

# Epigenetic inactivation of *TWIST2* in acute lymphoblastic leukemia modulates proliferation, cell survival and chemosensitivity

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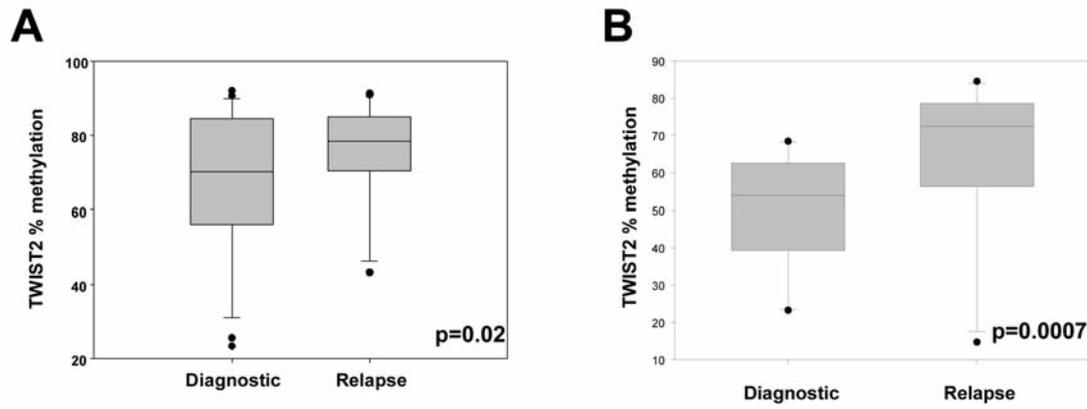
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Citation: Thathia SH, Ferguson S, Gautrey HE, van Otterdijk SD, Hili M, Rand V, Moorman AV, Meyer S, Brown R, and Strathdee G. Epigenetic inactivation of *TWIST2* in acute lymphoblastic leukemia modulates proliferation, cell survival and chemosensitivity. *Haematologica* 2012;97(3):371-378. doi:10.3324/haematol.2011.049593

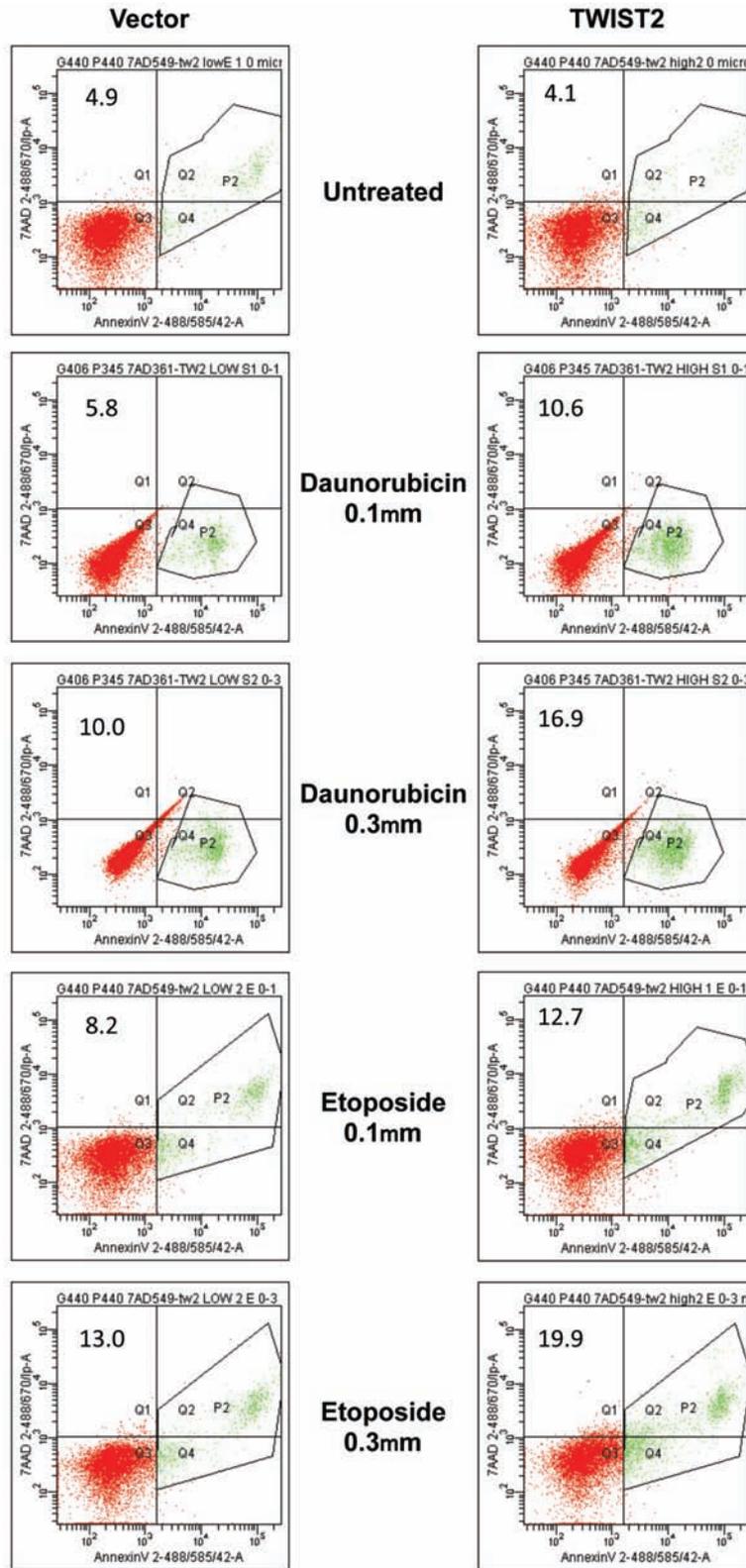
Online Supplementary Table S1. Analysis of *TWIST2* promoter methylation using pyrosequencing.

Sample	CpG1*	CpG2	CpG3	CpG4	Hypermethylated by COBRA
ALL 1	86,06	86,02	83,04	84,83	Yes
ALL 2	88,07	88,8	87,66	90,06	Yes
ALL 3	90	89,87	84,88	89,44	Yes
ALL 4	82,44	80,79	78,07	84,03	Yes
ALL 5	4,9	7,56	7,5	7,95	No
ALL 6	74,18	73,53	72,62	75,19	Yes
ALL 7	75,46	78,13	67,1	66,61	Yes
ALL 8	84,05	83,26	78,75	82,37	Yes
ALL 9	84,39	85,75	82,97	84,66	Yes
ALL 10	2,92	3,21	3,81	3,78	No
ALL 11	83,53	81,9	65,25	68,48	Yes
ALL 12	15	16,31	16,02	14,95	No
ALL 13	5,07	5,22	5,45	6,23	No
ALL 14	8,67	6,86	8,9	7,74	No
ALL 15	78,05	85,14	80,93	85,28	Yes
Child ALL 1	75,16	72,95	66,5	71,95	Yes
Child ALL 2	18,5	32,12	33,05	11,66	No
Child ALL 3	80,12	82,14	69,71	82,55	Yes
Child ALL 4	4,42	4,76	5,39	5,14	No
Child ALL 5	55,79	71,64	56,28	56,08	Yes
Child ALL 6	77,88	78,26	71,33	71,81	Yes
Child ALL 7	23,63	28,96	28,53	22,74	No
Child ALL 8	9,11	9,66	13,94	14,51	No
Child ALL 9	3,33	5	4,31	4,9	No
Child ALL 10	80,9	83,3	69,81	69,39	Yes
Child ALL 11	76,5	72,7	68,53	74,25	Yes
Child ALL 12	75,66	73,41	70,25	75,08	Yes
Child ALL 13	78,4	75,83	63,06	63,18	Yes
Child ALL 14	74,97	77,34	73,61	75,34	Yes
Child ALL 15	4,34	6,96	4,86	8,39	No
CLL 1	9,03	10,56	9,27	9,37	No
CLL 2	14,67	23,03	12,14	13,12	No
CLL 3	22,95	24,65	17,66	13,03	No
CLL 4	31,74	48,55	20,07	47,25	No
CLL 5	24,38	32,57	49,62	47,7	No
CLL 6	41,93	42,87	14,76	11,12	No
CLL 7	35,78	18,51	28,63	23,85	No
CLL 8	13,99	27,81	11,07	11,96	No
CLL 9	15,9	20,86	17,8	15,11	No
CLL 10	11,3	32,06	10,52	10,92	No
CLL 11	43,41	70,18	45,25	44,79	No
CLL 12	5,25	4,68	4,75	4,53	No
CLL 13	20,59	42,57	12,66	11,19	No
CLL 14	20,76	31,39	18,16	13,7	No
CLL 15	19,44	17,93	50,12	16,69	No
100% IVM	94,82	90,3	90,57	98,31	Yes
PBL1	2,33	2,91	2,36	4,12	No
PBL2	2,43	4,34	2,84	4,83	No
PBL3	2,73	4,55	2,37	4,72	No
PBL4	2,90	3,08	2,91	4,80	No
PBL5	3,24	4,69	3,52	5,46	No
PBL6	5,70	6,68	4,44	7,17	No
PBL7	3,80	4,33	3,81	5,47	No
PBL8	2,67	3,50	2,61	4,60	No
PBL9	2,73	4,58	2,73	4,47	No
PBL10	4,41	4,62	5,01	6,12	No

\*Methylation levels at individual CpG sites as defined by pyrosequencing analysis. Boxes colored red indicate methylation levels of 50% or greater, whereas blue is <50%. Samples were defined as hypermethylated by either COBRA or pyrosequencing when the majority of sites exhibited methylation levels of 50% or greater.



**Online Supplementary Figure S1.** *TWIST2* methylation is increased in relapse samples from patients with ALL. (A) Paired diagnostic and relapse samples (n=22) were analyzed for *TWIST2* methylation using pyrosequencing. Methylation levels in the paired diagnostic samples were found to be high (average 67%), however the paired relapse samples exhibited a further significant increase in methylation (average 74%,  $P=0.02$ , paired t-test). (B) Increased *TWIST2* methylation was restricted to samples exhibiting lower *TWIST2* methylation at diagnosis. Results of *TWIST2* methylation analysis using pyrosequencing are shown for paired samples in which the diagnostic samples had a methylation level of below 70% (n=10). In these cases there was a far more pronounced increase in *TWIST2* methylation in the paired relapse samples ( $P=0.0007$ , paired t-test).



**Online Supplementary Figure S2.** *TWIST2* expression results in increased sensitivity to chemotherapeutic agents. Nalm6 cells stably transfected with the pIRES-EGFP vector (Vector column) alone or the *TWIST2*-expressing vector (*TWIST2* column) were treated with the indicated dose of either etoposide or daunorubicin and assayed 24 h later for apoptosis (annexin V staining) by flow cytometry. The percentage of annexin V positive cells (cells in box P2) is indicated in each plot. Annexin V-positive cells appear lower down on plots following daunorubicin treatment due to the compensation required to account for autofluorescence of this drug.