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Clinical and germline risk factors for multiple treatment-related toxicities in pediatric acute lymphoblastic leukemia

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Running Heads: Multiple treatment related toxicities during ALL therapy

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Treatment-related toxicity causes morbidity in acute lymphoblastic leukaemia (ALL). A minority of patients suffer multiple treatment-related toxicities (mTRT). We characterised the incidence and risk factors for ALL mTRTs in 1240 patients between 1998-2013. The mTRT incidence was 10·7% with the most common mTRT combination being bone and neurotoxicity in 40%. There was no difference in leukemia-free (LFS), event-free (EFS), or overall survival (OS) following mTRT. Five clinical/laboratory factors (older age (≥10 years), female gender, high-risk leukemia, low albumin and elevated gamma glutamyl transferase (GGT) during induction therapy) and one germline *MUC16* single nucleotide polymorphism (SNP) (rs78342591, $P=2{\cdot}24x10^{-8}$) were associated with mTRT risk.

The burden of TRTs can be devastating for patients and clinicians. The occurrence of mTRTs has not been well studied but can impair chemotherapy delivery and may be associated with an increased relapse risk. It is unknown what predisposes individuals to mTRTs. Possible susceptibilities include organ dysfunction, delayed drug excretion, drug-drug interactions, genetic predisposition, constitutional syndromes and physiological factors such as age or gender. mTRT is likely exacerbated by intensive ALL therapy. Genome wide association studies (GWAS) have identified germline risk factors associated with TRT but have focused on individual TRT. In ERASE (Evaluation of Risk of ALL Treatment-related Side-Effects), we undertook a retrospective study of Australian paediatric ALL patients diagnosed between 1998-2013, including annotation of treatment, survival, TRTs and a germline GWAS (Supplementary Tables 1&2). This analysis focused on mTRTs, their impact on survival, and identifying clinical and germline factors associated with mTRT risk. The ERASE study including the GWAS have been published[1, 2]. mTRT was defined as experiencing ≥2 TRTs and controls as 0 or 1 documented TRT and who were followed for ≥18 months from diagnosis. The mTRT phenotype included bone (osteonecrosis or fractures), central or peripheral neurotoxicity, symptomatic VTE and insulin requirement. The mTRT GWAS cohort included 707 individuals, with 5 excluded due to lack of mTRT information, leaving 64 mTRT cases and 638 controls. The number of directly genotyped and imputed SNPs with a minor allele frequency (MAF)>0·05% was 10999498 and with a MAF>2% was 7780980.

The median age was 59 months (range 9-218 months) with a median follow-up of 78 months (range 3-186 months). The five-year OS, EFS and LFS of the ERASE cohort was 92% ±0·8%, 83·8% ±1·1% and 85·6% ±1·1%. mTRTs occurred in 133/1240 (10·7%) with the majority being CTCAE grade ≥2 severity (123/1240, 9·9%). The incidence of individual TRTs included neurotoxicity in 7·6% (94/1240), insulin requirement in 6·9% (85/1240), bone toxicity in 6·0% (75/1240) and VTE in 5·5% (68/1240) (Table 1). Bone and neurotoxicity was the most frequent combined mTRT. There was no difference in LFS, EFS or OS in mTRT patients (n=133), compared to controls (n=1107). The 5-year LFS was 88·8±2·9% (mTRT), versus 85·9±1·1% (control, P=0·276), 5-year EFS was 84·9±3·3% (mTRT) versus 84·2±1·2% (control, P=0·595) and 5-year OS was 89·1±2·9% (mTRT) versus 92·5±0·9% (no control, P=0·138) (Supplementary Figure 1).

Risk factors for mTRT were assessed using univariable and multivariable logistic regression analyses. Twenty six of 38 factors were significant in univariable analysis. Univariable associations with the mTRT phenotype included factors present at diagnosis and treatment during the early dose-intensive phases of chemotherapy (Table 2). Eighteen variables were carried into multivariable regression and 5 were independently associated with mTRT: age ≥10 years, female gender, high-risk ALL treatment, low serum albumin (<20g/L during induction/consolidation), elevated GGT (>5 x upper limit of normal during induction/consolidation) (Table 2).

The GWAS identified 28 candidate SNPs (P <$5x10^{-6}$), mapping to 8 genes including *MUC16, SMYD3, FAM155A, UQCRFS1, FMO1, PIGF, LOC105371611, LOC105372352* (Table 3). Most candidate SNPs (20/28) were associated with a reduced odds ratio of mTRT. Three SNPs, associated with increased mTRT risk fell within *MUC16* introns (rs78342591, rs62118276 and rs2341321). One reaching genome wide significance (rs78342591, P=$2·24x10^{-8}$) (Table 3). Four SNPs in *SMYD3* were associated with increased mTRT risk (Table 3). The *MUC16* rs78342591 risk allele (C) was examined with 640 individuals with informative data. Individuals with at least 1 rs78342591 risk allele C accounted for 17/64 (26·6%) of the GWAS cohort of children affected by mTRT. Four individuals were homozygous for the risk allele, with 50% (2/4) experiencing mTRT. Seventy-three individuals

were heterozygous for the risk allele (CT), with 20·5% (15/73) experiencing mTRT. In contrast, 563 patients were homozygous for the non-risk allele (TT) with 8·3% (47/563) experiencing mTRT. Splicing analysis using Introme predicted the introduction of a polypyrimidine tract-binding protein (PTB) binding site from the rs62118276 SNP.

The ERASE study collected mTRT data across 2 major ALL treatment platforms, creating an opportunity to undertake the first study of clinical and genetic risk factors for mTRT in pediatric ALL. At least 10% of ALL patients experienced mTRT, but mTRTs did not impact on ALL survival, a finding, whilst counterintuitive, aligns with the observation of Yeoh and co-workers who did not observe an increase in relapse risk in ALL patients experiencing treatment delay during the intensive phase of ALL therapy.[3]

The strongest independent risk factor for mTRT was older age (≥10 years), which is a risk factor for VTE, osteonecrosis, fractures, methotrexate neurotoxicity, vincristine-induced neuropathy and insulin requirement.[2, 4-8] The association between high-risk ALL and mTRT is likely correlated with dose intensity and/or cumulative chemotherapy dosing. Female gender was an independent significant risk factor for mTRT, but female gender has not consistently been identified as a TRT risk factor across different studies.[4, 5, 9] There was an association between hypoalbuminaemia and mTRT, independent of risk group and age, pointing to a link between therapy intensity and serum albumin, as low serum albumin often occurs during severe illness. Hypoalbuminemia is a likely consequence of treatment with asparaginase, malnourishment and/or underlying disease severity. A tentative association between albumin and osteonecrosis has been reported.[5] Hypoalbuminemia has been associated with delayed methotrexate clearance.[10] Treatment-related GGT elevation was associated with mTRT. Elevated GGT has been identified as a risk factor for symptomatic VTE[11] as well as decreased survival in multiple cancers including breast, ovarian, endometrial and melanoma treated with checkpoint inhibitors.

The mTRT GWAS identified 28 SNPs mapping to 8 genes with P values $<5x10^{-6}$. Six loci were associated with a reduced mTRT risk and two with increased mTRT risk. One SNP, rs78342591, reached genome-wide significance. *MUC16* encodes a large transmembrane, mucinous, glycoprotein normally found on bronchial, endometrial, ovarian and corneal epithelia.[12] Multiple *MUC16* functions have been identified including as an anti-microbial barrier, providing immune-protection from the innate immune system, enhancing metastasis and cancer cell proliferation and when knocked down promoting apoptosis and cell cycle arrest.[12] All *MUC16* SNPs identified in the GWAS are intronic, raising the hypothesis that the SNPs might influence *MUC16* splicing. Although Introme analysis did not link these SNPs with a high probability splicing change[13], the rs62118276 SNP is predicted to introduce a polypyrimidine tract-binding protein (PTB) binding site. PTB regulates alternative splicing by exon inclusion/exclusion. Within the Expression Atlas, *MUC16* is expressed within the liver and kidney but not within bone, postnatal brain, nerve cells, vascular endothelium, or haematopoietic cells. The role of *MUC16* in mTRT remains to be clarified. We hypothesise that dysregulated hepatic and renal *MUC16* expression following cytotoxic chemotherapy exposure results in dysregulated local cytokine production and inflammation increasing the risk of treatment-related toxicity.

This study has several limitations arising from the retrospective design and findings will require validation. Following the ERASE study, we are collecting data on two additional Australian ALL cohorts, one retrospective and one prospective, to replicate these findings. Data were collected by retrospective chart review, a resource- and time-intensive methodology which limits data collection to information easily and reproducibly documented in the medical record. With electronic medical records, automatically extracting adverse events in ALL patients has been demonstrated by the Children's Oncology Group[14], suggesting that automated collection of relevant TRTs in the future is feasible. There are substantial differences between the risk stratification and treatment algorithms used in different ALL treatment platforms. The data analysis was as recorded by the treating clinician and centre based on the local risk stratification and treatment allocation without adjusting for differences between protocols (Supplementary Table 2). Toxicities analysed in ERASE reflect those

occurring at a reasonable frequency (≈5%) and consistently captured. Replication followed by functional validation of the *MUC16* SNP on chemo-toxicity may provide clearer evidence regarding the mechanism of ALL mTRT.

Although blinatumomab is effective and well tolerated in patients with B-ALL[15], most ALL treatment platforms are adding blinatumomab to existing chemotherapy rather than substituting chemotherapy with blinatumomab suggesting that TRTs from conventional chemotherapy will continue to be a problem for the foreseeable future. Functional validation and biomarker studies may provide tools for early diagnosis and intervention in children who are at high-risk of mTRTs. This study provides clinically relevant information that can be used for counselling ALL patients and their families, regarding factors that may lead to increased risk of mTRT. Adolescent girls diagnosed with high-risk ALL have the highest chance of mTRT. Through improved understanding of clinical and germline factors associated with mTRT risk, it may be possible to devise strategies to reduce mTRTs.

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Table 1. Incidence of individual toxicities & combinations of multiple toxicities observed in the ERASE cohort (n= 133)		
Incidence of individual treatment related toxicity		
	Number affected	**% of mTRT cohort**
Neuropathy	94	70·7
Insulin requirement	85	63·9
Bone toxicity	75	56·4
Venous thromboembolism	68	51·1
Combinations of multiple treatment related toxicities		
	Number affected	**% of mTRT cohort**
Bone + neurotoxicity	53	39·8
Neurotoxicity + insulin requirement	22	16·5
VTE + neurotoxicity	13	9·8
Bone + neurotoxicity + insulin requirement	13	9·8
VTE + bone toxicity	12	9·0
Bone toxicity + insulin requirement	11	8·3
VTE + bone + neurotoxicity	5	3·8
VTE + insulin requirement	1	0·8
VTE + bone toxicity + insulin requirement	1	0·8
VTE + neurotoxicity + insulin requirement	1	0·8
VTE + bone + neurotoxicity + insulin requirement	1	0·8
Abbreviations: mTRT: multiple treatment related toxicities; VTE: venous thromboembolism		

Table 2. Univariable and multivariable analysis of risk factors associated with multiple treatment related toxicities.

Variable	Univariable			Multivariable		
	P	**OR**	**95% CI**	***P***	**OR**	**95% CI**
Sex (female)	0·345			0·029	1·80	1·06-3·04
Treatment Platform (BFM* vs COG)	<0·001	2·12	1·4-3·19			
T-immunophenotype	0·012	1·83	1·14-2·95			
Age ≥10 years	<0·001	6·84	4·68-10·0	<0·001	3·91	2·26-6·73
WCC at diagnosis	0·026	1·002	1·000-1·003			
CNS3 at diagnosis	0·005	3·29	1·43-7·59			
High-risk group (HR/VHR)[a]	<0·001	4·36	3·0-6·34	<0·001	2·87	1·59-5·16
Peak urate[b]	0·002	3·42	1·59-7·35			
Tumour lysis[b]	<0·001	2·86	1·74-4·70			
Bilirubin at diagnosis	<0·001	1·04	1·02-1·06			
GGT at diagnosis	<0·001	1·01	1·005-1·013			
Abnormal peak creatinine >2 x baseline[c,d]	0·005	2·70	1·34-5·45			
Peak bilirubin[d]	<0·001	1·01	1·01-1·02			
Peak bilirubin >3 x ULN[d]	<0·001	5·21	2·83-9·58			
Lowest albumin[d]	<0·001	0·9	0·87-0·93			
Lowest serum albumin <20g/L[d]	<0·001	2·66	1·71-4·13	0·026	1·95	1·08-3·52
Peak GGT[d]	<0·001	1·002	1·001-1·002			
Peak GGT >5 x ULN[d]	<0·001	4·98	3·07-8·09	<0·001	3·76	2·14-6·62
Peak ALT[d]	0·006	1·001	1·000-1·001			
Peak ALT >5 x ULN[d]	<0·001	2·04	1·37-3·03			
Confirmed infection[d]	0·002	1·88	1·26-2·81			
Positive blood culture[d]	0·024	1·56	1·06-2·29			
Weight at diagnosis (Z score, CDC)	0·01	1·24	1·05-1·46			
Weight at diagnosis >95th centile	0·009	1·86	1·16-2·97			
BMI at diagnosis >95th centile	0·001	2·34	1·41-3·87			
BMI at diagnosis (Z score, CDC)	0·003	1·26	1·08-1·47			
BMI at end of consolidation (Z score, CDC)	0·038	0·85	0·73-0·99			

Thirty-eight variables were assessed in univariable analysis, relating to baseline diagnostic factors (n=6), treatment response (n=1), biochemical parameters at baseline and during induction/consolidation (n=19), infection during induction/consolidation (n=3), and anthropometric values at diagnosis or during induction/consolidation (weight, body mass index "BMI")(n=9). Univariable and multivariable modelling was conducted with a Bonferroni correction for multiple comparisons so that a P<0·0013 (0·05/38) was considered significant. For risk modelling, individuals with incomplete data were excluded. Categorical variables were assessed using Pearson Chi-squared analysis. Variables with a significance level *P*<0·20 were assessed in multivariable modelling by backward elimination. The multivariable analyses were adjusted for age and sex. The least significant factor was removed at each stage, until all factors in the model were independently significant (2-tailed *P* <0·05) and the model was significant (overall model *P*<0·05, Hosmer-Lemeshow value *P*>0·05)

*BFM reference cohort. [a]High-risk group comprised high risk and very high-risk patients which were compared to the non-high-risk group comprising of standard, medium, average or low risk patients, as defined by their respective protocols. [b]Value during induction. [c]Peak creatinine value as compared to baseline creatinine at diagnosis, or 2 x the upper limit of normal if the presenting creatinine at diagnosis was above the normal range. [d] Values during induction/consolidation. OR, odds ratio; 95 CI, 95% confidence interval; COG, Children's Oncology Group; BFM, Berlin-Frankfurt-Munster; WCC, white cell count; CNS3, i.e., CNS disease; HR, high-risk; VHR, very high-risk; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; BMI, body mass index; CDC, Centres for Disease Surveillance and Control Growth Charts

Table 3. Top single nucleotide polymorphisms (SNPs) associated with mTRT phenotype in the ERASE cohort

Chromosome	Position	SNP	Non effect allele	Effect allele	MAF	*P*	OR	OR 95 CI (lower)	OR 95 CI (upper)	Gene[a]	Location
1	2774381	rs12567869	G	A	0·20	$1{\cdot}34x10^{-6}$	0·14	0·05	0·36	-	-
1	171204809	rs12405613	A	G	0·38	$3{\cdot}21x10^{-6}$	0·36	0·23	0·58	*LOC105371611*	intronic
1	171207408	rs7513485	T	C	0·38	$3{\cdot}40x10^{-6}$	0·36	0·23	0·58	*LOC105371611*	intronic
1	171208014	rs2421710	C	T	0·38	$3{\cdot}45x10^{-6}$	0·36	0·23	0·58	*LOC105371611*	intronic
1	171208094	rs2421711	C	T	0·38	$3{\cdot}46x10^{-6}$	0·36	0·23	0·58	*LOC105371611*	intronic
1	171209900	rs35152982	A	C	0·38	$3{\cdot}72x10^{-6}$	0·36	0·23	0·58	*LOC105371611*	intronic
1	171216717	rs7520777	C	G	0·40	$4{\cdot}51x10^{-6}$	0·37	0·23	0·58	*FMO1*	intronic
1	246355668	rs10924537	T	C	0·29	$1{\cdot}82x10^{-6}$	3·50	1·96	6·23	*SMYD3*	intronic
1	246355795	rs12407828	C	T	0·29	$3{\cdot}05x10^{-6}$	3·39	1·91	6·00	*SMYD3*	intronic
1	246356128	rs2333991	A	G	0·21	$4{\cdot}17x10^{-6}$	4·37	2·10	9·10	*SMYD3*	intronic
1	246356200	rs2333992	G	T	0·29	$3{\cdot}95x10^{-6}$	3·39	1·90	6·04	*SMYD3*	intronic
2	46807991	rs2276554	T	C	0·13	$4{\cdot}60x10^{-6}$	0·13	0·04	0·42	*PIGF*	intronic
4	25062315	rs11723040	T	A	0·27	$4{\cdot}29x10^{-6}$	0·28	0·15	0·51	-	-
5	60919428	rs56300029	C	T	0·28	$4{\cdot}45x10^{-6}$	2·87	1·83	4·50	-	-
7	80894904	rs117511099	G	A	0·02	$1{\cdot}11x10^{-6}$	$4{\cdot}75x10^{-30}$	$8{\cdot}43x10^{-57}$	$2{\cdot}67x10^{-3}$	-	-
7	80905279	rs117698731	C	T	0·02	$1{\cdot}52x10^{-6}$	$2{\cdot}62x10^{-30}$	$1{\cdot}89x10^{-57}$	$3{\cdot}63x10^{-3}$	-	-
13	108289235	rs67586898	ATATAT	A	0·36	$3{\cdot}53x10^{-6}$	0·33	0·20	0·55	*FAM155A*	intronic
14	53685357	rs61986921	C	A	0·23	$4{\cdot}09x10^{-6}$	0·28	0·15	0·52	-	-
14	53769396	rs4573847	A	G	0·16	$5{\cdot}65x10^{-7}$	0·14	0·06	0·38	-	-
16	13815870	rs179606	G	A	0·35	$1{\cdot}96x10^{-6}$	0·33	0·20	0·54	-	-
16	13818107	rs179609	G	T	0·35	$1{\cdot}91x10^{-6}$	0·33	0·20	0·54	-	-
19	9020185	rs78342591	T	C	0·08	**$2{\cdot}24x10^{-8}$**	5·89	3·23	10·74	***MUC16***	intronic
19	9027313	rs62118276	A	G	0·06	$6{\cdot}09x10^{-8}$	6·11	3·25	11·48	*MUC16*	intronic
19	9029511	rs2341321	A	G	0·06	$6{\cdot}63x10^{-8}$	5·88	3·16	10·94	*MUC16*	intronic
19	29693669	rs142959560	GA	G	0·15	$4{\cdot}46x10^{-6}$	0·15	0·05	0·42	-	-
19	29700027	rs71960487	GAC	G	0·15	$4{\cdot}46x10^{-6}$	0·15	0·05	0·42	*UQCRFS1*	intronic
19	29712441	rs35484580	C	T	0·15	$4{\cdot}53x10^{-6}$	0·15	0·05	0·42	*LOC105372352*	nc transcript variant
19	29744818	rs201622525	CTCT	C	0·12	$1{\cdot}61x10^{-6}$	0·07	0·02	0·31	-	-

There was one SNP at genome-wide significance ($<5x10^{-8}$) located within *MUC16*· In total, there were 28 SNPs below a *P* significance threshold $<5\ x10^{-6}$· The table is ordered according to chromosome and sequential position (assembly GRCh37/hg19) [a]The annotated gene was determined by cross referencing Refseq, ensembl 74 and UCSC database information (hg19, 2015 update), accessed through SNPnexus (2012 update)· The SNPnexus database (http://www·snp-nexus·org) is kept synchronised with the UCSC human genome annotation database (http://genome·ucsc·edu)· Where there was discrepancy or the gene was uncertain, a search was performed manually using NCBI dbSNP build 149· SNPs with a minor allele frequency (MAF) < 2% were excluded· Functional annotation was determined using NCBI dbSNP build 149· SNPs with an odds ratio (OR) of 0·00 were excluded as were SNPs with an OR 95% confidence interval that included 1· The 95% confidence interval for the OR is the range of values between "OR 95 CI (lower)" through to "OR 95 CI (upper)"· *P* value thresholds are: $<1x10^{-5}$ is suggestive of an association and $<5x10^{-8}$ is the threshold for genome-wide significance

Supplementary Information for Clinical and germline risk factors for multiple treatment related toxicities during pediatric acute lymphoblastic leukaemia therapy

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Supplementary Table 1. Characteristics of the ERASE multiple toxicity cohort		
DIAGNOSTIC INFORMATION	**NUMBER (n=1251)**	**% OF COHORT**
Male	696	55·6
DIAGNOSIS		
Pre-B ALL	1068	85·4
B-lymphoblastic lymphoma	14	1·1
T-ALL	110	8·8
T-lymphoblastic Lymphoma	39	3·1
Other (ALL/LL, not specified)	20	1·6
TREATMENT PROTOCOL		
AIEOP-BFM-based protocols	***(n=1033)***	
ANZCHOG Study 7	239	19·1
ANZCHOG Study 8	608	48·6
AIEOP-BFM-Study 9	40	3·2
BFM-95	125	10·0
COG A5971	21	1·7
COG-based protocols	***(n=218)***	
AALL0031	2	0·2
AALL0232	25	2
AALL0331	49	3·9
AALL0434	12	1·0
AALL08P1	2	0·2
AALL0932	17	1·4
AALL1131	4	0·3
CCG1882	1	0·1
CCG1952	16	1·3
CCG1961	36	2·9
CCG1991	54	4·3
The ERASE cohort of 1251 patients was derived from analysing 1438 records of consecutive patients treated for ALL at 6 Australian hospitals. Patients excluded (n=187) included clinical information not available (n=31), treatment center (n=7), time period (n=1), protocol exclusion (n=9), age exclusion (n=4), relapsed ALL therapy (n=4), premorbid condition exclusion (n=3), early death from relapse (n=8), early death from treatment* (n=24), < 18 months in CR1 and no toxicity (n=96) Patients without adequate clinical information to determine case or control status were excluded. *Early death from treatment: patients who experienced treatment-related mortality unrelated to target toxicities were excluded. Abbreviations: AIEOP: Associazione Italiana Ematologia Oncologia Pediatrica; ANZCHOG: Australian and New Zealand Children's Haematology & Oncology Group; BFM: international Berlin-Frankfurt-Munster study group; CCG: Children's Cancer Group; COG: Children's Oncology Group		

Supplementary Table 2: Overview of risk and response adapted risk classification systems used in patients participating in the ERASE study																								
Study	**ALL-BFM-95[1]**			**ANZCCSG Study VII[2]**		**ANZCHOG Study VIII[3]**				**AIEOP-BFM Study 9[4]**			**COG stratification for ALL[5]**											
Risk Group	**Standard**	**Medium**	**High**	**Standard**	**High**	**Standard**	**Medium**	**High**	**Very High**	**Standard**	**Medium**	**High**	**Low**	**T**	**Average**			**High**			**Very High**			
	No HR features	No HR features		No HR features		No HR features	No HR features			No HR features	No HR features			T cell low risk		T cell intermediate			T cell high					
NCI Risk Group	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	SR		SR		SR	SR	HR	HR	HR	HR (≥13y)	SR or HR	
Age	1-6y	1-6y or older		1-10y	>10y	Not used	Not used	Not used	Not used				1-9·99	1-9·99	1-9·99	Any		1-9·99	>10·0	>10·0				
White cell count	<20x10^9/l	>20x10^9/l	-	<50x10^9/l	>50x10^9/l and ETV6::RUNX1 negative				≥100x10^9/l and PPR				<50	<50	<50	<50		<50	>50	>50				
Immunophenotype	B-ALL	T-ALL	B or T	B or T					T-ALL and PPR Pro-B ALL and PPR				B	T	B			B		B	B or T			
ALL genetics			BCR::ABL1 KMT2a::AFF1	ETV6::RUNX1	BCR::ABL1, KMT2a::AFF1, TCF3::PBX1 hypodiploidy			*BCR::ABL1* KMT2a::AFF1	BCR::ABL1 KMT2a::AFF1			KMT2a::AFF1 hypodiploidy		No *BCR::ABL*		No *BCR::ABL*		KMT2a-r with RER		KMT2a-r with RER	*BCR::ABL* Hypodiploidy *KMT2a*-r with a SER		
CNS Status													1	1	2	1-3		CNS3		CNS3				
Extramedullary disease													No	No	No			No	No	Testicular				
Steroid Pre-treatment																		Yes	No	Yes				
COG favourable genetics Triple trisomy 4, 10, 17 OR ETV6::RUNX1													Yes		Yes		Yes	No	Any	No	Any	Any	Any	

Supplementary Table 2: Overview of risk and response adapted risk classification systems used in patients participating in the ERASE study																							
Study	**ALL-BFM-95[1]**			**ANZCCSG Study VII[2]**		**ANZCHOG Study VIII[3]**				**AIEOP-BFM Study 9[4]**			**COG stratification for ALL[5]**										
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	No HR features	No HR features		No HR features		No HR features	No HR features			No HR features	No HR features			T cell low risk		T cell intermediate			T cell high				
NCI Risk Group	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	SR		SR		SR	SR	HR	HR	HR	HR (≥13y)	SR or HR
COG unfavourable characteristics· CNS3, hypodiploidy, iAMP21, Induction failure or MLL rearrangement	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	No		No	No	No	No	No	No	No	No	Yes
Day 8 Prednisone response Peripheral blasts <or≥ $1{\cdot}0x10^9/l$	PGR	PGR	PPR	Not used	Not used	PGR	PGR	PPR	PPR & immunophenotype or PPR	PGR	PGR	PPR											
Day 8 peripheral blood MRD													<0·01%		≥0·01%	<1%	Any	≥1%	Any	Any	Any	Any	Any
Day 8 or 15 BM Response					M3				M3 (HR ALL only)			≥10%	M1	M1	M1	M1-3		D15 M2/3	M1				
End Induction Morphologic Response	M1	M1	M2 or M3	M1	M2 or M3	M1	M1	M2 or M3	M2 or M3	M1	M1	M2 or M3	M1	M1	M1	M1		M2	M1	M2	M3 or M2		
End induction MRD response				Not used	Not used	MRD negative	low positive			MRD negative	MRD positive		<0·1%	<0·1%	<0·1%	<1%	≥0·01%	≥1%	<0·1%	≥1%	≥1%	<0·01%	Any

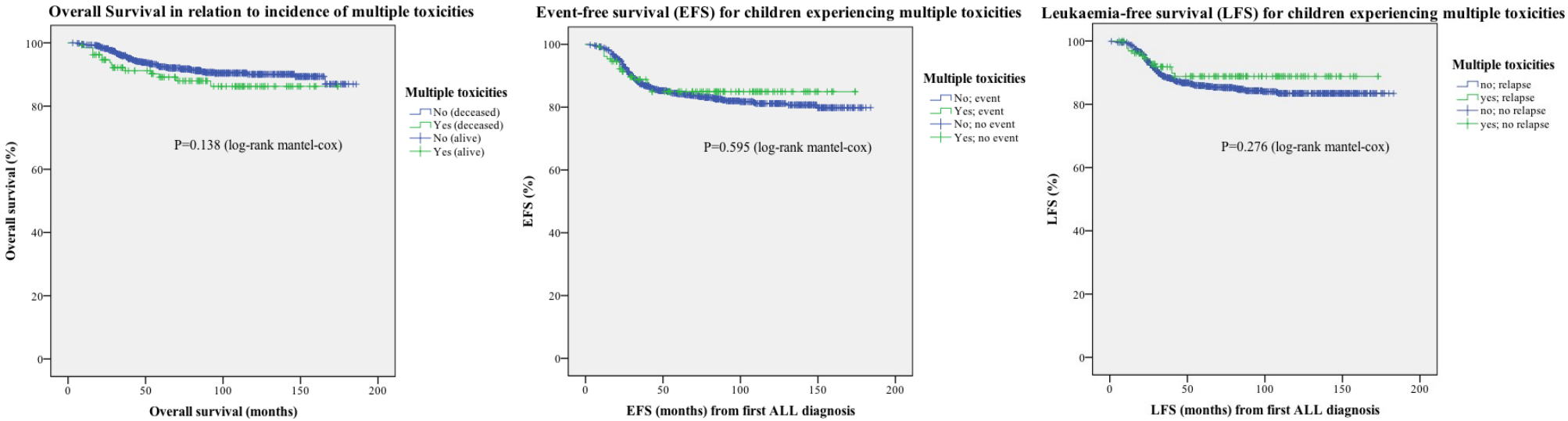
Supplementary Table 2: Overview of risk and response adapted risk classification systems used in patients participating in the ERASE study

Study	**ALL-BFM-95[1]**			**ANZCCSG Study VII[2]**		**ANZCHOG Study VIII[3]**				**AIEOP-BFM Study 9[4]**			**COG stratification for ALL[5]**										
Risk Group	**Standard**	**Medium**	**High**	**Standard**	**High**	**Standard**	**Medium**	**High**	**Very High**	**Standard**	**Medium**	**High**	**Low**	**T**	**Average**			**High**			**Very High**		
	No HR features	No HR features		No HR features		No HR features	No HR features			No HR features	No HR features			T cell low risk		T cell intermediate			T cell high				
NCI Risk Group	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	SR		SR		SR	SR	HR	HR	HR	HR (≥13y)	SR or HR
Post consolidation MRD response						MRD negative	$<10^{-3}$	Consolidation $\geq 10^{-3}$		MRD negative	MRD$<10^{-3}$	MRD$\geq 10^{-3}$ OR B-ALL with slow early response (End induction MRD $\geq 10^{-3}$ and consolidation MRD positive $<10^{-3}$)	Not used		Not used	Not used	Not used	M1 marrow and MRD <1%	Not used	M1 marrow & MRD <0·1%	M2/3 and/or MRD ≥1%	Not used	Not used

COG risk classification based on the AALL08B1 Classification System[5]· Patients on the ERASE study were treated on the following CCG and COG protocols: AALL0031[6, 7], AALL0232[8], AALL0331[9], AALL0434[10], AALL08P1[11], AALL0932[12, 13], AALL1131[12], CCG1882[14], CCG1952[15], CCG1961[16] and CCG1991[17].

BCR::ABL1 – not eligible· **Abbreviations:** NCI / Rome Consensus criteria for B-ALL: Standard risk: Age 1·0-9·99 years and WBC < 50,000/µl· High Risk: Age <1y OR ≥10y OR WBC ≥50,000/µl· MRD: measurable residual disease. RER: rapid early response· SER: slow early response

Supplementary Figure 1: Overall, event-free and leukaemia-free survival in ALL patients in the ERASE cohort



Overall, event-free and leukaemia-free survival in the ERASE cohort. Five-year OS for children who experienced multiple toxicities was 89·1±2·9% compared to 92·5±0·9% for children who did not experience multiple toxicity. Five-year EFS for children who experienced multiple toxicities was 84·9±3·3% compared to 84·2±1·2% for children who did not experience multiple toxicities. Five-year LFS for children who experienced multiple toxicity was 88·8±2·9% compared to 85·9±1·1%.

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