Supplementary Materials: CDC25 Inhibition in Acute Myeloid Leukemia–A Study of Patient Heterogeneity and the Effects of Different Inhibitors

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**Table S1.** Clinical and biological characteristics of the 79 AML patients included in the study.

| Patient characteristics | Cell morphology | Cell genetics |
|-------------------------|-----------------|--------------|
| **Age**                 | **FAB classification** | **Cytogenetic** |
| Median (years)          | M0              | 6            |
| Range (years)           | M1              | 21           |
|                         | M2              | 11           |
| **Gender**              | **CD34 expression** | **Flt3 mutations** |
| Females                 | M3              | 2            |
| Males                   | M4              | 18           |
|                         | M5              | 15           |
|                         | n.d.           | 6            |
| **Secondary AML**       | **CD34 expression** | **NPM1 mutations** |
| MDS                     | CD34 expression | ITD          |
| Chemotherapy            | Negative (≤ 20 %) | Wild-type    |
| CM(M)L                  | Positive (> 20 %) | n.d.         |
|                         | n.d.           | 5            |
| **AML relapse**         |                 |              |
|                         | 6              | 6            |

1 Thirteen patients had secondary AML and six patients relapsed AML (two of them relapse of secondary AML).
2 n.d.: not determined
3 15 patients had an adverse cytogenetic abnormalities (i.e. −5; −7; +8; complex karyotype) whereas the nine patients in the favorable risk group had inv(16) (six patients), t(15;17) (two patients) and t(8;21) (one patient).
4 One of the patients has an additional point mutation at D835.
Table S2. Single genes identified in the bioinformatical comparison of responders versus non-responders to CDC25 inhibitors. A total of 81 annotated genes were identified, and only 46 of them have a known function. A major subset of 18 genes encode proteins that are directly involved in cell cycle regulation (1 gene), the function of microtubuli/cytoskeleton (9 genes) and intracellular organelle formation/trafficking (4); 4 additional genes are involved in protein ubiquitination that is important for regulation of cell cycle progression/CDC25 modulation. These genes may thus represent a functional entity that is important for cell cycle progression/mitosis. The remaining genes are transcriptional regulators together with a DNA ligase (6 molecules), surface membrane molecules or surface membrane receptors (7 molecules), and molecules involved in intercellular communication (2 genes) or intracellular signaling (3 molecules). Thus, none of the molecules involved in regulation of the early steps of the cell cycle showed differential expression, but several genes important for mitosis/cell division differed between the two groups.

| DOWNREGULATED IN RESPONDERS TO CDC25 INHIBITORS |
|-----------------------------------------------|
| FJX1  Four jointed box 1 | Important for embryonic development in Drosophila, unknown function in humans. |
| MUC4  MUCIN 4 | Encoding an integral membrane glycoprotein found on the cell surface. |
| SAS6  SAS-6 centriolar assembly protein | SAS6 is necessary for centrosome duplication and functions during procentriole formation; SAS6 functions to ensure that each centriole seeds the formation of a single procentriole per cell cycle. |
| ACOT1  Acyl-CoA thioesterase 1 | Cell metabolism. |
| LOC643176 | |
| ABCA5  ATP-binding cassette, sub-family A, member 5 | A membrane-associated protein that is a member of the superfamily of ATP-binding cassette transporters (ABC1 subfamily). These proteins transport various molecules across extra- and intracellular membranes. |
| LOC642169 | |
| DOK7  Docking protein 7 | The encoded protein can induce autophosphorylation of receptor kinase; the protein may also be implicated in breast cancer development [1, 2]. |
| ANO7  ANOCTAMIN 7 | The protein is a member of the anoctamin family; these proteins are proposed to have eight transmembrane domains with both termini facing the cytoplasm and a C-terminal domain of unknown function. |
| GPBP1L1  GC-rich promoter binding protein 1-like 1 | |
| KIR2DL5A  Killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 5 | Killer cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins classified by the number of extracellular immunoglobulin domains (2D or 3D) and by whether they have a long (L) or short (S) cytoplasmic domain. KIR proteins with the long cytoplasmic domain transduce inhibitory signals upon ligand binding. |
| LOC728667 | |
| DNAI2  Dynein, axonemal, intermediate chain 2 | Belongs to the dynein intermediate chain family and part of the dynein complex of respiratory cilia and sperm flagella (see also FLJ20397). Cytoplasmic dynein is a large minus-end-directed microtubule motor complex, involved in many different cellular processes including intercellular trafficking, organelle positioning, and microtubule organization. Furthermore, dynein plays essential roles during cell division where it is implicated in multiple processes including centrosome separation, chromosome movements, spindle organization, spindle positioning, and mitotic checkpoint silencing [3]. |
| COL3A1  Collagen, type III, alpha 1 | This gene encodes the extracellular fibrillar pro-alpha1 chains of type III collagen. |
| FAM134C  Family with sequence similarity 134, member C | A nuclear respiratory factor-I (NRF-1) transcriptional target expressed in neuroblastoma cells, regulates cellular outgrowth. |
| Gene Symbol | Description |
|-------------|-------------|
| PIP4K2C | Phosphatidylinositol-5-phosphate 4-kinase, type II, gamma |
| LOC728125 | The protein has similarity to phosphatidylinositol 5-phosphate 4-kinase II alpha and II beta isoforms, may play a role in phosphatidylinositol bisphosphate synthesis in the endoplasmic reticulum. |
| DCAF10 (WDR32) | DDB1 and CUL4 associated factor 10 |
| Ceorf59 | AGPAT4 intronic transcript |
| AGPAT4 | 1-acylglycerol-3-phosphate O-acyltransferase 4 |
| LOC730118 | This gene encodes a member of the 1-acylglycerol-3-phosphate O-acyltransferase family. This integral membrane protein converts lysophosphatidic acid to phosphatidic acid, the second step in de novo phospholipid biosynthesis. |
| DCDC5 | Doublecortin domain containing 5 |
| FLJ45983 | GATA3 antisense RNA 1 |
| LOC441131 | The gene can show altered methylation in breast cancer. |
| GPR37L1 | G protein-coupled receptor 37 like 1 |
| LOC647711 | G protein-coupled receptor 37 like 1 |
| LOC388955 | The gene can show altered methylation in breast cancer. |
| LOC100131373 | A member of the forkhead family of transcription factors. |
| CHRNA2 | Cholinergic receptor, nicotinic, alpha 2 |
| KALRN | Kalirin, RhoGEF kinase |
| SOX9 | SRY (sex determining region Y)-box 9 |
| LOC652054 | A DNA binding and transcription-regulating protein |
| LOC653507 | Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, neurotransmitter receptor. |
| LOC126075 | May have a function in vesicle trafficking. |
| LOC731052 | A DNA binding and transcription-regulating protein |
| LOC650013 | Possibly involved in chromatin remodelling/transcriptional regulation [6]. |
| LOC732134 | Possibly important for platelet function/thrombus formation? [7]. |
| LOC728792 | Possibly involved in ubiquitination [4]. |
| LOC730130 | Possibly involved in ubiquitination [4]. |

**UPREGULATED IN RESPONDERS TO CDC25 INHIBITION**

| Gene Symbol | Description |
|-------------|-------------|
| LOC730130 | A member of the forhead family of transcription factors. |
| LOC131857 | A member of the forhead family of transcription factors. |
| FOXE1 | Forkhead box E1 provided |
| LOC126075 | Cholinergic receptor, nicotinic, alpha 2 |
| INO80E | INO80 complex subunit |
| LOC650013 | A DNA binding and transcription-regulating protein |
| LOC731052 | Possibly involved in chromatin remodelling/transcriptional regulation [6]. |
| LOC732134 | Possibly important for platelet function/thrombus formation? [7]. |
| LOC728792 | Possibly important for platelet function/thrombus formation? [7]. |
| Gene Symbol | Gene Name and Description |
|-------------|---------------------------|
| FLJ20397    | Dynein, axonemal, assembly factor 5 |
| LOC653346   | FLJ20397 Dynein, axonemal, assembly factor 5 |
| WDR23       | DDB1 and CUL4 associated factor 11 |
| RAB4A       | RAB4A, member RAS oncogene family |
| MAP2        | Microtubule-associated protein 2 |
| LOC727860   | LOC727860 |
| LOC389676   | LOC389676 |
| NIN         | Ninein (GSK3B interacting protein) |
| LNNR3       | Liver neoplastic nodule remodelling QTL 3 |
| FBXO48      | F-box protein 48 |
| ATG4A       | Autophagy related 4A, cysteine peptidase |
| LOC729260   | LOC729260 |
| MACROD2     | MACRO domain containing 2 |
| ITH3        | Inter-alpha-trypsin inhibitor heavy chain 3 |
| ZGRF1 (C4orf21) | Zinc finger, GRF-type containing 1 |
| LOC100128717 | LOC100128717 |
| LIG3        | Ligase III, DNA, ATP-dependent |
| OGFOD3 (C17orf101) | 2-oxoglutarate and iron-dependent oxygenase domain containing 3 |
| LOC100132932 | LOC100132932 |
| SCG82A2 (MGB1) | Secretoglobin, family 2A, member 2 |
| LOC85391    | LOC85391 |

The protein is essential for the preassembly or stability of axonemal dynein arms (see also DNAI2 above). Cytoplasmic dynein is a large minus-end-directed microtubule motor complex, involved in many different cellular processes including intracellular trafficking, organelle positioning, and microtubule organization. Furthermore, dynein plays essential roles during cell division where it is implicated in multiple processes including centrosome separation, chromosome movements, spindle organization, spindle positioning, and mitotic checkpoint silencing [3].

This gene encodes a WD repeat-containing protein that interacts with the COP9 signalosome, a macromolecular complex that interacts with cullin-RING E3 ligases and regulates their activity by hydrolysing cullin-Nedd8 conjugates. Cullin-Ring E3 ubiquitin ligases are important for actin cytoskeletal organization [8].

Belongs to the Ras superfamily of small GTPases, it is associated with early endosomes and is involved in their sorting/recycling and recycling of receptors from endosomes to the plasma membrane.

Belongs to the microtubule/cytoskeletal-associated protein family, possibly involved in microtubule assembly.

This gene encodes a protein important for centrosomal function and for positioning and anchoring of microtubules.

Unknown

This is a member of the F-box protein family (Fbxs subclass) that is characterized by an approximately 40 amino acid motif referred to as the F-box; these proteins constitute one of the four subunits of the ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box) involved in phosphorylation-dependent ubiquitination.

This gene encodes a member of the autophagin protein family that is important for autophagy, the protein also being designated as a member of the C-54 family of cysteine proteases.

The protein can mediate chemoresistance in breast cancer cells [9].

This gene encodes the heavy chain subunit of the pre-alpha-trypsin inhibitor complex; this complex may stabilize the extracellular matrix through its ability to bind hyaluronic acid.

Unknown

This gene is a member of the DNA ligase family, encoding a protein that catalyses the joining of DNA ends, being involved in excision repair and being located in both mitochondria and nucleus.

Unknown

The protein can be expressed in breast cancer cells.
| Gene       | Description                                                                                                                                                                                                 |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| FEZ1       | Fasciculation and elongation protein zeta 1. CLASP2 and NEK1 proteins are present in a centrosomal complex and participate in cell cycle and cell division mechanisms, and they co-localize with FEZ1 in a perinuclear region and interact with endogenous gamma-tubulin [10]. |
| BOLA2      | BolA family member 2. The protein belongs to the family of BolA-like proteins probably involved in cell proliferation or cell-cycle regulation.                                                                 |
| LOC100130703 |                                                                                                                                                                                                          |
| LOC643302  |                                                                                                                                                                                                          |
| GPR173     | G-protein-coupled receptor 173. This gene encodes a member of the G-protein coupled receptor 1 family.                                                                                                       |
| MAP2K3     | Mitogen-activated protein kinase 3. The protein belongs to the MAP kinase family. It phosphorylates and thus activates MAPK14/p38-MAPK.                                                                     |
| MDF1       | MyoD family inhibitor. This protein is a transcription factor.                                                                                                                                           |
| THAP8      | THAP domain containing 8. Unknown.                                                                                                                                                                         |
| DMD        | Dystrophin. Dystrophin is a large cytoskeletal protein, it is part of the dystrophin-glycoprotein complex (DGC) that bridges the inner cytoskeleton (F-actin) and the extra-cellular matrix, it seems to interact with microtubules and have a role in cell division/cell cycle regulation, and it is expressed in hematopoietic cells [11, 12]. |
| HSPC157    | Long intergenic non-protein coding RNA 33. Unknown.                                                                                                                                                       |
| CCKAR      | Cholecystokinin A receptor. This gene encodes a G-protein coupled receptor.                                                                                                                                  |
| CAB39L     | Calcium binding protein 39-like. CABL39L can activate the LKB1 tumour suppressor [13].                                                                                                                      |
| DMRTA1     | Doublesex and mab-3 related transcription factor like family A1. The protein is a Pax6 activated transcription factor [14].                                                                                   |
| TRIM34     | Tripartite motif containing 34. The protein is a member of the tripartite motif (TRIM) family, the TRIM motif including three zinc-binding domains, a RING, B-box type 1 and B-box type 2 domain, and a coiled-coil region. Unknown functions. |
| AKAP2      | A kinase (PRKA) anchor protein 2. The encoded protein binds to the regulatory subunit of protein kinase A and is found associated with the actin cytoskeleton.                                                   |
| TMEM52B    | Transmembrane protein 52B. The protein is possibly involved in protein ubiquitination [15].                                                                                                                |
| LOC650577  |                                                                                                                                                                                                          |
References to Table S2

1. Fitzgerald, L. M.; Browne, E. P.; Christie, K. D.; Punska, E. C.; Simmons, L. O.; Williams, K. E.; Pentecost, B. T.; R. M. J.; Otis, C. N.; Arcaro, K. F., ELF5 and DOK7 regulation in anti-estrogen treated cells and tumors. Cancer Cell Int 2016, 16, 8.

2. Heyn, H.; Carmona, F. J.; Gomez, A.; Ferreira, H. J.; Bell, J. T.; Sayols, S.; Ward, K.; Stefansson, O. A.; Moran, S.; Sandova1, J.; Eyfjord, J. E.; Spector, T. D.; Esteller, M., DNA methylation profiling in breast cancer discordant identical twins identifies DOK7 as novel epigenetic biomarker. Carcinogenesis 2013, 34, (1), 102-108.

3. Raaijmakers, J. A.; Medema, R. H., Function and regulation of dynein in mitotic chromosome segregation. Chromosoma 2014, 123, (5), 407-422.

4. Bennett, E. J.; Rush, J.; Gygi, S. P.; Harper, J. W., Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. Cell 2010, 143, (6), 951-965.

5. Meyer, R. C.; Giddens, M. M.; Schaefer, S. A.; Hall, R. A., GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. Proc Natl Acad Sci U S A 2013, 110, (23), 9529-9534.

6. Chen, L.; Cai, Y.; Jin, J.; Florens, L.; Swanson, M. P.; Conaway, J. W.; Conaway, R. C., Subunit organization of the human INO80 chromatin remodeling complex: an evolutionarily conserved core complex catalyzes ATP-dependent nucleosome remodeling. J Biol Chem 2011, 286, (13), 11283-11289.

7. Zhang, Y.; Tong, Y.; Zhang, Y.; Ding, H.; Zhang, H.; Geng, Y.; Zhang, R.; Ke, Y.; Han, J.; Yan, Z.; Zhou, L.; Wu, T.; Hu, F. B.; Wang, D.; Cheng, J., Two novel susceptibility SNPs for ischemic stroke using exome sequencing in Chinese Han population. Mol Neurobiol 2014, 49, (2), 852-862.

8. Hudson, A. M.; Mannix, K. M.; Cooley, L., Actin Cytoskeletal Organization in Drosophila Germline Ring Canals Depends on Kelch Function in a Cullin-RING E3 Ligase. Genetics 2015, 201, (3), 1117-1131.

9. Moltseni, M.; Cidado, J.; Croessmann, S.; Cravero, K.; Cimino-Mathews, A.; Wong, H. Y.; Scharpf, R.; Zabransky, D. J.; AbuKhdeir, A. M.; Garay, J. P.; Wang, G. M.; Beaver, J. A.; Cochran, R. L.; Blair, B. G.; Rosen, D. M.; Erlanger, B.; Argani, P.; Hurley, P. J.; Lauring, J.; Park, B. H., MACROD2 overexpression mediates estrogen independent growth and tamoxifen resistance in breast cancers. Proc Natl Acad Sci U S A 2014, 111, (49), 17606-17611.

10. Lanza, D. C.; Meirelles, G. V.; Alborghetti, M. R.; Abrile, C. H.; Lenz, G.; Kobarg, J., FEZ1 interacts with CLASP2 and NEK1 through coiled-coil regions and their cellular colocalization suggests centrosomal functions and regulation by FKC. Mol Cell Biochem 2010, 338, (1-2), 35-45.

11. Teniente-De Alba, C.; Martinez-Vieyra, I.; Vivanco-Calixto, R.; Galvan, I. J.; Cisneros, B.; Cerecedo, D., Distribution of dystrophin- and utrophin-associated protein complexes (DAPC/UAPC) in human hematopoietic stem/progenitor cells. Eur J Haematol 2011, 87, (4), 312-322.

12. Villarreal-Silva, M.; Centeno-Cruz, F.; Suarez-Sanchez, R.; Garrido, E.; Cisneros, B., Knockdown of dystrophin Dp71 impairs PC12 cells cycle: localization in the spindle and cytokinesis structures implies a role for Dp71 in cell division. PLoS One 2011, 6, (8), e23504.

13. Fan, D.; Ma, C.; Zhang, H., The molecular mechanisms that underlie the tumor suppressor function of LKB1. Acta Biochim Biophys Sin (Shanghai) 2009, 41, (2), 97-107.

14. Sun, J.; Rockowitz, S.; Xie, Q.; Ashery-Padan, R.; Zheng, D.; Cvekl, A., Identification of in vivo DNA-binding mechanisms of Pax6 and reconstruction of Pax6-dependent gene regulatory networks during forebrain and lens development. Nucleic Acids Res 2015, 43, (14), 6827-6846.

15. Abdelmohsen, K.; Srikanth, S.; Yang, X.; Lal, A.; Kim, H. H.; Kuwano, Y.; Galban, S.; Becker, K. G.; Kamara, D.; de Cabo, R.; Gorospe, M., Ubiquitin-mediated proteolysis of HuR by heat shock. Embo j 2009, 28, (9), 1271-1282.
Table S3. Constitutive cytokine release by primary human AML cells – the effect of 10 µM NSC95397. The release of 28 soluble mediators was tested for primary human AML cells derived from 79 patients. The second column of the table shows the levels for AML cells cultured in medium alone, whereas the third column shows the levels when NSC95397 was added. The table is divided into three subgroups: cytokines that are released at high/detectable levels for a large majority of patients both in drug-containing cultures and drug-free controls (upper part); mediators that are released for most patients when leukemic cells are cultured in medium alone but at lower/undetectable levels in the presence of NSC95397 (middle); and mediators that are released only by a subset of patients also in drug-free control cultures (lower part). The right column shows the results from correlation analyses for mediator levels in drug-free control cultures and cultures with NSC95397.

| Mediator | Cytokine level medium only | 10 µM NSC95397 | Statistical analyses |
|----------|---------------------------|----------------|---------------------|
|          | No.¹ Median level (pg/mL) | Range (pg/mL) | No.¹ Median level (pg/mL) | Range (pg/mL) | r-value | p-value |
| MMP-2    | 79 1,288 148 – 9,485 | | 79 1,548 172 – 12,808 | | 0.652 | <0.001 |
| CCL5     | 79 39 7.4 – 2,481 | | 79 59 3.9 – 3,106 | | 0.659 | <0.001 |
| HGF      | 79 42 5.9 – 884 | | 79 29 0.1 – 721 | | 0.756 | <0.001 |
| IL-1RA   | 79 841 6.4 – > 10,700 | | 77 1,296 n.d.² – > 10,700 | | 0.855 | <0.001 |
| CXCL5    | 78 95 n.d.² – > 14,500 | | 75 150 n.d. – > 14,500 | | 0.785 | <0.001 |
| CXCL8    | 79 535 0.3 – > 18,500 | | 77 1,312 n.d. – > 18,500 | | 0.783 | <0.001 |
| CXCL10   | 79 12 1.4 – 24,642 | | 73 4.3 n.d. – > 25,000 | | 0.672 | <0.001 |
| CXCL11   | 79 83 25 – 246 | | 70 26 n.d. – 95 | | 0.178 | 0.036 |
| VEGF     | 79 11 2.8 – 96 | | 70 7.1 n.d. – 71 | | 0.542 | <0.001 |
| IL-1β    | 79 2.6 1.2 – 1,008 | | 70 2.8 n.d. – 18,555 | | 0.777 | <0.001 |
| CCL3     | 79 200 119 – > 30,000 | | 69 156 n.d. – > 30,000 | | 0.641 | <0.001 |
| CCL4     | 79 92 21 – 11,744 | | 68 61 n.d. – > 90,000 | | 0.753 | <0.001 |
| MMP-1    | 79 33 26 – > 7,100 | | 62 5.4 n.d. – > 7,100 | | 0.713 | <0.001 |
| CXCL1    | 79 79 41 – 22,772 | | 58 44 n.d. – > 65,000 | | 0.887 | Ns³ |
| TNFα     | 79 3.0 0.8 – 1,346 | | 52 1.8 n.d. – 5,462 | | 0.715 | <0.001 |
| TIMP-1   | 79 1,812 572 – 34,789 | | 49 852 n.d. – 102,004 | | 0.756 | <0.001 |
| HB-EGF   | 78 11 n.d. – 71 | | 38 n.d. n.d. – 64 | | 0.408 | <0.001 |
| Tie-2    | 79 183 21 – 248 | | 27 n.d. n.d. – 140 | | 0.016 | Ns |
| GM-CSF   | 79 12 8.7 – 555 | | 26 n.d. n.d. – 4,484 | | 0.545 | <0.001 |
| IL-33    | 79 8.5 6.6 – 22 | | 25 n.d. n.d. – 7.6 | | 0.418 | <0.001 |
| IL-10    | 79 1.6 0.6 – 79 | | 19 n.d. n.d. – 196 | | 0.578 | <0.001 |
| bFGF     | 79 19 10 – 44 | | 17 n.d. n.d. – 43 | | 0.275 | 0.007 |
| CXCL2    | 69 16 n.d. – 11,594 | | 72 15 n.d. – 11,430 | | 0.351 | <0.001 |
| CCL2     | 67 110 n.d. – 9,807 | | 61 170 n.d. – 6,964 | | 0.745 | <0.001 |
| Ang-1    | 61 58 n.d. – 2,135 | | 67 98 n.d. – 1,545 | | 0.758 | <0.001 |
| MMP-9    | 47 1,545 n.d. – 261,935 | | 45 1,192 n.d. – 316,386 | | 0.777 | <0.001 |
| IL-6     | 37 n.d. n.d. – 19,311 | | 60 3.7 n.d. – > 19,500 | | 0.738 | <0.001 |
| G-CSF    | 11 n.d. n.d. – 10,262 | | 15 n.d. n.d. – > 10,500 | | 0.749 | <0.001 |

¹ Number of patients with detectable release.
² n.d. below detection limit;
³ Ns not significant.