Akt is activated in chronic-lymphocytic-leukemia cells and delivers a pro-survival signal: therapeutic potential of Akt inhibition

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Supplementary Methods

Knockdown of Akt1 with siRNA

Here, 1×10^7 CLL cells were transfected with siRNA duplexes targeting three different regions of the mRNA for human Akt1. To do this, CLL cells were mixed with 100 mL transfection solution (Amaxa) and a total of 2 mg of siRNA duplexes (IDT) or with 2 mg of non-specific control siRNA (IDT) before nucleofection using program X-01. Cells were subsequently cultured at 5×10^6/mL for 72 h, after which levels of Akt1 and apoptosis or p-GSK-3a and GSK-3a, MCL1 and BCL2 were measured as described in the main text.

The sequences of siRNA duplexes used for transfection to knock down human Akt1 were:-

i, 5'-CCCUCAGAACAAUCCGAUUCACGTA-3';
ii, 5'-AGAGGAGCAAGGUUUAAAUUUGUTA-3';
iii, 5'-GGCAGCACGUGUACGAGAAGAAGCT-3'.

Non-specific control:-
5'-CUUCCUCUCUUUCUCUCCCUUGUCA-3'

Supplementary Table 1. CLL patients' characteristics.

No.	Sex	Age	Prior therapy	Stage (Binet)	WBC (10^9/L)	CD38*	IgVH status¶	Chromosomal abnormalities	p53#	LC50±SD† (μM)
1	M	50	yes	A	35	78	1.65	13q-	N	0.46±0.11
2	F	79	no	A	31.8	3	14.01	13q-	N	1.01±0.21
3	M	43	no	A	153	29	1.75	None	D	0.59±0.17
4	M	65	no	A	183	40	1.35	13q-	D	0.71±0.05
5	M	56	no	A	135	nd‡	0	None	D	0.73±0.14
6	M	60	no	B	377	18	0	+12	N	0.95±0.1
7	F	60	yes	A	45	nd	3.06	None	N	0.52±0.07
8	M	65	no	B	194	nd	2.46	13q-	D	0.64±0.08
9	M	45	yes	C	28.2	nd	0	17p-	D	0.96±0.05
10	M	77	yes	C	108	53	0	11q-	D	0.76±0.15
11	F	70	yes	A	18	3	0.34	17p-	D	1.62±0.2
12	M	56	no	A	138	57	0	17p-,13q-	D	1.04±0.04
13	F	62	no	A	31.5	22	7.08	13q-	N	0.34±0.05
14	F	61	yes	nd	183	nd	0.34	+12	N	0.9±0.14
15	F	79	no	C	128	28	10.47	13q-, +12	N	0.53±0.18
16	M	62	no	A	45	40	0	+12	N	0.26±0.12
17	M	57	no	C	171	7	0	11q-	N	0.65±0.52
18	M	54	no	B	257	1	0.27	13q-	N	0.23±0.06
19	M	74	yes	B	91	76	0	17p-	D	1.14±0.12
20	F	57	no	A	95	nd	8.15	13q-	N	0.25±0.04
21	F	73	no	A	63	8	0.68	13q-	D	0.95±0.1
22	F	40	no	A	90.8	5	nd	13q-	N	0.44±0.17
23	M	39	no	A	82	6	4.47	13q-	D	1.08±0.2
24	M	47	no	A	153	29	1.75	None	N	nd
25	M	76	no	B	217	28	2.43	None	N	nd
26	M	76	no	B	86	28	0.69	None	N	nd

**CD38 expression is expressed as percent positive cells.31 ¶ IgVH status refers to IgVH mutation expressed as the percent deviation from the germ-line sequence.31. # p53 refers to functional probing of the ATM-p53-p21 pathway using ionising radiation5, 52. N = functionally normal p53 response, D = functional impairment of the ATM-p53-p21 pathway associated with mutations in TP53 or ATM. † LC50 values were derived from dose-response experiments using the Akt inhibitor A-443654. ‡ nd: unknown/not done.*

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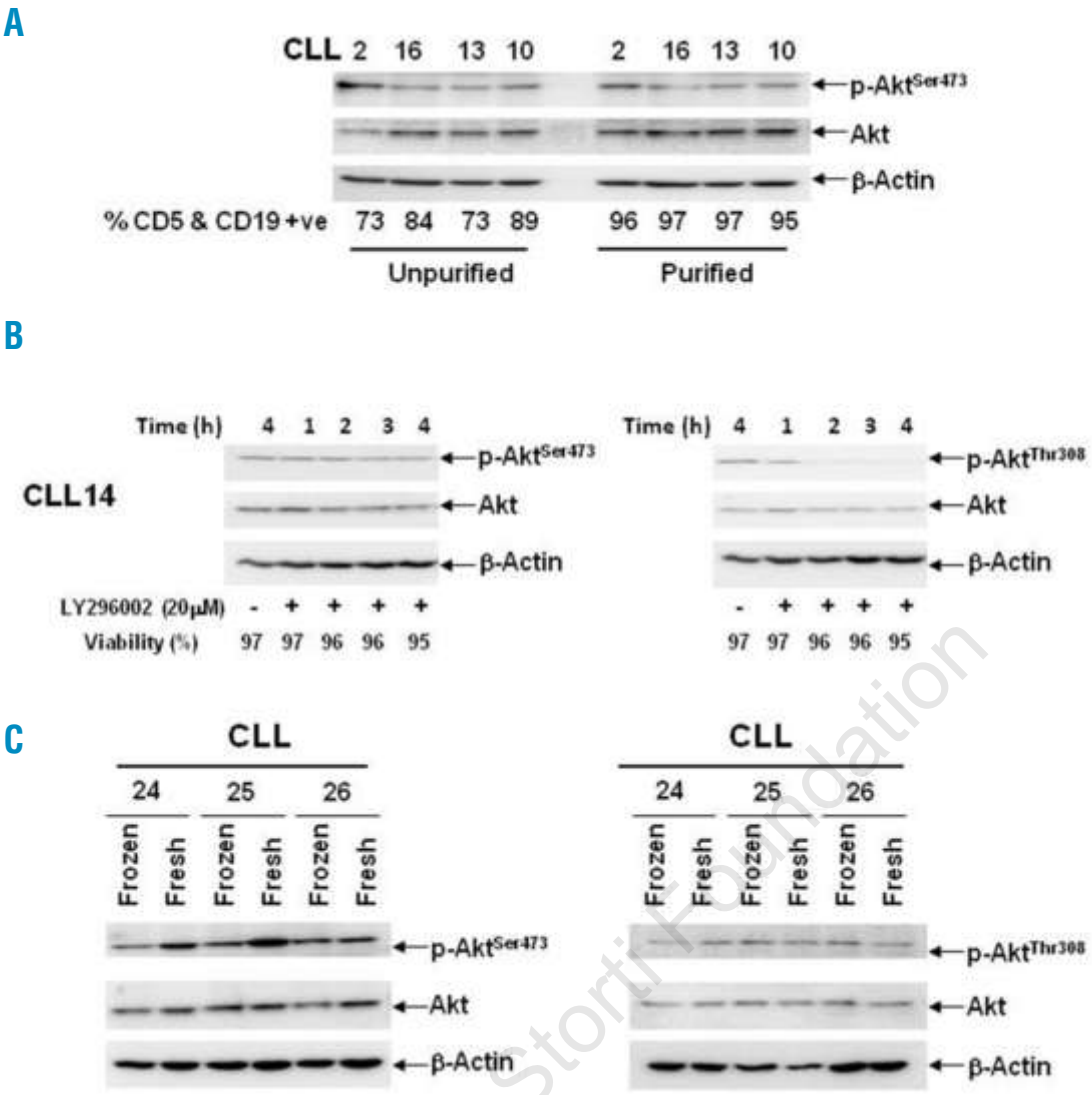

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Supplementary Figure S1. Phospho-Akt levels are similar in CLL preparations before and after purification and in both fresh and frozen samples. In (A), phospho-Akt levels were compared in CLL samples before and after depletion of contaminating cells (*see Text*) (n=8, four representative clones shown). In (A), (B) and (C), material extracted from $1x10^6$ CLL cells in lysis buffer containing 1% Triton X-100 was sonicated before SDS-PAGE. In (B), cells (4×10^6/mL) were incubated with a specific PI-3K inhibitor (LY296002) for 1-4 h, and Western blotted for p-Akt1 (Ser473) (left panel, rabbit antibody) or p-Akt1 (Thr308) (right panel, mouse monoclonal antibody). Viability was measured by Trypan blue exclusion. In (C), the effect of freezing/thawing on p-Akt (as detected by two different antibodies) in unstimulated CLL cells is shown.