mucositis oral*	2		2
Musculoskeletal and connective tissue disorder	4		4	*Musculoskeletal and connective tissue disorder*	3		3
Nausea	2		2	*Nausea*	3		3
Nervous system disorders - Other				*Nervous system disorders - Other*	1		1
Neutrophil count decreased	13	24	37	*Neutrophil count decreased*	9	25	34
Oral pain	1		1	*Oral pain*			
Pain	1		1	*Pain*			
Pancreatitis				*Pancreatitis*		1	1
Platelet count decreased	12	19	31	*Platelet count decreased*	18	22	40
Respiratory failure				*Respiratory failure*		2	2
Seizure				*Seizure*		1	1
Sepsis				*Sepsis*		1	1
Sinus tachycardia	1		1	*Sinus tachycardia*			
Thromboembolic event	2		2	*Thromboembolic event*	1	3	4
Urinary retention	1		1	*Urinary retention*			
Urticaria	1		1	*Urticaria*			
Vomiting	2		2	*Vomiting*	2		2
Weight gain	1		1	*Weight gain*			

Weight loss	1		1	*Weight loss*	1		1
White blood cell decreased	2	1	3	*White blood cell decreased*	3	2	5
Total	**139**	**46**	**185**	**Total**	**141**	**65**	**206**

Table S2. Selected Post-Hoc Adverse Event Analysis

	Achieved Protocol M/HR1 by Day 94 (n=34)		Not Achieved Protocol M/HR1 by Day 94 (n=48)		P-values	
Toxicity	**Events (n=)**	**Patients (n=, %)**	**Events (n=)**	**Patients (n=, %)**	**Events**	**Patients**
All Grades	185	32 (94)	206	41 (85)	0.019	0.141
Grade 4 only	46	19 (56)	65	31 (65)	0.996	0.133
Hepatic*	11	6 (18)	19	11 (23)	0.499	0.160
Asparaginase-related**	16	12 (35)	30	18 (38)	0.302	0.170
Grade 4 neutropenia	24	17 (50)	26	19 (40)	0.348	0.116
Anemia	32	16 (47)	14	11 (23)	<0.001	0.014
Grade 4 non-hematologic***	2	2 (6)	15	12 (25)	0.013	0.018

*Hepatic toxicity defined as alanine aminotransferase increased, alkaline phosphatase increased, blood bilirubin increased, GGT increased, hepatic failure, hepatobiliary disorders – other.

**Asparaginase-related toxicity defined as alanine aminotransferase increased, alkaline phosphatase increased, blood bilirubin increased, fibrinogen decreased, GGT increased, hepatic failure, hepatobiliary disorders – other, hyperammonemia, hyperglycemia, hypertriglyceridemia, pancreatitis, thromboembolic event

***Grade 4 non-hematologic toxicity defined as all grade 4 events that did not include anemia, neutrophil count decreased, platelet count decreased, white blood cell decreased.

Table S3. Factors Associated with Day 79 MRD Negativity

Day 79 MRD Status		Negative (N, %)		Positive (N, %)		*p*-value	OR* (for +)	95%CI
Sex	Female	10	55.6%	8	44.4%	0.76	0.85	0.29-2.50
	Male	31	59.6%	21	40.4%			
Age	≤Median	20	55.6%	16	44.4%	0.598	0.77	0.30-2.01
	>Median	21	61.8%	13	38.2%			
Phenotype	B	28	54.9%	23	45.1%	0.115	0.37	0.11-1.31
	T	13	76.5%	4	23.5%			
Time to M/HR1	≤Median	25	61.0%	16	39.0%	0.627	1.27	0.48-3.33
	>Median	16	55.2%	13	44.8%			

*Odds ratio for a positive MRD result at day 79

Table S4. Assessment of MRD Response through HR Therapy

	Mean Change in Absolute MRD Value Relative to Day 79 Result*		
HR Block	1	2	3
No Relapse/Alive	**-** 1.58×10^{-3}	**-** 1.2×10^{-3}	**-** 1.2×10^{-3}
Relapse/Died	**-** 1.72×10^{-3}	**+** 9.5×10^{-4}	**+** 6.4×10^{-3}
***p*-value**	0.929	0.032	0.038

* (-) indicates an absolute fall in MRD value, (+) indicates an absolute increase in MRD value

Table S5. Survival Outcomes Following SCT

Time	**Transplant**	**DFS** (*p*=0.763)		**OS** (*p*=0.876)	
		%	95%CI	%	95%CI
1 year	No	84.7	75.6-93.9	85.5	76.7-94.3
	Yes	95.0	85.4-104.6	100.0	100.0-100.0
3 years	No	69.5	57.1-81.8	75.0	64.0-86.0
	Yes	75.0	56.0-94.0	75.0	56.0-9402

Figure S1. ALL06 Treatment Protocol Summary

Standard/Medium Risk Protocol

Prephase

- Prednisone PO/IV 60mg/m^2/day in two divided doses days 1-7
- IT MTX 12mg day 1

Protocol I: Induction
- Vincristine IV 1.5mg/m^2 (max 2 mg) days 8, 16, 22, 29
- Prednisone PO/IV 60mg/day/m^2 in three divided dose days 8-28 then tapered
- Daunorubicin IV 30mg/m^2 days 8, 15, 22, 29
- Pegylated asparaginase IM/IV 1000 IU/m^2 days 8, 22
- IT MTX 12mg days 15, 33[(a)]

Protocol I: Consolidation
- Cyclophosphamide IV 1000mg/m^2 days 36, 64
- Mercaptopurine PO 60mg/m^2/day, days 36-63
- Cytarabine IV/SC 75mg/m^2 days 38-41, 45-48, 52-55, 59-62
- IT MTX 12mg days 43, 57

Protocol M
- Mercaptopurine PO 25mg/m^2/day PO days 1-56.
- High dose MTX IV 5g/m^2 as a continuous infusion over 24 hours, days 8, 22, 36, 50 with leucovorin rescue

Protocol II: Induction[(b)]
- Dexamethasone PO 10mg/m^2/day in 2-3 divided doses, days 1-21 then tapered.
- Vincristine IV 1.5mg/m^2 (max 2 mg) days 8, 15, 22, 29
- Doxorubicin IV 25mg/m^2 days 8, 15, 22, 29
- Pegylated asparaginase IM/IV 1000iU/m2 day 1

Protocol II: Consolidation
- Thioguanine PO 60mg/m^2/day, days 36-49
- Cyclophosphamide IV 1000mg/m2 day 36
- Cytarabine IV/SC 75mg/m^2 days 38-41, 45-47

Maintenance [(c)]
- Mercaptopurine PO 50mg/m^2 Daily
- MTX 20mg/m^2/weekly

High Risk Protocol

HR Block 1[(d)]
- Dexamethasone PO/IV 20mg/m^2/day, days 1-5
- Vincristine IV 1.5mg/m^2 (max 2 mg) days 1, 6
- Cytarabine IV 2000mg/m^2 twice daily day 5
- High dose MTX IV 5000mg/m^2 as continuous infusion day 1 with leucovorin rescue
- Cyclophosphamide IV 200mg/m^2 twice daily on days 2-4
- Pegylated asparaginase 1000iU/m^2 on day 6
- IT MTX 12mg/cytarabine 30mg/hydrocortisone 50mg on day 1

HR Block 2
- Dexamethasone PO/IV 20mg/m^2/day, days 1-5
- Vindesine IV 3mg/m^2/day (max dose 5mg) days 1,6[(e)]
- Daunorubicin IV 30mg/m^2 day 5
- High dose MTX IV 5g/m^2 as continuous infusion given on day 1 with leucovorin rescue
- Ifosfamide IV 800mg/m^2 twice daily on days 2-4
- Pegylated asparaginase IV/IM 1000iu/m^2 on day 6
- IT MTX 12mg/cytarabine 30mg/hydrocortisone 50mg on day 1

HR Block 3
- Dexamethasone PO/IV 20mg/m^2/day, days 1-5
- Cytarabine IV 2000mg/m^2 twice daily on days 1,2
- Etoposide IV 100mg/m^2 twice daily on days 3-5
- Pegylated asparaginase IV/IM 1000iU/m^2 on day 6
- IT MTX 12mg/cytarabine 30mg/hydrocortisone 50mg on day 1

PO, oral; IV, intravenous; IT, intrathecal; SC, subcutaneous; MTX, methotrexate

(a) In case of CNS involvement additional IT MTX was given on days 18, 27

(b) Cranial irradiation (18Gy) was considered for initial CNS involvement, HR/VHR patients not proceeding to SCT and T-ALL with presenting WCC >100 x 10^9/L

(c) To a total of 2 years of therapy

(d) MHR/HR/VHR patients received a minimum of HR1 and 2 if MRD^{neg} following HR1 before proceeding to Protocol II or SCT if suitable donor available

(e) Vincristine IV (1.5mg/m2 to a max of 2mg) was substituted if drug was unavailable

Figure S2. Impact of Risk Group and Phenotype on Outcomes

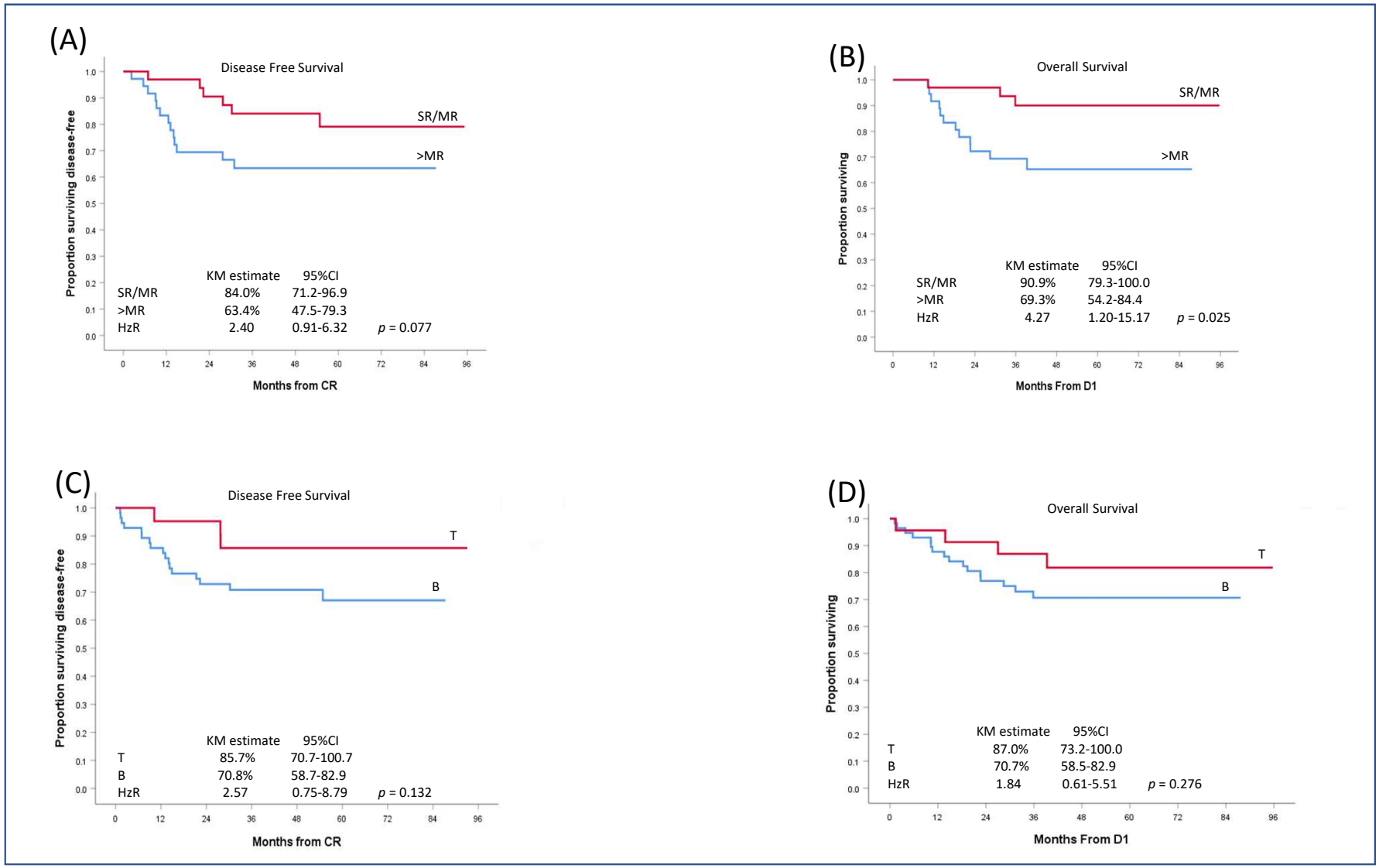
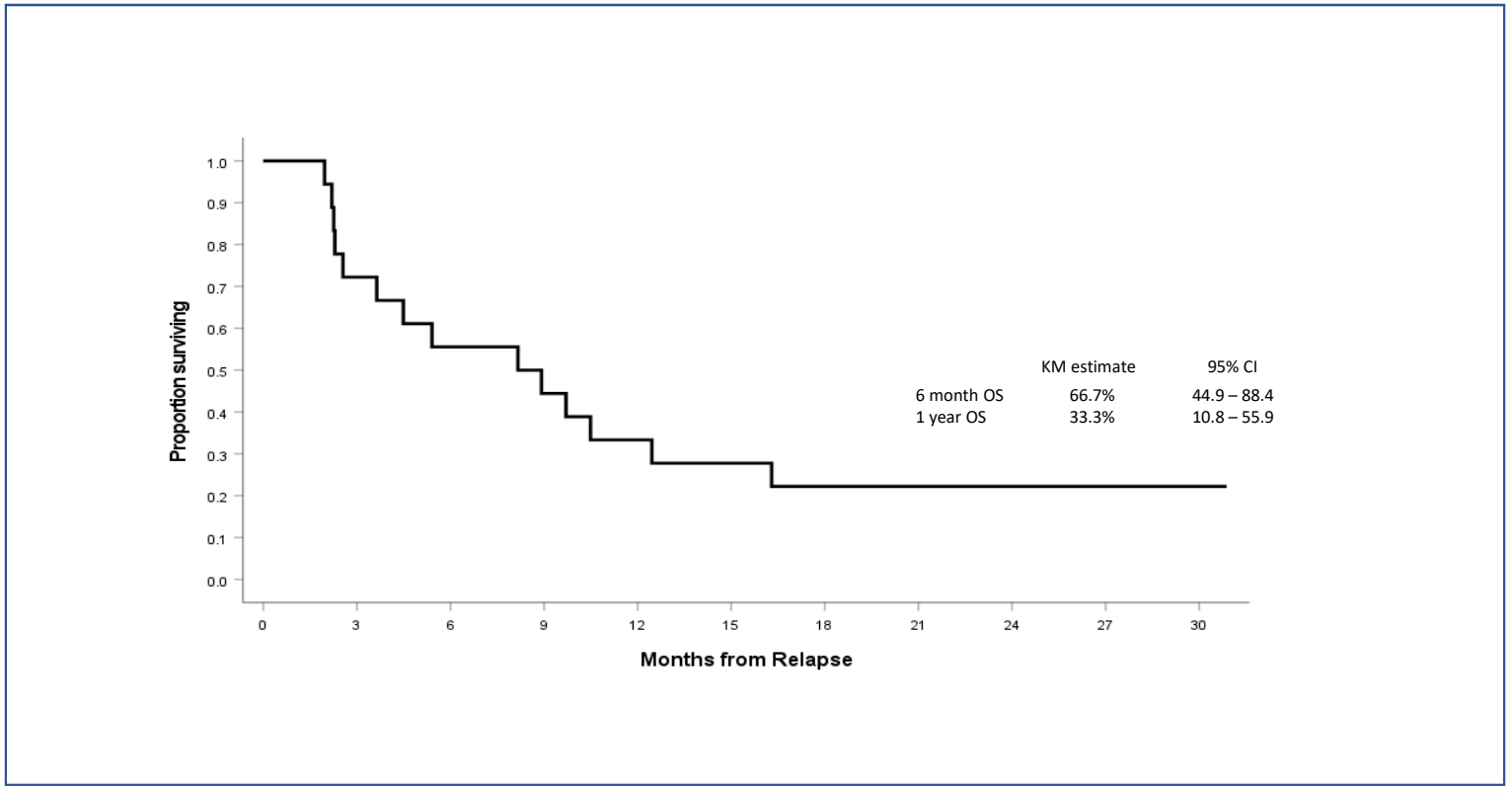


Figure S3. Overall Survival Following Relapse



Supplementary Figure Legends

Figure S1. **ALL06 Treatment Protocol Summary.** Standard/Medium Risk and High Risk protocols are shown. Patients considered MHR/HR or VHR proceeded to a minimum of HR1 and 2 if MRD^{neg} following HR1 before proceeding to SCT if a suitable donor was available or to Protocol II. MHR patients who were MRD positive after HR2 proceeded to HR3 with a further MRD sample collected on recovery prior to proceeding to HR4. If MRD^{neg} after HR3, MHR patients proceeded to Protocol II. MHR patients who remained MRD positive were offered SCT if a suitable donor was identified or completed HR4-6 if no donor was available before proceeding to Protocol II. HR/VHR patients who were MRD positive after HR3 were offered SCT if a suitable donor could be identified or completed HR4-6 before proceeding to Protocol II.

Figure S2. **Impact of Risk Group and Phenotype on Outcomes.** (A) Risk group higher than MR was associated with 3-year DFS of 63.4% (95% CI, 47.5-79.3) versus 84.0% (95%CI, 71.2-96.9) in the SR/MR cohort (HzR 2.40, p=0.077) and (B) 3-year OS of 69.3% (95%CI, 54.2-84.4) versus 90.9% (95%CI, 79.3-100.0) in the SR/MR cohort (HzR 4.27, p=0.025). (C) T-cell phenotype was associated with a 3-year DFS of 85.7% (95%CI 70.7-100.7) versus 70.8% for B-cell phenotype (HzR 2.57, p=0.132) and (D) 3-year OS of 87.0% (95%CI, 73.2-100.0) versus 70.7% (95%CI, 58.5-82.9) in B-cell phenotype (HzR 1.84, p=0.276)

Figure S3. Overall Survival Following Relapse. Kaplan-Meier (KM) estimate of overall survival following relapse. Estimated 6 month overall survival 66.7% (95%CI, 44.9-88.4) and 1 year overall survival 33.3% (95%CI, 10.8-55.9). As of last follow up, 22% of relapsed patients remain alive.