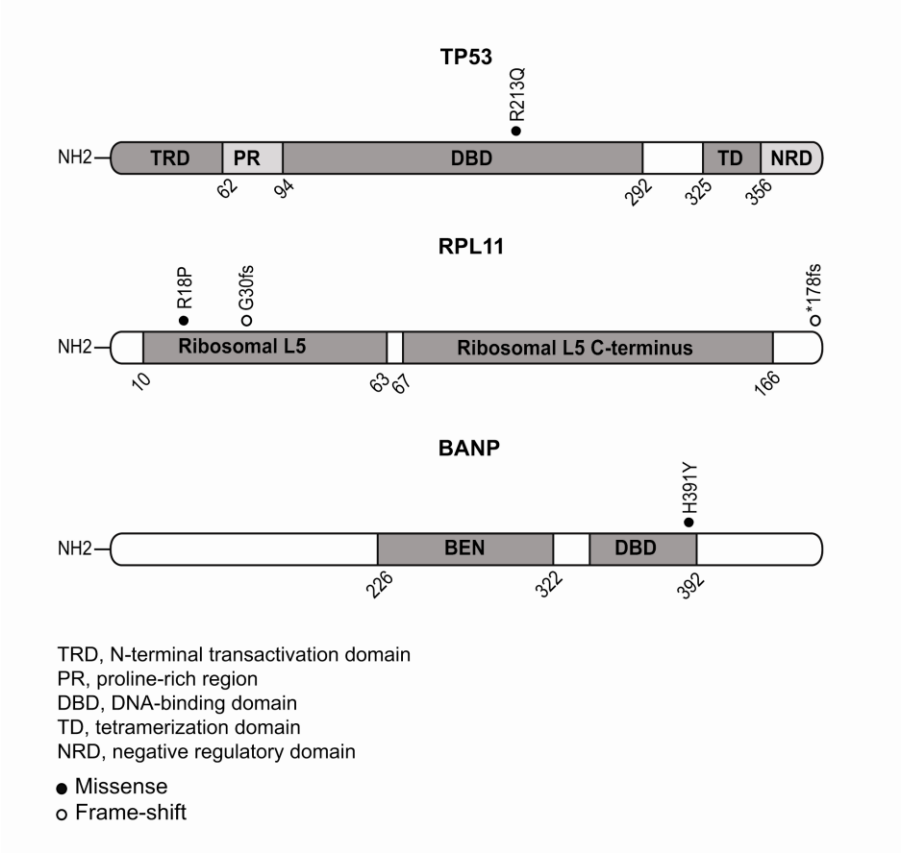
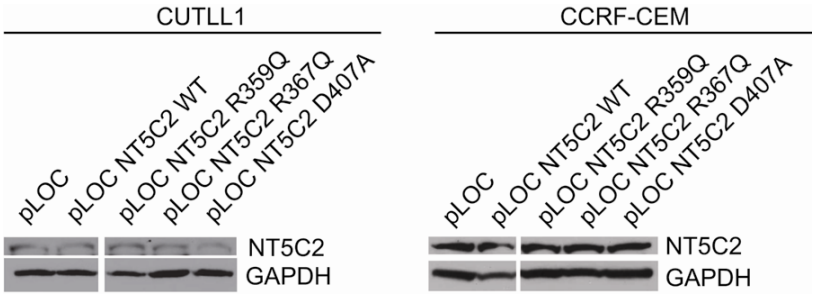
Activating mutations in the *NT5C2* nucleotidase gene drive chemotherapy resistance in relapsed ALL

Gannie Tzoneva, Arianne Perez Garcia, Zachary Carpenter, Hossein Khiabanian, Valeria Tosello, Maddalena Allegretta, Elisabeth Paietta, Janis Racevskis, Jacob M. Rowe, Martin S. Tallman, Maddalena Paganin, Giuseppe Basso, Jana Hof, Renate Kirschner-Schwabe, Teresa Palomero, Raul Rabadan and Adolfo Ferrando

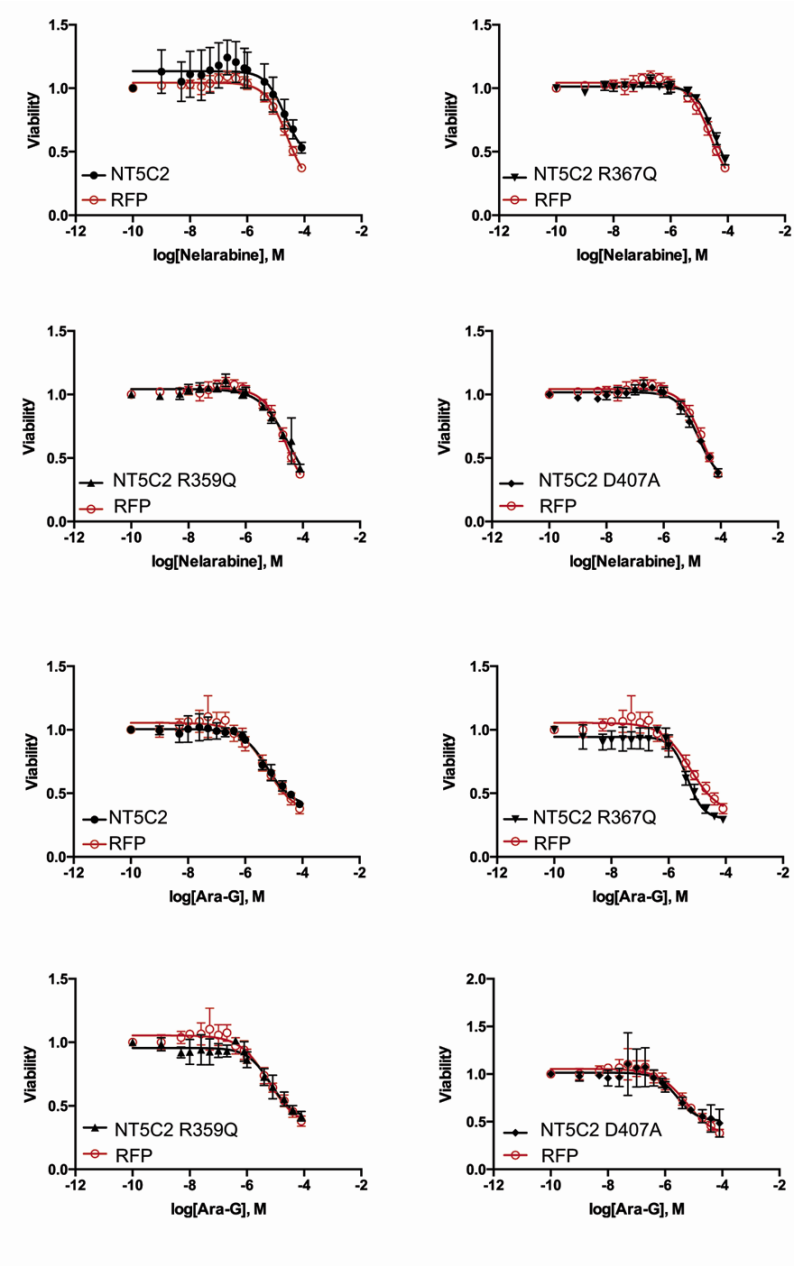


Supplementary Figure 1. *TP53*, *RPL11* and *BANP* mutations in relapsed pediatric T-ALL.

Schematic representation of the structure of the TP53, RPL11 and BANP proteins. Missense mutations identified in these genes in relapsed pediatric patients are indicated with filled circles. Open circles represent frameshift mutations.



Supplementary Figure 2. Western blot analysis of NT5C2 expression in CCRF-CEM and CUTLL1 cells transduced with wild type and mutant NT5C2 expressing lentiviruses.



Supplementary Figure 3. Nelarabine and ara-G dose response cell viability curves in CCRF-CEM cells expressing wild type and mutant forms of NT5C2 compared to a red fluorescent protein empty vector control (RFP). Data is shown as means ± s.d.

Supplementary Table 1. Summary of patient samples and molecular assays

Cohort	N	Source	Analyses
T-ALL diagnostic/remission/relapse samples*	5	AIEOP (University of Padua)	Whole exome sequencing
T-ALL diagnostic/remission/relapse Samples*	18	AIEOP (University of Padua)	*TP53, BANP, RPL11, NRAS, NT5C2* mutation analysis
T-ALL relapse samples*	80	AIEOP (University of Padua)(n=13) BFM (Charité-Universitätsmedizin Berlin) (n=67)	*NT5C2* mutation analysis
B-precursor relapse ALL samples	35	AIEOP (University of Padua)	*NT5C2* mutation analysis
Diagnostic T-ALL samples	23	ECOG	*NT5C2* mutation analysis
Diagnostic B-precursor ALL samples	27	AIEOP (University of Padua)	*NT5C2* mutation analysis

* A total of 103 Relapse T-ALL samples were analyzed for *NT5C2* mutations: 36 (5+18+13) AIEOP (University of Padua) samples + 67 BFM (Charité-Universitätsmedizin Berlin) samples

Supplementary Table 2. Mutations identified by exome sequencing in diagnosis and relapse T-ALL

Sample ID	Gene	Position (in coding sequence)	Transcript ID	Predicted amino acid change	Mutated at diagnosis	Mutated at relapse
T-ALL 11	*CASQ1*	1033C>A	ENST00000368079	Q339K	yes	no
	CD163L1	526G>A	ENST00000396630	W176*	yes	no
	CTTNBP2	1966C>T	ENST00000160373	L656F	yes	no
	NOS1	1777T>G	ENST00000317775	Y593D	yes	no
	DCST1	492C>A	ENST00000423025	N164K	yes	yes
	SYT16	992A>G	ENST00000430451	E331G	yes	yes
	AFF1	985G>T	ENST00000307808	V329L	no	yes
	NT5C2	1075A>C	ENST00000404739	K359Q	no	yes
T-ALL 16	*ACP2*	121C>T	ENST00000256997	R41C	yes	yes
	COL4A2	2816G>T	ENST00000360467	R939I	yes	yes
	CTCF	1208-1delG	ENST00000264010	splicing	yes	yes
	FBXW7	1040G>A	ENST00000296555	R347H	yes	yes
	IGFL1	320G>A	ENST00000437936	R107H	yes	yes
	MAPK13	688G>A	ENST00000211287	D230N	yes	yes
	NKTR	4339C>T	ENST00000232978	R1447*	yes	yes
	SEPSECS	1003G>A	ENST00000302922	V335M	yes	yes
	VCPIP1	1280T>A	ENST00000310421	I427N	yes	yes
	WT1	1091C>A	ENST00000452863	S364*	yes	yes
	A4GALT	974G>A	ENST00000249005	R325Q	no	yes
	CCKBR	1306C>T	ENST00000334619	L436F	no	yes
	CNN3	521C>G	ENST00000370206	A174G	no	yes
	GPR151	701G>A	ENST00000311104	T234I	no	yes
	PTGS1	1744delC	ENST00000362012	P582fs	no	yes
	SPAG17	6141+2A>G	ENST00000336338	splicing	no	yes
	TP53	638G>A	ENST00000269305	R213Q	no	yes
T-ALL 38	*AKAP1*	926delG	ENST00000337714	S309fs	yes	no
	C17orf80	1017C>T	ENST00000268942	T306M	yes	yes
	DNM2	235+1G>C	ENST00000355667	splicing	yes	yes
	TBCK	599C>A	ENST00000361687	T200N	yes	yes
	ROCK1	1638+1G>T	ENST00000399799	splicing	no	yes
T-ALL 47	*CAPN9*	1403G>A	ENST00000354537	R468Q	yes	no
	CMBL	482C>A	ENST00000296658	S161Y	yes	no
	EPHA3	1621C>T	ENST00000336596	Q541*	yes	no
	PRKCSH	1199G>C	ENST00000412601	G400A	yes	no
	SHROOM3	116G>T	ENST00000296043	G39V	yes	no
	SLC12A5	1167A>T	ENST00000243964	E556V	yes	no
	USP7	640G>C	ENST00000344836	V214L	yes	no
	ZMYND11	527A>G	ENST00000402736	E175G	yes	no
	BANP	1171C>T	ENST00000393208	H391Y	no	yes
	KDR	3686G>A	ENST00000263923	R1229Q	no	yes
	MLLT4	2957A>G	ENST00000392108	Y986C	no	yes
	MLLT4	3350G>A	ENST00000392108	R1116H	no	yes
	RBM47	210delC	ENST00000381793	G70fs	no	yes
	SOX6	1633C>T	ENST00000316399	R545C	no	yes
T-ALL 49	*CDC42EP1*	757delC	ENST00000249014	P253fs	yes	no
	DNM2	2308C>T	ENST00000359692	R770*	yes	no
	EXTL1	939G>A	ENST00000374280	W313*	yes	no
	SACS	2785C>T	ENST00000382292	R929C	yes	no
	SERPINB4	1057G>A	ENST00000341074	E353K	yes	no
	WEE1	1210A>G	ENST00000299613	M404V	yes	no
	ASPM	8957C>T	ENST00000367409	R2853W	yes	yes
	SHROOM3	5279T>A	ENST00000296043	L1760Q	yes	yes
	INSR	320C>T	ENST00000302850	T107M	no	yes
	MAGEH1	557G>A	ENST00000342972	R186Q	no	yes
	NRAS	38G>T	ENST00000369535	G13V	no	yes
	RPL11	53G>C	ENST00000374550	R18P	no	yes
	SATB1	1219G>A	ENST00000338745	E407K	no	yes
	SYT10	413C>A	ENST00000228567	P138H	no	yes
	VSTM2A	46G>A	ENST00000407838	V16I	no	yes
	IFNA10	114delG	ENST00000357374	L38fs	no	yes

Supplementary Table 3. Genomic regions of LOH in diagnostic and relapsed T-ALLs analyzed by whole exome sequencing.

Sample	Chromosome	Start	End	Cytoband	Sample
T-ALL 15	12	116408538	133768553	12q24.21 - 12q24.33	Relapse
T-ALL 16	6	30954873	31323353	6p21.33	Diagnosis
T-ALL 17	1	949608	54644859	1p36.33 - 1p32.3	Diagnosis
T-ALL 17	1	949608	120437718	1p36.33 - 1p12	Relapse
T-ALL 17	9	117713	32425910	9p24.3 - 9p21.1	Diagnosis
T-ALL 21	9	214864	21816758	9p24.3 - 9p21.3	Diagnosis

Supplementary Table 4. *NT5C2* mutations in ALL

Sample ID	Diagnosis	Exon	Predicted amino acid change	Ref. Seq NM_012229
Relapse T-ALL 4	T-ALL	13	R367Q	1100G>A
Relapse T-ALL 11	T-ALL	13	K359Q	1076A>C
Relapse T-ALL 17	T-ALL	13	R367Q	1100G>A
Relapse T-ALL 17	T-ALL	9	R238L	713G>T
Relapse T-ALL 22	T-ALL	9	R238W	712C>T
Relapse T-ALL 29	T-ALL	11	R291W	871C>T
Relapse T-ALL 35	T-ALL	13	R367Q	1100G>A
Relapse T-ALL 37	T-ALL	15	D407A	1219A>C
Relapse T-ALL 30	T-ALL	17	Q523*	1567C>T
Relapse pre-B ALL 16	pre-B ALL	13	R367Q	1100G>A
Relapse T-ALL B11	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B15	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B30	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B37	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B39	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B44	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B48	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B52	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B53	T-ALL	13	R367Q	1100G>A
Relapse T-ALL B9	T-ALL	9	R238W	712C>T
Relapse T-ALL B29	T-ALL	9	R238W	712C>T
Relapse T-ALL B64	T-ALL	9	R238W	712C>T
Relapse T-ALL B9	T-ALL	13	L375F	1123C>T
Relapse T-ALL B64	T-ALL	13	L375F	1123C>T

Supplementary Table 5. Correlative clinical data on *NT5C2* mutated relapsed T-ALLs treated in AIEOP clinical trials.

Sample	Age (years)	Gender	Initial ALL Treatment	Duration of first complete remission (months)	Karyotype
T-ALL 4	6	M	AIEOP LAL91	24.9	46,XY
T-ALL 11	NA	M	AIEOP LAL95	3.6	46,XY(50%)/46,XY, del9(p22), -19, +der(19), t(9;19)(q22;q12)(20%)/47,XY, +8(30%)
T-ALL 17	8	M	AIEOP LAL95	11.6	NA
T-ALL 29	6	M	AIEOP LAL91	25.0	NA
T-ALL 30	9	M	AIEOP LAL2000	18.5	NA
T-ALL 35	6	F	AIEOP LAL2000	14.5	NA
T-ALL 37	11	M	AIEOP LAL2000	26.1	NA

Clinical protocols are described in Conter *et al.* J. Clin Oncol 1997; 15:2786-91 (AIEOP LAL91); Lo Nigro *et al.* Medical and Pediatric Oncol 2000; 35:449-455 (AIEOP LAL95) and Cario *et al.* Blood 2010; 115:5393-5397 (AIEOP LAL2000)

Supplementary Table 6. Correlative clinical and molecular data on *NT5C2* mutated relapsed T-ALLs in BFM based protocols

Sample	Age at initial diagnosis (years)	Gender	Initial ALL treatment	Duration of first complete remission (months)	Time of relapse (on/off treatment)	Site of relapse	Immunophenotype	*TP53* mutation and/or deletion
Relapse T-ALL B9	12.08	male	ALL-BFM 95, high risk arm	12.93	on treatment	isolated BM	cortical T-ALL	no
Relapse T-ALL B11	7.67	female	ALL-BFM 95, high risk arm	9.33	on treatment	combined BM/CNS	T-ALL	no
Relapse T-ALL B15	6.75	male	ALL-BFM 95, high risk arm	19.40	on treatment	isolated BM	cortical T-ALL	no
Relapse T-ALL B29	14.33	male	COALL 06-97, high risk arm	10.47	on treatment	combined BM/other	T-ALL	no
Relapse T-ALL B30	12.17	male	ALL-BFM 2000, intermediate risk arm	11.93	on treatment	isolated BM	cortical T-ALL	no
Relapse T-ALL B37	2.92	male	ALL-BFM 2000, intermediate risk arm	20.23	on treatment	combined BM/Testis	cortical T-ALL	no
Relapse T-ALL B39	5.08	male	NHL-BFM 95	16.07	on treatment	isolated BM	cortical T-ALL	no
Relapse T-ALL B44	4.67	male	Euro-LB 02	19.13	on treatment	combined BM/other	pre-T ALL	no
Relapse T-ALL B48	15.25	male	ALL-BFM 2000, high risk arm	15.77	on treatment	isolated BM	T-ALL	no
Relapse T-ALL B52	13.67	female	ALL-BFM 2000, intermediate risk arm	13.63	on treatment	combined BM/CNS	T-ALL	no
Relapse T-ALL B53	11.00	male	ALL-BFM 2000, intermediate risk arm	15.17	on treatment	isolated BM	cortical T-ALL	no
Relapse T-ALL B64	2.58	female	Euro-LB 02	14.80	on treatment	combined BM/CNS/other	T-ALL	no

Clinical protocols are described in Möricke et al. Blood 2008; 111:4477-4489 (NHL/ALL-BFM 95); Escherich *et al.* Hematologica 2011; 96:854-862 (COALL 06-97); Scherey *et al.* Pediatric Blood Cancer 2010; 54:952-958 (ALL-BFM2000); Oschlies *et al.* A, J Surg Pathol 2011; 35:836-44 (Euro-LB 02)

Supplementary Table 7. Correlative clinical data on rescue treatment for NT5C2 mutated relapsed T-ALLs in BFM based protocols

Sample	Relapse treatment protocol	Relapse risk group/treatment arm	Cytological response	Stem cell transplantation in second complete remission	Event	Death
Relapse T-ALL B9	ALL-REZ BFM 96	S4 / high risk	early	no	second relapse	yes
Relapse T-ALL B11	ALL-REZ BFM 96	S4 / high risk	normal	yes	secondary malignancy	yes
Relapse T-ALL B15	ALL-REZ BFM 96	S4 / high risk	nonresponse	no	cytological nonresponse	yes
Relapse T-ALL B29	ALL-REZ BFM 2002 pilot	S4 / high risk	nonresponse	no	cytological nonresponse	yes
Relapse T-ALL B30	ALL-REZ BFM 2002 pilot	S4 / high risk	late	yes	second relapse	yes
Relapse T-ALL B37	ALL-REZ BFM 2002	S4 / high risk	early	yes	in CCR	no
Relapse T-ALL B39	ALL-REZ BFM 2002	S4 / high risk	normal	yes	treatment related death in CR	yes
Relapse T-ALL B44	ALL-REZ BFM 2002	S4 / high risk	early	yes	second relapse	yes
Relapse T-ALL B48	ALL-REZ BFM 2002	S4 / high risk	nonresponse	no	cytological nonresponse	yes
Relapse T-ALL B52	ALL-REZ BFM 2002	S4 / high risk	early	yes	second relapse	yes
Relapse T-ALL B53	ALL-REZ BFM 2002	S4 / high risk	late	no	second relapse	yes
Relapse T-ALL B64	ALL-REZ BFM 2002	S4 / high risk	n. a.	no	early death in induction therapy	yes

Clinical protocols are described in Von Stackelberg *et al.* Med Pediatr Oncol 2002; 39: 236 (ALL-REZ BFM 96) and at clinicaltrials.gov (http://clinicaltrials.gov/show/NCT00114348) (ALL-REZ BFM 2002)

Supplementary Table 8. Association of *NT5C2* mutations with clinical characteristics, response, outcome and genetics in T-ALL patients treated in BFM based protocols.

		wildtype		mutation		
Parameter	**Variable**	**n**	**%**	**n**	**%**	***p***
Total		**55**	100.0	**12**	100.0	
Clinical characteristics						
Gender	male	**41**	74.5	**9**	75.0	1.000
	female	**14**	25.5	**3**	25.0	
Age at relapse diagnosis	< 5 years	**9**	16.4	**2**	16.7	1.000
	≥ 5 and < 10 years	**17**	30.9	**4**	33.3	
	≥ 10 years	**29**	52.7	**6**	50.0	
Age at initial diagnosis	< 5 years	**13**	23.6	**3**	25.0	0.662
	≥ 5 and < 10 years	**21**	38.2	**3**	25.0	
	≥ 10 years	**21**	38.2	**6**	50.0	
Time of relapse[1]	very early	**25**	45.5	**9**	75.0	0.079
	early	**16**	29.1	**3**	25.0	
	late	**14**	25.5	**0**	0.0	
Time of relapse[1]	very early or early	**41**	74.5	**12**	100.0	**0.058**
	late	**14**	25.5	**0**	0.0	
Time of relapse	on treatment	**30**	54.5	**12**	100.0	**0.002**
	off treatment	**25**	45.5	**0**	0.0	
Duration of CR1	median (years)	1.5		1.21		0.540
Site of relapse[2]	BM isolated	**41**	74.5	**6**	50.0	0.160
	BM combined	**14**	25.5	**6**	50.0	
Immunophenotype at relapse	T-ALL	**22**	40.0	**5**	41.7	0.365
	pre-T ALL	**15**	27.3	**1**	8.3	
	cortical T-ALL	**18**	32.7	**6**	50.0	
Treatment, response and outcome after relapse						
Relapse treatment	ALL-REZ BFM 95/96	**20**	36.4	**3**	25.0	0.532
	ALL-REZ BFM 2002	**35**	63.6	**9**	75.0	
HSCT after relapse	no	**25**	45.5	**6**	50.0	1.000
	yes	**30**	54.5	**6**	50.0	
Cytological response[3]	early	**20**	39.2	**4**	36.4	0.597
	normal	**12**	23.5	**2**	18.2	
	late	**3**	5.9	**2**	18.2	
	non-response	**16**	31.4	**3**	27.3	
	no data	4		1		
Induction of 2nd remission	2nd CR achieved	**36**	65.5	**8**	66.7	1.000
	no 2nd CR achieved	**19**	34.5	**4**	33.3	
Event	in CCR	**15**	27.3	**1**	8.3	0.267
	any event	**40**	72.7	**11**	91.7	
Event	in CCR	**15**	27.3	**1**	8.3	0.234
	death in CR	**7**	12.7	**1**	8.3	
	secondary malignancy	**0**	0.0	**1**	8.3	
	second relapse	**14**	25.5	**5**	41.7	
	cytological nonresponse	**16**	29.1	**3**	25.0	
	death in induction	**3**	5.5	**1**	8.3	
Event	in CCR	**15**	27.3	**1**	8.3	0.341
	early event (death in ind., non-resp.)	**21**	38.2	**7**	58.3	
	late event (2nd rel., 2nd mal., death in CR)	**19**	34.5	**4**	33.3	
Genetics at relapse						
TP53 mutation and/or deletion	no	**49**	89.1	**12**	100.0	0.582
	yes	**6**	10.9	**0**	0.0	
PTEN mutation	no	**51**	92.7	**10**	83.3	0.291
	yes	**4**	7.3	**2**	16.7	
CDKN2A/2B deletion	no	**17**	30.9	**3**	25.0	1.000
	yes	**38**	69.1	**9**	75.0	
SIL/TAL fusion	no	**52**	94.5	**9**	75.0	0.066
	yes	**3**	5.5	**3**	25.0	
NOTCH1 mutation	no	**25**	48.1	**5**	41.7	0.757
	yes	**27**	51.9	**7**	58.3	
	no data	3		0		

Supplementary Table 8 continued

FBXW7 mutation	no	**40**	76.9	**10**	83.3	1.000
	yes	**12**	23.1	**2**	16.7	
	no data	3		0		
Frontline treatment						
Treatment protocol	ALL-BFM[4]	**32**	61.5	**8**	66.7	0.241
	COALL[5]	**14**	26.9	**1**	8.3	
	BFM - lymphoblastic lymphoma[6]	**6**	11.5	**3**	25	
	no data	3		0		
Treatment intensity	high-risk treatment[7]	**19**	59.4	**5**	55.6	1.000
	non high-risk treatment[8]	**13**	40.6	**4**	44.4	
	no data	23		3		

[1)]time of relapse: very early, <18 months after initial diagnosis of ALL; early, ≥18 months after initial diagnosis of ALL; late, ≥6 months after completion of primary treatment. [2)]site of relapse: isolated BM, no evidence of extramedullary disease; combined BM, more than 5% leukemic cells in the BM combined with CNS, testis, or other extramedullary disease. [3)]cytologic response: early, remission after first induction course; normal, remission after second induction course; late, remission after 1st R2 block/day 15 protocol II-IDA; non-response, no remission after 1st R1 block/day 29 protocol II-IDA. Cytologic remission was defined as less than 5% leukemic blasts in regenerating bone marrow, no peripheral blasts cells and no extramedullary involvement. [4)]ALL-BFM includes patients treated according to protocols ALL-BFM 90, ALL-BFM 95 and ALL-BFM 2000. [5)]COALL includes patients treated according to protocols CoALL 05-92, CoALL 06-97 and CoALL 07-03. [6)]BFM - lymphoblastic lymphoma includes patients treated according to protocols NHL-BFM 90, NHL-BFM 95 and Euro-LB 02. [7)]high-risk treatment includes patients treated according to the high risk arm of protocols ALL-BFM 90, ALL-BFM 95, ALL-BFM 2000, CoALL 06-97 and CoALL 07-03. [8)]non high-risk treatment includes patients treated according to the standard risk or intermediate risk arm of protocols ALL-BFM 90, ALL-BFM 95 and ALL-BFM 2000.

Abbreviations: BM, bone marrow; CR2, second complete remission; CCR, complete continuous remission; MRD, minimal residual disease in bone marrow aspiration after induction phase (week 5); HSCT, hematopoietic stem cell transplantation.

Supplementary Table 9. Association of frontline treatment with *NT5C2* mutations

		***NT5C2* wild type**		***NT5C2* mutation**		
Parameter	**Variable**	**n**	**%**	**n**	**%**	***p*[7]**
Total number of patients investigated		**55**		**12**		
Frontline treatment						
Treatment protocol	total no. of patients without data[1]	**3**		**0**		
	total no of patients with data	**52**	100.0	**12**	100.0	
	ALL-BFM[2]	**32**	61.5	**8**	66.7	0.241
	COALL[3]	**14**	26.9	**1**	8.3	
	BFM - lymphoblastic lymphoma[4]	**6**	11.5	**3**	25	
Treatment intensity	total no. of patients without data[1]	**23**		**3**		
	total no of patients with data	**32**	100.0	**9**	100.0	
	high-risk treatment[5]	**19**	59.4	**5**	55.6	1.000
	non high-risk treatment[6]	**13**	40.6	**4**	44.4	

[1]Patients without data regarding frontline treatment were excluded from the statistical analysis.

[2]ALL-BFM includes patients treated according to protocols ALL-BFM 90, ALL-BFM 95 and ALL-BFM 2000.

[3]COALL includes patients treated according to protocols CoALL 05-92, CoALL 06-97 and CoALL 07-03.

[4]BFM - lymphoblastic lymphoma includes patients treated according to protocols NHL-BFM 90, NHL-BFM 95 and Euro-LB 02.

[5]high-risk treatment includes patients treated according to the high risk arm of protocols ALL-BFM 90, ALL-BFM 95, ALL-BFM 2000, CoALL 06-97 and CoALL 07-03.

[6]non high-risk treatment includes patients treated according to the standard risk or intermediate risk arm of protocols ALL-BFM 90, ALL-BFM 95 and ALL-BFM 2000.

[7]Equality of categorical variables was analyzed by Fisher's exact test (2-sided).

Supplementary Table 10. Multivariate binary logistic regression with time of relapse (on/off treatment) as outcome variable

Variable		No of patients[1]	Regression coefficient beta	$e^{ß}$ (odds ratio)	*p*-Value	Nagelkerkes R^2
Model with NT5C2 mutation and frontline protocol						
NT5C2	wt	12		1	<0.001	
	mutation	52	20.961	1 268 199 318		
frontline protocol	BFM - lymphoblastic lymphoma	9		1	0.803	0.246
	ALL-BFM	40	0.378	1.462		
	COALL	15	0	1		
Best Model						
NT5C2	wt	12		1	<0.001	0.238
	mutation	52	20.971	1 281 238 668		

[1)]The total number of patients included in the analysis is 64 of 67 ALL-REZ BFM T-ALL relapse patients. Three patients were excluded because of missing data on frontline treatment.

Supplementary Table 11. **Nucleoside analog IC50 values (μM) of T-ALL cell lines expressing relapse associated *NT5C2* mutant alleles**

6-mercaptopurine			6-thioguanine		
	CCRF-CEM	CUTLL1		CCRF-CEM	CUTLL1
RFP*	3.07	0.71	RFP*	0.77	1.18
NT5C2 WT[#]	3.54	0.41	NT5C2 WT[#]	1.10	1.39
NT5C2 R367Q	5.57	> 10	NT5C2 K367Q	> 10	2.6
NT5C2 R359Q	6.47	> 10	NT5C2 R359Q	> 10	3.37
NT5C2 D407A	5.67	> 10	NT5C2 D407A	10	2.56

* RFP, empty vector control
[#] NT5C2 WT, wild type NT5C2