Supplementary Material for:

Ras pathway mutations are highly prevalent in relapsed childhood acute lymphoblastic leukaemia, may act as relapse-drivers and confer sensitivity to MEK inhibition.

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Table S1. Clinical characteristics of patients in the study cohort compared to the total trial cohort.

Parameter	Category	Representativeness				
		Total cohort		Study cohort		
		n	%	n	%	p -value
	Total	329		206		
Gender	Male	191	58.0	126	61.0	0.47
	Female	138	41.9	80	38.8	
Age at initial diagnosis	<5 years	173	52.6	103	50.0	0.81
	5 - 10 years	79	24.0	54	26.0	
	≥10 years	77	23.4	49	23.8	
	Median, 95%CI (years)	4.64	4.25-5.46	4.99	4.36-6.11	0.46
Age at relapse diagnosis	<5 years	58	17.6	29	14.1	0.56
	5 - 10 years	137	41.6	89	43.2	
	≥10 years	134	40.7	88	42.7	
	Median, 95%CI (years)	8.44	7.56-9.56	9	7.79-9.99	0.54
Time point of relapse	Very early	61	18.5	44	21.4	0.45
	Early	83	25.2	43	20.9	
	Late	185	56.2	119	57.8	
Relapse during (on)/after (off) initial treatment	On-therapy	97	29.5	62	30.1	0.88
	Off-therapy	232	70.5	144	69.9	
Time between initial and relapse diagnosis	Median, 95%CI (years)	2.73	2.56-2.86	2.79	2.54-2.97	0.89
Site of relapse	BM isolated	256	77.8	163	79.1	0.72
	BM combined	73	22.2	43	20.9	
CNS involvement	No	280	85.1	177	85.9	0.800
	Yes	49	14.9	29	14.1	
Testicular involvement	No	311	94.5	195	94.7	0.94
	Unilateral	12	3.7	8	3.9	
	Bilateral	6	1.8	3	1.5	
Immunophenotype	pro-B ALL	24	7.5	12	6.0	0.87
	common ALL	228	70.8	140	70.4	
	prae-B ALL	60	18.6	39	19.6	
	biphenotypic	10	3.1	8	4.0	
	unknown	7		7		
PBC	<1/µl	45	14.0	22	10.9	0.13
	≥1-<10.000/µl	225	69.9	134	66.3	
	≥10.000/µl	52	16.1	46	22.8	
	unknown	7		4		
	Median, 95%CI (cells/µl)	864	509-1262	1860	1013-2404	0.013
Strategic group	S2	211	64.1	131	63.6	0.45
	S3	57	17.3	31	15	
	S4	61	18.5	44	21.4	
Outcome	CCR	94	45.6	157	47.7	0.91
	2^{nd} relapse	55	26.7	91	27.7	
	TRD	15	7.3	25	7.6	
	Secondary malignancy	2	0.97	5	1.5	
	Non-Response	33	16	42	12.8	
	Induction death	7	3.4	9	2.7	
Cytologic response	after 1st course (F1)	129	40.7	86	43.4	0.53
	after 2nd course (F2)	112	35.3	56	28.3	
	after 3rd course	26	8.2	17	8.6	
	after 4th course	8	2.5	6	3.0	
	non-response (no CR)	42	13.3	33	16.7	
	unknown	12		8		
MRD after F2	$<10^{-4}$	59	32.4	43	37.4	0.79
	10^{-4} - $<10^{-3}$	27	14.8	17	14.8	
	10^{-3} - $<10^{-2}$	42	23.1	26	22.6	
	$\geq 10^{-2}$	54	29.7	29	25.2	
	unknown	147		91		

Abbreviations: BM, bone marrow; PBC, peripheral blast cell count; CI, confidence interval; CCR, continuous complete remission; TRD, therapy related death; CR, clinical remission, MRD, minimal residual disease.
Definitions: Time point of relapse: Very early, <18 months after diagnosis; Early, >18 months after diagnosis <6 months after regular completion of initial treatment; Late, >6 months after regular completion of initial treatment.

Table S2. Clinical details of UK diagnostic patients as well as mutation status determined by DHPLC and p-ERK levels as measured by western analyses.

UK cohort	Total	80
Gender	Male	43 (54%)
	Female	37 (46%)
Age at Diagnosis	Infants < 2 years	7 (9%)
	2-5 years	31 (39%)
	5-10 years	15 (19%)
	> 10 years	27 (33%)
Mutations	Wild type	48 (60%)
	NRAS	15 (19%)
	KRAS	11 (14%)
	PTPN11	2 (2.5%)
	FLT3	1 (1.2%)
	CBL	1 (1.2%)
	KRAS/PTPN11	1 (1.2%)
	CBL/FLT3	1 (1.2%)
p-ERK positivity	+ve	36 (45%)
	-ve	44 (55%)
	Range	0.01-1.12
	Median	0.29

Table S3. Clinical details of UK diagnostic patients used in the *KRAS* allele specific PCR screen.

UK Cohort	Total	111
Gender	Male	59 (53%)
	Female	53 (47%)
Age at Diagnosis	Infants < 2yrs	7 (6.3%)
	2-5 years	33 (29.5%)
	5-10 years	35 (31.2%)
	> 10 years	37 (33%)

Table S4. Clinical details of patients used in the *in vivo* study.

Patient ID	Sex	Age at Diagnosis	Cytogenetics	Mutation	End of induction MRD
L779	M	5.5 yrs	High hyperdiploid	*NRAS* (Q61R)	Indeterminate
L897*	M	16.8 yrs	Failed	*KRAS* (G12D)	High Risk
L848	M	2.5 yrs	t(12;21)	Wild type	Low Risk
L920	F	4.4 yrs	Failed	Wild type	Low Risk

*****patient suffered on- treatment CNS relapse

Table S5. Mutations identified in the study.

Gene, exon	Number	Mutation
NRAS, exon 1	24	G12A (3), G12C (1), G12D (8), G12S (2), G12V (2), G13D (7), G13R (1)
NRAS, exon 2	7	Q61L (1),Q61R (1),Q61H (3), Q61K (2)
KRAS, exon 1	30	G12A (1), G12C (2), G12D (11), G12S (1), G12V (7), G13D (4), V14I (2), A18D (1), Q22K (1)
FLT3, exon 14	3	Y589D (1), ins600FREYEYD (1), ins601REYEYDL (1)
FLT3, exon 20	7	delI836M37 (4), D839G (1), Y842H (1), D835H + subclone delI836M37 (1)
PTPN11, exon 3	7	G60V (1), D61H (1), E69K (1), A72A (1), A72V(1), E76K (1), del61ins61IPQP(1)
PTPN11, exon 13	2	G503A (2)

Table S6. RAS pathway mutations in relapsed B lineage ALL and clinical characteristics.

Parameter	Category	All RAS pathway genes					NRAS					KRAS					PTPN11					FLT3				
		negative		positive			negative		positive			negative		positive			negative		positive			negative		positive		
		n	%	n	%	p -value	n	%	n	%	p -value	n	%	n	%	p -value	n	%	n	%	p -value	n	%	n	%	p -value
	Total	128		78			176		30			176		30			197		9			196		10		
Gender	Male	73	57	53	68	0.12	108	61	18	60	0.89	102	58	24	80	**0.022**	120	61	6	67	0.73	120	61	6	60	1.00
	Female	55	43	25	32		68	37	12	40		74	42	6	20		77	39	3	33		76	39	4	40	
Age at initial diagnosis	<5 years	62	48	41	53	0.33	86	49	17	57	0.20	88	50	15	50	0.27	100	51	3	33	**0.016**	96	49	7	70	0.40
	5 - 10 years	38	30	16	21		50	28	4	13		49	28	5	17		48	24	6	67		53	27	1	10	
	≥10 years	28	22	21	27		40	23	9	30		39	22	10	33		49	25	0	0		47	24	2	20	
	Median, 95%CI (years)	5.2	4.1 - 7.0	4.9	4.2 - 6.2	0.65	5.1	4.3 - 6.3	4.6	3.6 - 8.0	0.94	5.0	4.3 - 6.1	5.0	2.8 - 10.4	0.95	4.9	4.3 - 6.4	5.4	2.2 - 6.4	0.49	5.1	4.4 - 6.4	4.4	2.3 - 8.7	0.42
Age at relapse diagnosis	<5 years	15	12	14	18	0.46	24	14	5	17	0.89	23	13	6	20	0.57	27	14	2	22	0.72	27	14	2	20	0.82
	5 - 10 years	57	45	32	41		76	43	13	43		78	44	11	37		85	43	4	44		85	43	4	40	
	≥10 years	56	44	32	41		76	43	12	40		75	43	13	43		85	43	3	33		84	43	4	40	
	Median, 95%CI (years)	9.2	8.0 - 10.2	8.2	6.8 - 10.2	0.37	9.4	7.9 - 10.0	7.5	6.2 - 11.4	0.54	9.0	7.8 - 10.0	8.6	5.9 - 12.7	0.63	8.9	7.6 - 10.0	9.5	4.9 - 10.8	0.72	9.0	7.8 - 10.0	8.6	5.3 - 11.9	0.76
Time point of relapse	Very early	26	20	18	23	**0.01**	38	22	6	20	**0.017**	34	19	10	33	**0.039**	43	22	1	11	0.72	43	22	1	10	0.83
	Early	19	15	24	31		31	18	12	40		34	19	9	30		42	21	1	11		41	21	1	20	
	Late	83	65	36	46		107	61	12	40		108	61	11	37		112	57	7	78		112	57	7	70	
Relapse during (on)/after (off) initial treatment	On-therapy	34	27	28	36	0.16	49	28	13	43	0.087	50	28	12	40	0.20	60	31	2	22	0.73	61	31	1	10	0.29
	Off-therapy	94	73	50	64		127	72	17	57		126	72	18	60		137	70	7	78		135	69	9	90	
Time between initial and relapse diagnosis	Median (years)	2.9	2.7 - 3.2	2.5	2.1 - 3.0	0.23	2.9	2.6 - 3.2	2.4	1.7 - 2.8	0.15	2.8	2.6 - 3.1	2.3	1.5 - 3.1	**0.047**	2.8	2.5 - 3.0	3.4	1.7 - 5.6	0.27	2.8	2.5 - 2.9	3.8	2.2 - 5.2	0.071
Site of relapse	BM isolated	104	81	59	76	0.34	143	81	20	67	0.069	139	79	24	80	0.90	156	79	7	78	1.00	155	79	8	80	1.00
	BM combined	42	19	19	24		33	19	10	33		37	21	6	20		41	21	2	22		41	21	2	20	
CNS involvement	No	114	89	63	81	0.097	155	88	22	73	**0.032**	153	87	24	80	0.31	169	86	8	89	1.00	168	86	9	90	1.00
	Yes	14	11	15	19		21	12	8	27		23	13	6	20		28	14	1	11		28	14	1	10	
Testicular involvement	No	120	94	75	96	0.87	167	95	28	93	0.44	165	94	30	100	0.76	187	95	8	89	0.40	185	94	10	100	1.00
	Unilateral	6	5	2	3		7	4	1	3		8	5	0	0		7	4	1	11		8	4	0	0	
	Bilateral	2	2	1	1		2	1	1	3,3		3	2	0	0		3	2	0	0		3	2	0	0	
Immunophenotype	pro-B ALL	9	7	3	4	0.57	10	6	2	7	0.8	11	7	1	4	0.19	12	6	0	0	0.55	12	6	0	0	0.60
	common ALL	88	72	52	68		118	69	22	76		123	72	17	59		133	70	7	78		133	70	7	70	
	prae-B ALL	21	17	18	24		34	20	5	17		29	17	10	35		38	20	1	11		37	20	2	20	
	biphenotypic	5	4	3	4		8	5	0	0		7	4	1	3		7	4	1	11		7	4	1	10	
	unknown	5		2			6		1			6		1			7		0			7		0		
PBC	<1/µl	15	12	7	9	0.601	20	12	2	7	0.64	17	10	5	17	0.54	22	12	0	0	0.87	22	12	0	0	0.26
	≥1-<10.000/µl	84	67	49	65		115	66	18	64		114	67	19	63		126	66	7	78		127	67	6	60	
	≥10.000/µl	26	21	20	26		38	22	8	29		40	23	6	20		44	23	2	22		42	22	4	40	
	unknown	3		2			3		2			6		0			5		0			5		0		
	Median, 95%CI	1299	796 - 2359	1939	910 - 3425	0.29	1800	966 - 2366	2204	886 - 3566	0.53	1573	906 - 2358	2015	459 - 7418	0.90	1682	936 - 2346	1976	999 - 43840	0.56	1686	895 - 2351	1673	536 - 25209	0.32
Strategic group	S2	87	68	44	56		115	65	16	53	0.15	117	67	14	47	0.11	124	63	7	78	0.88	123	63	8	80	0.72
	S3	15	12	16	21		23	13	8	27		25	14	6	20		30	15	1	11		30	15	1	10	
	S4	26	20	18	23		38	22	6	20		34	19	10	33		43	22	1	11		43	22	1	10	
Outcome	CCR	61	48	34	44	0.40	82	47	12	40	0.63	83	47	11	37	0.31	89	45	5	56	1.00	88	45	6	60	0.85
	2nd relapse	28	22	33	42		43	24	12	40		45	26	10	33		52	26	3	33		53	27	2	20	
	TRD	10	8	5	6		13	7	2	7		13	7	2	7		15	8	0	0		14	7	1	10	
	Secondary malignancy	2	2	0	0		2	1	0	0		2	1	0	0		2	1	0	0		2	1	0	0	
	Non-Response	23	18	10	13		29	16	4	13		29	17	4	13		32	16	1	11		32	16	1	10	
	Induction death	4	3	3	4		7	4	0	0		4	2	3	10		7	4	0	0		7	4	0	0	
Cytologic response	after 1st course (F1)	60	49	26	35	0.079	74	44	12	40	0.26	80	47	6	22	**0.023**	82	43	4	44	0.83	82	44	4	40	0.70
	after 2nd course (F2)	29	24	27	36		47	28	9	30		46	27	10	37		52	28	4	44		51	27	5	50	
	after 3rd course	9	7	8	11		15	9	2	7		11	6	6	22		17	9	0	0		17	9	0	0	
	after 4th course	2	2	4	5		3	2	3	10		5	3	1	4		6	3	0	0		6	3	0	0	
	non-response (no CR)	23	19	10	13		29	17	4	13		29	17	4	15		32	17	1	11		32	17	1	10	
	unknown	5		3			8		0			5		3			8		0			8		0		
MRD after F2	$<10^{-4}$	25	33	18	45	0.68	36	36	7	50	0.37	40	39	3	25	0.08	39	36	4	57	0.38	38	36	5	63	0.58
	10^{-4} - $<10^{-3}$	12	16	5	13		15	15	2	14		16	16	1	15		16	15	1	14		16	15	1	13	
	10^{-3} - $<10^{-2}$	18	24	8	20		22	22	4	29		25	24	1	23		24	22	2	29		25	23	1	13	
	$\geq 10^{-2}$	20	27	9	23		28	28	1	7		22	21	7	25		29	27	0	0		28	26	1	13	
	unknown	53		38			75		16			73		18			89		2			89		2		
ETV6-RUNX1	positive	27	79	4	5	0.001	28	16	3	10	0.58	30	17	1	3	0.055	31	16	0	0	0.19	31	16	0	0	0.17
	negative	99	21	74	95		146	84	27	90		144	83	29	97		164	84	9	100		163	84	10	100	
	unknown	2		0			2		0			2		0			2		0			2		0		
MLL-AFF1	positive	5	4	1	1	0.41	5	3	1	3	1.00	6	3	0	0	0.30	6	3	0	0	0.59	6	3	0	0	1.00
	negative	121	96	77	99		169	97	29	97		168	97	30	100		168	97	9	100		188	97	10	100	
	unknown	2		0			2		0			2		0			2		0			2		0		
BCR-ABL	positive	5	4	0	0	0.16	5	3	0	0	1.00	5	3	0	0	0.35	5	3	0	0	0.63	5	3	0	0	1.00
	negative	121	96	78	100		169	97	30	100		169	97	30	100		169	97	9	100		189	97	10	100	
	unknown	2		0			2		0			2		0			2		0			2		0		
High hyperdiploid	positive	17	13	24	31	0.002	33	19	8	27	0.32	31	18	10	33	**0.046**	37	19	4	44	0.08	39	20	2	20	1.00
	negative	111	87	54	69		143	81	22	73		145	82	20	67		160	81	5	56		157	80	8	80	
iAMP21	positive	6	5	4	5	1.00	7	4	3	10	0.16	10	6	0	0	0.18	10	5	0	0	0.49	9	5	1	10	0.40
	negative	122	95	74	95		169	96	27	90		166	94	30	100		187	95	9	100		187	95	9	90	
***TP53* alteration**	positive	16	13	7	9	0.43	20	12	3	10	1.00	20	12	3	10	1.00	22	12	1	11	1.00	23	12	0	0	0.61
	negative	108	87	69	91		151	88	26	90		151	88	26	90		169	89	8	89		167	88	10	100	
	unknown	4		2			5		1			5		1			6		0			6		0		
***IKZF1* deletion**	positive	37	30	28	37	0.310	54	32	11	38	0.52	56	33	9	31	1.00	61	32	4	44	0.48	61	32	4	40	0.73
	negative	87	70	48	63		111	68	18	62		115	67	20	69		130	68	5	56		129	68	6	60	
	unknown	4		2			5		1			5		1			6		0			6		0		

Abbreviations: BM, bone marrow; PBC, peripheral blast cell count; CI, confidence interval; CCR, continuous complete remission; TRD, therapy related death; CR, clinical remission, MRD, minimal residual disease. Definitions: Time point of relapse; Very early, <18 months after diagnosis; Early, >18 months after diagnosis <6 months after regular completion of initial treatment; Late, >6 months after regular completion of initial treatment.

Table S7. Multivariate analysis of overall survival for *KRAS* mutations – final model

Analysis	Variable		n	Hazard ratio	95%CI	P-value
Univariate						
	KRAS	Wildtype	176	1.00		
		Mutation	30	1.64	1.00 - 2.71	0.052
Multivariate						
Model 1	*KRAS*	Wildtype	176	1.00		
		Mutation	30	0.96	0.56 - 1.62	0.87
	Time point of relapse	Late	119	1.00		
		Early	43	3.05	1.79 - 5.19	<0.001
		Very early	44	8.28	4.98 - 13.75	<0.001
Model 2	*KRAS*	Wildtype	171	1.00		
		Mutation	29	0.99	0.58 - 1.67	0.96
	Time point of relapse	Late	114	1.00		
		Early	42	3.23	1.89 - 5.51	<0.001
		Very early	44	8.29	4.98 - 13.80	<0.001
	IKZF1 deletion	Negative	135	1.00		
		Positive	65	1.75	1.15 - 1.67	0.009

Table S8. Allele specific PCR analyses of 'wildtype' diagnostic samples.

Patient Number	Mutation at relapse	Gender	Cytogenetics	Mutation level at diagnosis	Assay sensitivity
1	KRAS G12D	F	HHD	1.00E-03	5.00E-04
2	KRAS G12V	M	Negative	1.00E-03	5.00E-04
3	KRAS G12V	M	+/++21	Negative	1.00E-03
4	KRAS G12V	M	Negative	1.00E-01	1.00E-03
5	KRAS G12V	F	Negative	1.00E-03	1.00E-03
6	KRAS G12V	F	Negative	1.00E-02	1.00E-03
7	NRAS G12D	F	Negative	1.00E-02	1.00E-02
8	NRAS G12D	F	Not Known	Negative	1.00E-02
9	NRAS G12V	M	+/++21	Negative	1.00E-04
10	NRAS G12D	M	Negative	5.00E-04	5.00E-04
11	NRAS G13D	M	HHD	Negative	1.00E-03
12	NRAS G12D	M	+/++21	1.00E-02	1.00E-02
13	NRAS G12D	F	Negative	Negative	1.00E-02
13	NRAS G13D	F	Negative	Negative	1.00E-02
14	NRAS G13D	F	HHD	Negative	1.00E-03

Table S9. Clinical characteristics of persisting versus gained Ras pathway mutations.

Parameter	Category	Mutation status between initial and relapse diagnosis				
		Gain at relapse		Persisting		
		n	%	n	%	p -value
	Total	25		22		
Gender	Male	15	60	17	77	0.23
	Female	10	40	5	23	
Age at initial diagnosis	<5 years	13	52	10	48	0.42
	5 - 10 years	3	12	6	29	
	≥10 years	9	36	5	24	
Age at relapse diagnosis	<5 years	6	24	4	18	0.70
	5 - 10 years	8	32	10	46	
	≥10 years	11	44	8	36	
Time point of relapse	Very early	9	36	3	14	0.23
	Early	7	28	9	41	
	Late	9	36	10	45	
Site of relapse	BM isolated	20	80	15	68	0.50
	BM combined	5	20	7	32	
CNS involvement	No	21	84	16	73	0.48
	Yes	4	16	6	27	
Immunophenotype	pro-B ALL	0	0	0	0	
	common ALL	17	71	19	86	0.14
	prae-B ALL	7	29	2	9	
	biphenotypic	0	0	1	5	
	unknown	1				
PBC	<1/µl	1	4	3	14	0.36
	≥1-<10.000/µl	16	67	11	50	
	≥10.000/µl	7	29	8	36	
	unknown	1				
Strategic group	S2	11	44	14	64	0.22
	S3	5	20	5	23	
	S4	9	36	3	14	
Outcome	CCR	10	40	11	11	0.21
	2nd relapse	8	32	6	27	
	TRD	1	4	2	9	
	Secondary malignancy	0	0	0	0	
	Non-Response	6	24	1	5	
	Induction death	0	0	2	9	
ETV6-RUNX1	positive	1	4	2	9	0.59
	negative	24	96	20	91	
High hyperdiploid	positive	7	28	7	32	1.00
	negative	18	72	15	68	

Abbreviations: BM, bone marrow; PBC, peripheral blast cell count; CCR, continuous complete remission; TRD, therapy related death; CR. Definitions: Time point of relapse: Very early, <18 months after diagnosis; Early, >18 months after diagnosis <6 months after regular completion of initial treatment; Late, >6 months after regular completion of initial treatment.

Table S10. Enrichment of *KRAS* G12D and G12V subpopulations during therapy.

	Diagnosis (%)	Day 28
Leukaemia cells (%)	96.0	3.72
KRAS G12D mutated cells (%)	8.06 (SD, 3.47)	3.66 (SD, 1.32)
G12D mutated leukaemia (%)	**8.39 (SEM, 2.09)**	**98.39 (SEM, 20.5)**
KRAS G12V mutated cells (%)	2.32 (SD, 2.26)	0.48 (SD, 0.07)
G12V mutated leukaemia (%)	**2.42 (SEM, 1.36)**	**12.93 (SEM, 1.07)**

TAQMAMA PCR for G12D and G12V was performed on diagnostic and days 28 samples from one patient and % mutated cells were related to levels of leukaemia as quantified by FLOW MRD. There was a significant increase in the % of mutant leukaemic cells within the total leukaemic population at day 28 compared to levels in the diagnostic sample for both *KRAS* G12D* and *KRAS* G12V**.

Table S11. Clonal size of *NRAS/KRAS* mutations in paired 1st and 2nd relapse samples.

Patient ID	Strategic group at first relapse	Source	Leukemic cells (%)	Source	Leukemic cells (%)	Gene/Exon	Mutation		Clonal size / Allele frequency			
							Codon	Exon position nucleotide	1st relapse	assessed by	2nd relapse	assessed by
1	S2	BM	91	BM	87	*NRAS* ex1	G12A	52 G>C	Dominant clone	1	Dominant clone	1
2	S2	BM	92	BM	97	*NRAS* ex2	Q61H	72 A>C	Dominant clone	1	Dominant clone	1
10	S2	BM	91	BM	94	*KRAS* ex1	G13D	49 G>A	Dominant clone	1	Dominant clone	1
14	S2	BM	95	BM	98	*KRAS* ex1	G12D	46 G>A	Dominant clone	1	Dominant clone	1
11	S2	BM	93	BM	96	*KRAS* ex1	G12V	46 G>T	Dominant clone	1+2	Small subclone	1+2
3	S2	BM	66	BM	94	*NRAS* ex1	G12A	52 G>C	Dominant clone	1	Dominant clone	1
4	S2	BM	98	BM	97	*NRAS* ex1	G13D	55 G>A	Dominant clone	1	Dominant clone	1+2
12	S2	BM	90	Blood	unknown	*KRAS* ex1	G12D	46 G>A	Dominant clone	1+2	Dominant clone	1+2
5	S3	BM	97	Mammary tissue	+++	*NRAS* ex1	G13D	55 G>A	Dominant clone	1+2	Dominant clone	1+2
6	S3	BM	81	BM	<50	*NRAS* ex1	G12D	52 G>A	Dominant clone	1	Possibly dominant*	1+2
7	S3	BM	85	BM	97	*NRAS* ex1	G13D	55 G>A	Dominant clone	1	Dominant clone	1
8	S4	BM	64	BM	84	*NRAS* ex2	Q61R	71 A>G	Small subclone	1	Dominant clone	1
9	S4	BM	86	BM	30	*NRAS* ex1	G12D	52 G>A	Dominant clone	1+2	Possibly dominant*	1+2
13	S4	BM	89	BM	59	*KRAS* ex1	G12D	46 G>A	Dominant clone	1+2	Dominant clone	1+2
15	S4	BM	91	BM	83	*NRAS* ex2	Q61L	71 A>T	Dominant clone	1	Dominant clone	1
16	S4	BM	90	Lymph node tissue	+++	*KRAS* ex1	G12V	46 G>T	Dominant clone	1+2	Dominant clone	1+2

Abbreviations: BM, bone marrow.

Clonal size was estimated from peak heights of sequencing chromatograms (1) or allele specific PCR (2) in paired 1st and 2nd relapse in relation to the leukemic burden.* in these samples with high levels of contaminating normal cells, clonal size was difficult to interpret.

Figure S1. *NRAS/KRAS* mutation status in presentation and relapse pairs in the S3/S4 risk group.

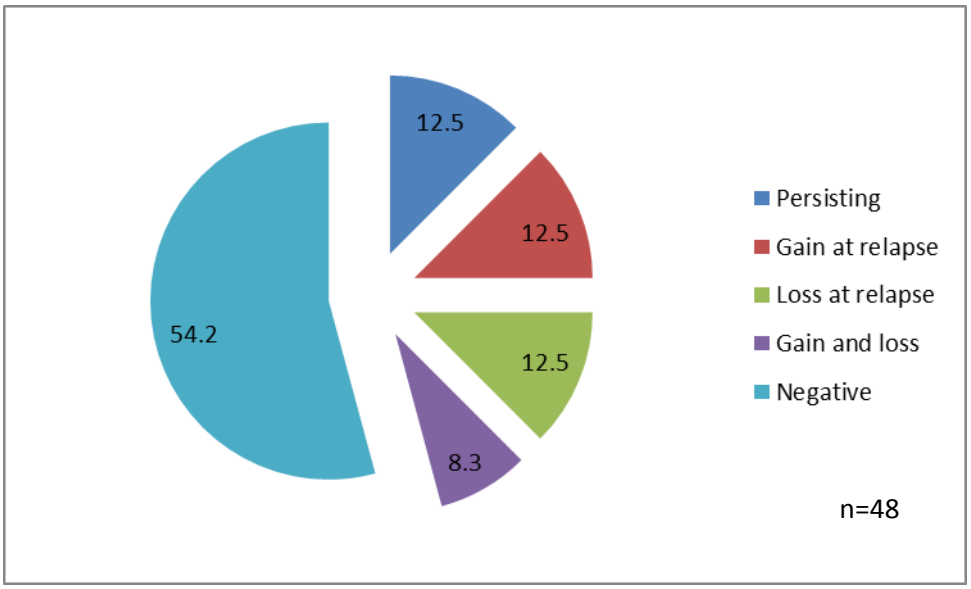
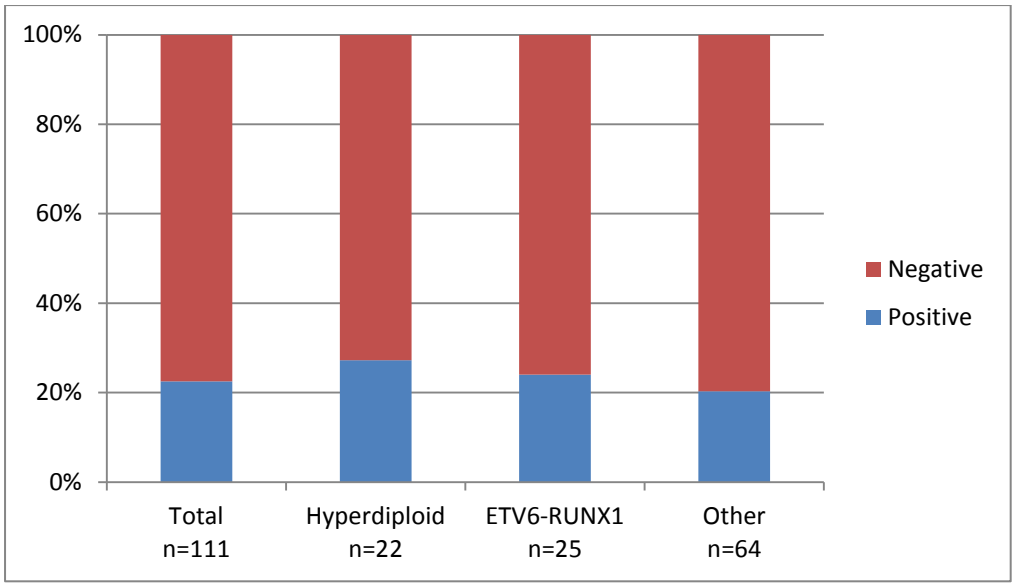
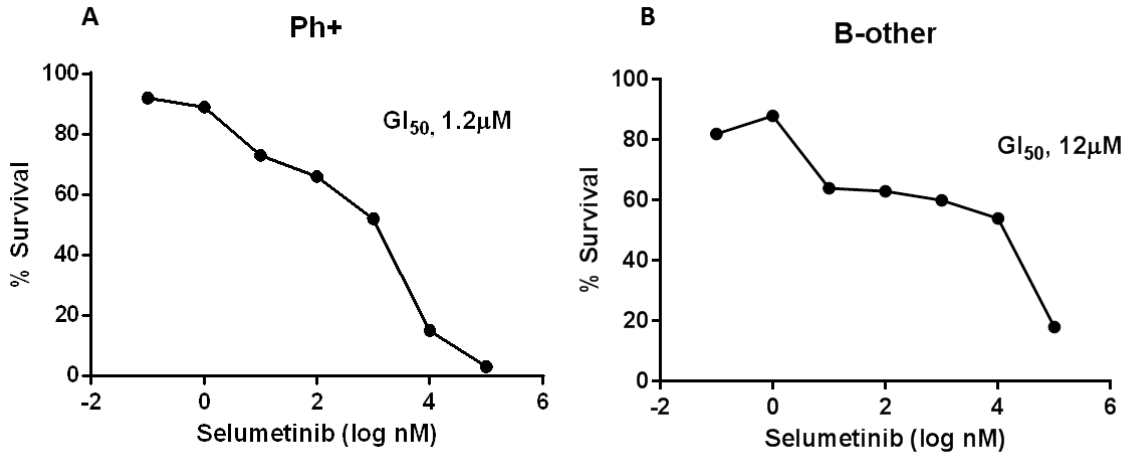


Figure S2. Incidence of low level *KRAS* mutations in a non-relapsing diagnostic cohort, negative for RAS pathway mutations by DHPLC.



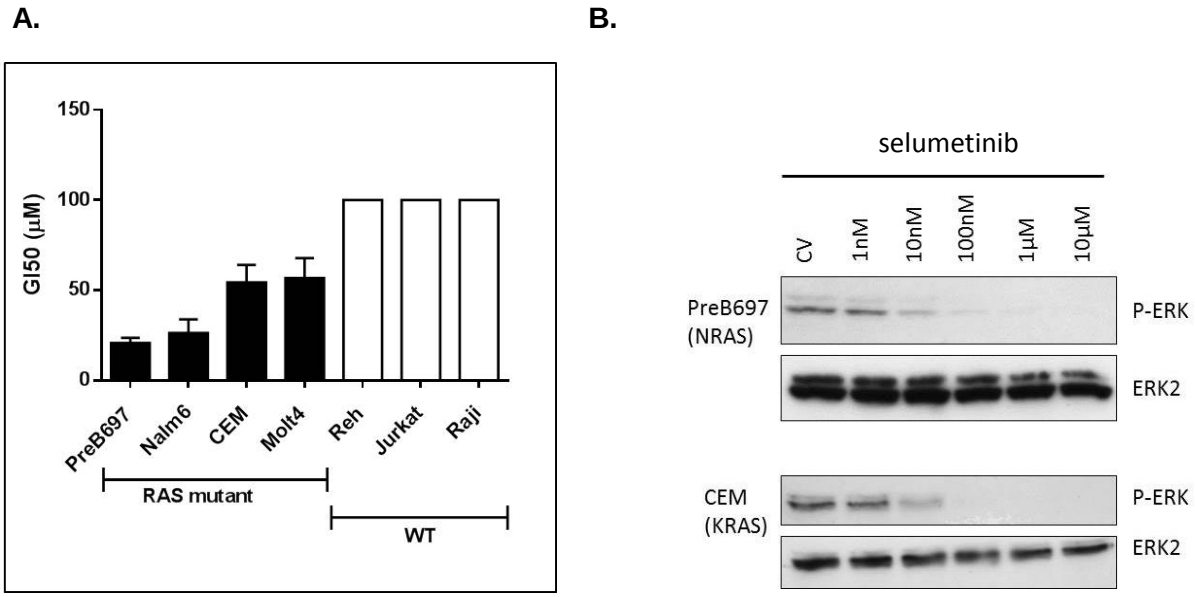
Histogram showing the percentage of diagnostic samples positive and negative by allele specific PCR for *KRAS* G12D and/or G13D and/or G12V, in relation to cytogenetic subgroup.

Figure S3. Selumetinib sensitivity in p-ERK positive, Ras pathway wildtype ALL.



Leukemic cells from diagnostic samples from a Ph+ (A) and B-other (B) ALL were treated with 0.1nM to 100µM selumetinib or control vehicle (DMSO) for 4 days and the cell viability assessed using CellTiter Aqueous One reagent. The GI_{50} values were calculated using GraphPad Prism.

Figure S4: ALL cells lines show increased sensitivity to selumetinib.



A. MTS cell viability assays for selumetinib in a range of RAS mutant and wild-type ALL cell lines. The GI50 values for all three wild type cell lines were found to be >100μM whereas the cell lines with mutated RAS were lower. Error bars are SEM from triplicate experiments.
B. PreB697 (*NRAS* mutation) and CEM cells (*KRAS* mutation) were incubated with increasing concentrations of selumetinib for 30 minutes, washed in PBS and blotted for p-ERK and ERK2 expression. ERK phosphorylation was inhibited in a dose dependent manner in both cell lines.