SUPPLEMENTARY APPENDIX

The PDK1 master kinase is over-expressed in acute myeloid leukemia and promotes PKC-mediated survival of leukemic blasts

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Supplemental Methods

Western blot analysis

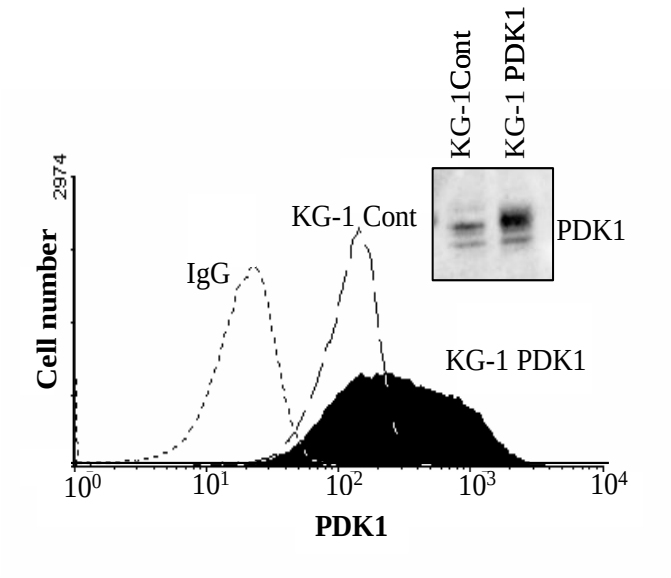
For membrane protein fractionation, samples were extracted on ice in homogenization buffer (sucrose 0.25M,*N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid–KOH 10mM, magnesium acetate 1mM, ethylenediaminetetraacetic acid 0.5mM, ethyleneglycoltetraacetic acid 0.5mM, β-mercaptoethanol 10mM, 20 μg/mL DNase, pH 7.2) and membrane fractions pelleted by centrifugation at 16 000*g* for 1 hour at 4°C. Cytosolic proteins (supernatant) were aspirated and the membrane pellet was solubilized in homogenization buffer containing 1% Triton X-100. Lysates were quantified for protein concentration using Bradford's reagent and run on 4-12% SDS gels using the Nupage blotting system (Life Technologies). Blots were probed with the following antibodies: PDK1, pSGK, Total SGK pAKT^{308}, pAKT^{473}, total AKT, pS6$^{235/236}$, total S6, pPKC^{514} (Cell Signalling, Danvers, MA), ACTIN (Abcam), CD45 (BD Biosciences) and PKCα (Santa Cruz Biotechnology, CA). Recombinant protein standards for PDK1, PKCα and AKT (Nventa, San Diego, CA) were included on each gel to allow quantification of these proteins using AIDA image analyser v4.22 (Raytest, Straubenhardt, Germany). Patients overexpressing PDK1 (relative to normal bone marrow CD34^{+} cells) were denoted PDK1^{Hi} and the remainder statistically in the normal range were termed PDK1^{Norm}

Supplementary Table 1

	Total	PDK1Norm	PDK1 High	p-value
Overall AML15	66	38	28	
Age				
0-14	0	0	0	0.2 †
15-29	9	4	5	
30-39	15	8	7	
40-49	16	10	6	
50-59	17	10	7	
60-69	9	6	3	
70+	0	0	0	
Median (range)	46.5 (18-67)	48 (22-67)	44 (18-62)	
Sex				
Female	31	19	12	0.6
Male	35	19	16	
Diagnosis				
de Novo	65	37	28	0.4
Secondary	1	1	0	
WBC				
<10	6	3	3	0.17 †
10-49.9	28	19	9	
50-99.9	16	9	7	
100+	16	7	9	
Median (range)	47.3 (1.7-255.8)	42 (5.5-209)	64.3 (1.7-255.9)	
Performance Status				
WHO 0	33	18	15	0.6 §
WHO 1	24	16	8	
WHO 2	4	3	1	
WHO 3	5	1	4	
Cytogenetics				
Favourable	14	8	6	0.8 §
Intermediate	42	23	19	
Adverse	2	1	1	
Unknown	8	6	2	
FAB Type				
M4	41	26	15	0.5
M5	25	14	11	
Induction treatment				
ADE	22	8	14	0.05
DA/DAT	27	18	9	
FLAG-Ida	17	12	5	

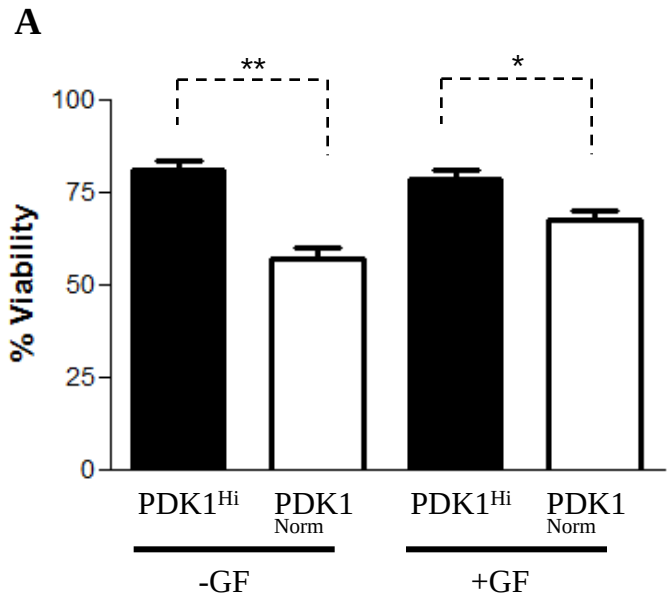
Supplementary Table 1. Patient variables in analysis by PDK1 status. AML patient material was derived from the UK MRC/NCRI AML15 national trials. Categorical variables were compared using chi-squared tests, and Mantel-Haenszel tests (§). Continuous variables were analyzed by Wilcoxon rank sum tests (†). ADE (Daunorubicin, Cytarabine, Etoposide), DA/DAT (Daunorubicin, Cytarabine/Daunorubicin, Cytarabine, Thioguanine), FLAG-Ida (Fludarabine, Cytarabine, Idarubicin, G-CSF).

Supplementary Fig. S1



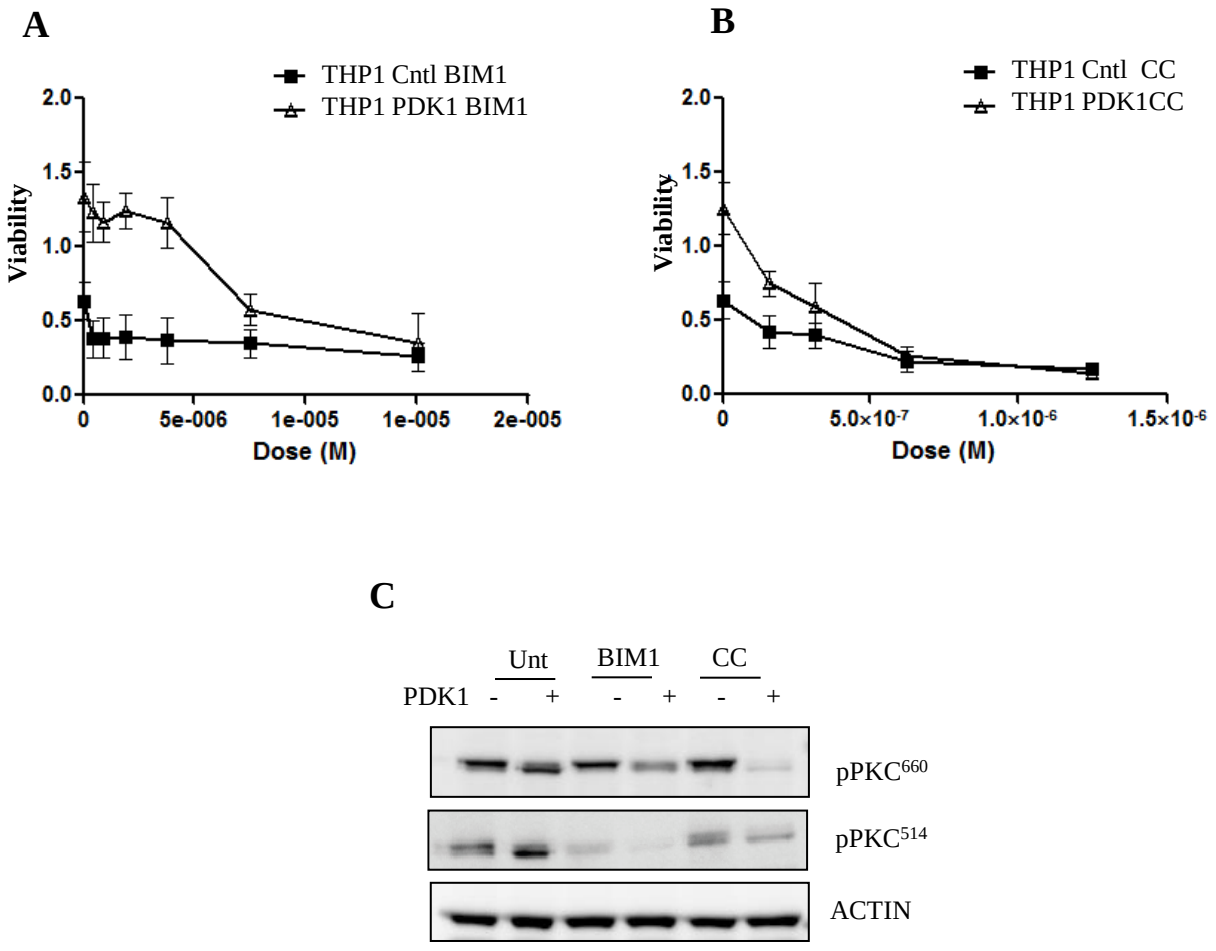
Supplementary Figure S1. Validation of intracellular PDK1 staining using KG-1 cells overexpressing PDK1 (KG-1 PDK1) compared to KG-1 empty vector control cells. Cytometric analysis showed a 4.5-fold overexpression of PDK1 in these cells compared to the empty vector control and this was confirmed by western blot analysis (inset).

Supplementary Fig. S2



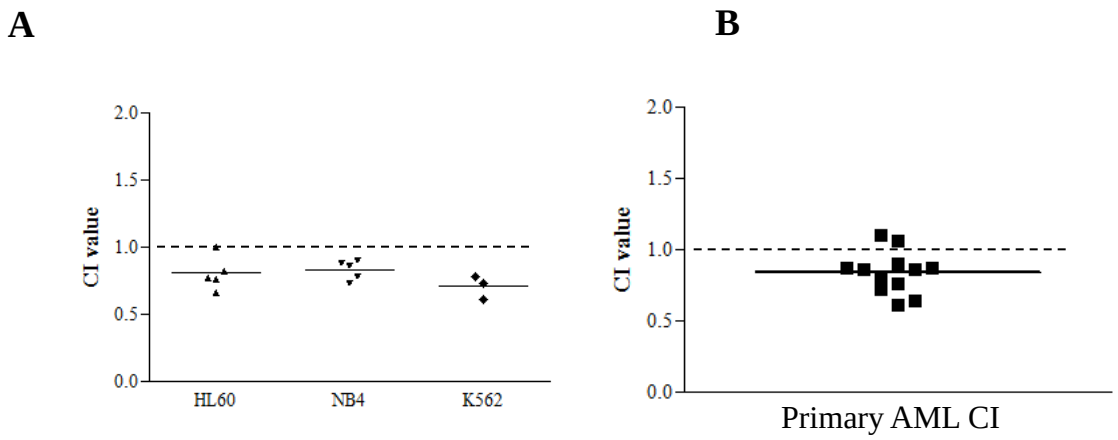
Supplementary Figure 2. Addition of cytokine enriched media (+GF) does not overcome advantage of PDK1^{Hi} cell survival. Cell viability by trypan blue exclusion at 48 hours with or without growth factors (IL3, GCSF, SCF, GM-CSF 20ng/ml). *P<0.05, **P<0.001

Supplementary Fig. S3



Supplementary Figure 3. (A) Dose-response data showing effect on viability of BIM1 (Bisindolylmalelimide) and (**B**) CC (Chelerythrine) in THP1^{Cntl} and THP1^{PDK1} overexpressing cell lines. Viability measured as OD at 495nm by MTS assay under serum deprived conditions. (**C**) Western blot analysis of PKC phosphorylation in response to 7.5µM and 0.3µM BIM1 and CC respectively.

Supplementary Fig. S4



Supplementary Figure 4. BX-795 shows moderate synergy with Cytarabine. Combination index (CI) values for synergy between BX-795 and AraC; (values<1.0 indicate synergy) in (**A**) AML cell lines (**B**) Primary AML samples n=12 using fixed molar ratios of 1:2.5 (BX-795:AraC).