new tumor suppressor gene and provide explanations for previous results.[11] The *NFIC* gene is already considered as a potential tumor suppressor gene.[12] The fact that the *NFIA*-deleted PV case had a JAK2 V617F mutation shows that several alterations may already coexist at this early stage of leukemogenesis. Deletion in a PV is coherent with the known function of NFIA in erythropoiesis.[7–9] The calculated number of *NFIA* gene copy suggests that the deletion was present at the heterozygous state in almost all cells or homozygous in around half the cells. The moderate JAK2 V617F allele burden suggests that the mutation may have occurred on the background of the 1p31.3 deletion. We cannot exclude that the *NFIA* mutation is not present in the germ line. However, *NFIA* haplo-insufficiency has a deleterious effect when occurring in germinal cells in the mouse.[3] Increased expression of *NFIB* mRNA in granulocytes has been described in PV,[13] which may suggest a compensatory or antagonist function of NFIB.

Our work suggests that NFIA could be added to the increasing list of transcription regulators whose alteration may have a function in leukemogenesis.

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References

1 Gronostajski RM. Roles of the NFI/CTF gene family in transcription and development. *Gene* 2000; **249**: 31–45.
2 das Neves L, Duchala CS, Tolentino-Silva F, Haxhiu MA, Colmenares C, Macklin WB *et al.* Disruption of the murine nuclear factor I-A gene (Nfia) results in perinatal lethality, hydrocephalus, and agenesis of the corpus callosum. *Proc Natl Acad Sci USA* 1999; **96**: 11946–11951.
3 Lu W, Quintero-Rivera F, Fan Y, Alkuraya FS, Donovan DJ, Xi Q *et al.* NFIA haploinsufficiency is associated with a CNS malformation syndrome and urinary tract defects. *PLoS Genet* 2007; **3**: e80.
4 Wong YW, Schulze C, Streichert T, Gronostajski RM, Schachner M, Tilling T. Gene expression analysis of nuclear factor I-A deficient mice indicates delayed brain maturation. *Genome Biol* 2007; **8**: R72.
5 Fazi F, Rosa A, Fatica A, Gelmetti V, De Marchis ML, Nervi C *et al.* A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis. *Cell* 2005; **123**: 819–831.
6 Rosa A, Ballarino M, Sorrentino A, Sthandier O, De Angelis FG, Marchioni M *et al.* The interplay between the master transcription factor PU 1 and miR: 424 regulates human monocyte/macrophage differentiation. *Proc Natl Acad Sci USA* 2007; **104**: 19849–19854.
7 Lee HJ, Young WJ, Shih CY, Chang C. Suppression of the human erythropoietin gene expression by the TR2 orphan receptor, a member of the steroid receptor superfamily. *J Biol Chem* 1996; **271**: 10405–10412.
8 Lin YL, Wang YH, Lee HJ. Transcriptional regulation of the human TR2 orphan receptor gene by nuclear factor 1-A. *Biochem Biophys Res Commun* 2006; **350**: 430–436.
9 Tanabe O, Shen Y, Liu Q, Campbell AD, Kuroha T, Yamamoto M *et al.* The TR2 and TR4 orphan nuclear receptors repress Gata1 transcription.. *Genes Dev* 2007; **21**: 2832–2844.
10 Adélaïde J, Finetti P, Bekhouche I, Repellini L, Geneix J, Sircoulomb F *et al.* Integrated profiling of basal and luminal breast cancers. *Cancer Res* 2007; **67**: 11565–11575.
11 Schuur ER, Kruse U, Iacovoni JS, Vogt PK. Nuclear factor I interferes with transformation induced by nuclear oncogenes. *Cell Growth Differ* 1995; **6**: 219–227.
12 Eeckhoute J, Carroll JS, Geistlinger TR, Torres-Arzayus MI, Brown M. A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. *Genes Dev* 2006; **20**: 2513–2526.
13 Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol* 2002; **30**: 229–236.

Clioquinol inhibits the proteasome and displays preclinical activity in leukemia and myeloma

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The 26S proteasome comprises the 19S proteasome that performs a regulatory function, and the 20S proteasome that is responsible for the enzymatic degradation of proteins. The 20S proteasome comprises α- and β-subunits that form the outer and inner rings of this complex, respectively.[1] The α-subunits form an antechamber that gives the proteasome its barrel shape and regulates the flow of substrates into the proteolytic chamber in the center of the barrel.[1] The β-subunits form the proteolytic chamber of the 20S proteasome and perform the peptidase function of the complex.[1] Bortezomib (Velcade) is an intravenous proteasome inhibitor that is approved for the treatment of multiple myeloma and mantle cell lymphoma.[2,3] Mechanistically, Bortezomib binds the active sites of the enzymes in the β-subunits located in the proteolytic chamber of the 20S proteasome.[4] Molecules that inhibit the proteasome through a different mechanism would be useful probes to understand the activity of this enzyme complex and potentially new therapeutic agents.

5–chloro-7–iodo-quinolin-8-ol (clioquinol) (Figure 1a) is a copper-binding halogenated 8-hydroxyquinoline that was used in the 1950s–1970s as an oral antiparasitic agent for the treatment and prevention of intestinal amebiasis, but its mechanism of action as an anti-parasitic agent was unknown. Recent studies in solid tumor cell lines have demonstrated that clioquinol, when supplemented with copper, can inhibit the

proteasome,[5] but its mechanism of action has not been explored fully. Here, we investigated the effects of clioquinol on the proteasome in hematologic malignancies.

LP-1 and OCI-MY5 myeloma cell lines were treated with increasing concentrations of clioquinol, and 24 h after treatment the abundance of ubiquitinated proteins in the cells was measured by immunoblotting. At low micromolar concentrations, clioquinol increased the amount of ubiquitinated proteins, and copper supplementation of the media was not required to observe this effect (Figure 1b). To assess the spectrum of activity of clioquinol as a proteasome inhibitor, we examined the effects of clioquinol on the activity of the chymotrypsin-like proteasomal enzyme in cell lines and primary patient samples. Leukemia, myeloma and solid tumor cell lines were treated with increasing concentration of clioquinol for 24 h. After treatment, cells were harvested and lysed, and the chymotrypsin-like activity of the proteasome was measured by monitoring the rate of cleavage of the fluorescent substrate Suc-LLVY-AMC (Figure 1c). Clioquinol inhibited the rate of Suc-LLVY-AMC cleavage in myeloma and leukemia cell lines but not in the tested solid tumor cells. Thus, the results in leukemia and myeloma cells are different than in solid tumor lines, where, consistent with previous reports, clioquinol is ineffective without copper supplementation.[5]

Given the effects of clioquinol on leukemia cell lines, we next examined the effects of clioquinol on the activity of the proteasome in primary acute myeloid leukemia (AML) cells and normal hematopoietic cells. Primary normal and malignant cells were treated with increasing concentrations of clioquinol for 24 h. After incubation, the enzymatic activity of the proteasome was measured as above. Clioquinol inhibited proteasome activity in the primary malignant AML cells preferentially over primary normal hematopoietic cells (Figure 1d). It can be noted that clioquinol was a preferential inhibitor of proteasomal enzymes as it did not inhibit the activity of other enzymes such as catalase or luciferase (data not shown).

Clioquinol binds copper, and prior reports in solid tumor cell lines demonstrated that its inhibition of the proteasome was copper dependent.[5] Therefore, we assessed the role of copper in influencing the activity of clioquinol in leukemia and myeloma cells. Leukemia and myeloma cell lines and patient samples were incubated with increasing concentrations of clioquinol, copper and equimolar concentrations of clioquinol and copper. Cell lysates were prepared and the chymotrypsin-like activity of the proteasome was measured by monitoring the rate of cleavage of the fluorescent substrate Suc-LLVY-AMC. The addition of copper alone to intact cells did not affect the function of proteasome (Figure 2a), most likely because it was

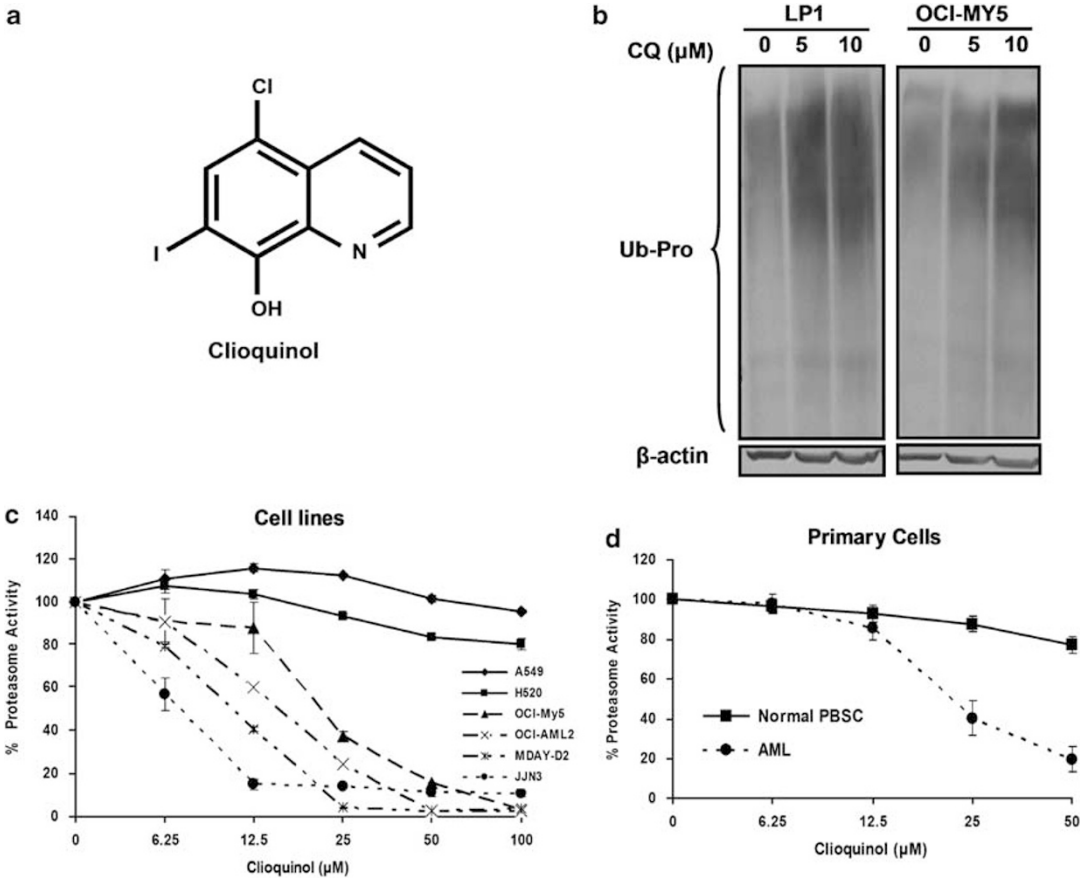

Figure 1 Clioquinol inhibits the proteasome. (**a**) Chemical structure of clioquinol. (**b**) LP1 and OCI-MY5 multiple myeloma cells were treated with increasing concentrations of clioquinol (CQ). After 24 h of incubation, cells were harvested and total proteins were isolated. The abundance of ubiquitinated proteins (Ub-Pro) and β-actin expression was measured by SDS-polyacrylamide gel electrophoresis immunoblotting with anti-ubiquitin and anti-β-actin antibodies, respectively. (**c**) Solid tumor (A549, H520), leukemia (OCI-AML2, MDAY-D2) or myeloma (OCI-MY5, JJN3) cell lines were treated with increasing concentrations of clioquinol. Twenty hours after treatment, cells were lysed and the preferential chymotrypsin-like substrate Suc-LLVY-AMC was added. The generation of free AMC was measured over time as described earlier.[6] (**d**) Primary acute myeloid leukemia (AML) blasts ($n = 3$) or normal peripheral blood stem cells (PBSCs) ($n = 3$) were obtained from the peripheral blood of consenting patients with AML or donors of PBSC for allotransplantation, respectively. Mononuclear cells were isolated by Ficol separation and treated with increasing concentrations of clioquinol. Twenty hours after treatment, the chymotrypsin-like enzymatic activity was measured as above.

bound by intracellular copper-chelating proteins or exported from the cell. However, supplementing clioquinol with copper increased the inhibition of the proteasome above clioquinol alone (Figure 2a).

To further explore the effects of copper on the proteasome, we added copper, clioquinol or a combination of copper and clioquinol to extracts of leukemia and myeloma cell lysates. In cell lysates, where active mechanisms to regulate levels of free intracellular copper are no longer functional, copper directly inhibited the chymotrypsin-like activity of the proteasome. In cell lysates, clioquinol also inhibited the proteasome, although to a lesser degree than copper, but the combination of clioquinol and copper produced no greater inhibition than copper alone did (Supplementary Figure 1).

As copper enhances clioquinol's activity as a proteasome inhibitor, we evaluated the effects of copper supplementation on clioquinol's effects on the proteasome in primary malignant and normal hematopoietic cells. Primary AML and normal hematopoietic cells were treated with increasing concentrations of clioquinol with and without an equimolar concentration of copper. Adding copper to the media enhanced the ability of clioquinol to inhibit the proteasome in both primary AML and normal hematopoietic cells. However, copper supplementation negated the differential inhibition between malignant and normal cells. Thus, our results demonstrate that clioquinol inhibits the proteasome through a copper-dependent mechanism. However, the results also suggest that supplementing clioquinol with copper may increase toxicity (Figure 2b).

As clioquinol alone inhibited the proteasome when added to leukemia and myeloma cell lines and extracts, it suggests that this compound may also inhibit the proteasome through a copper-independent mechanism. However, as leukemia and myeloma cells and extracts contain copper and other heavy metals, it may be that a complex of clioquinol-copper was

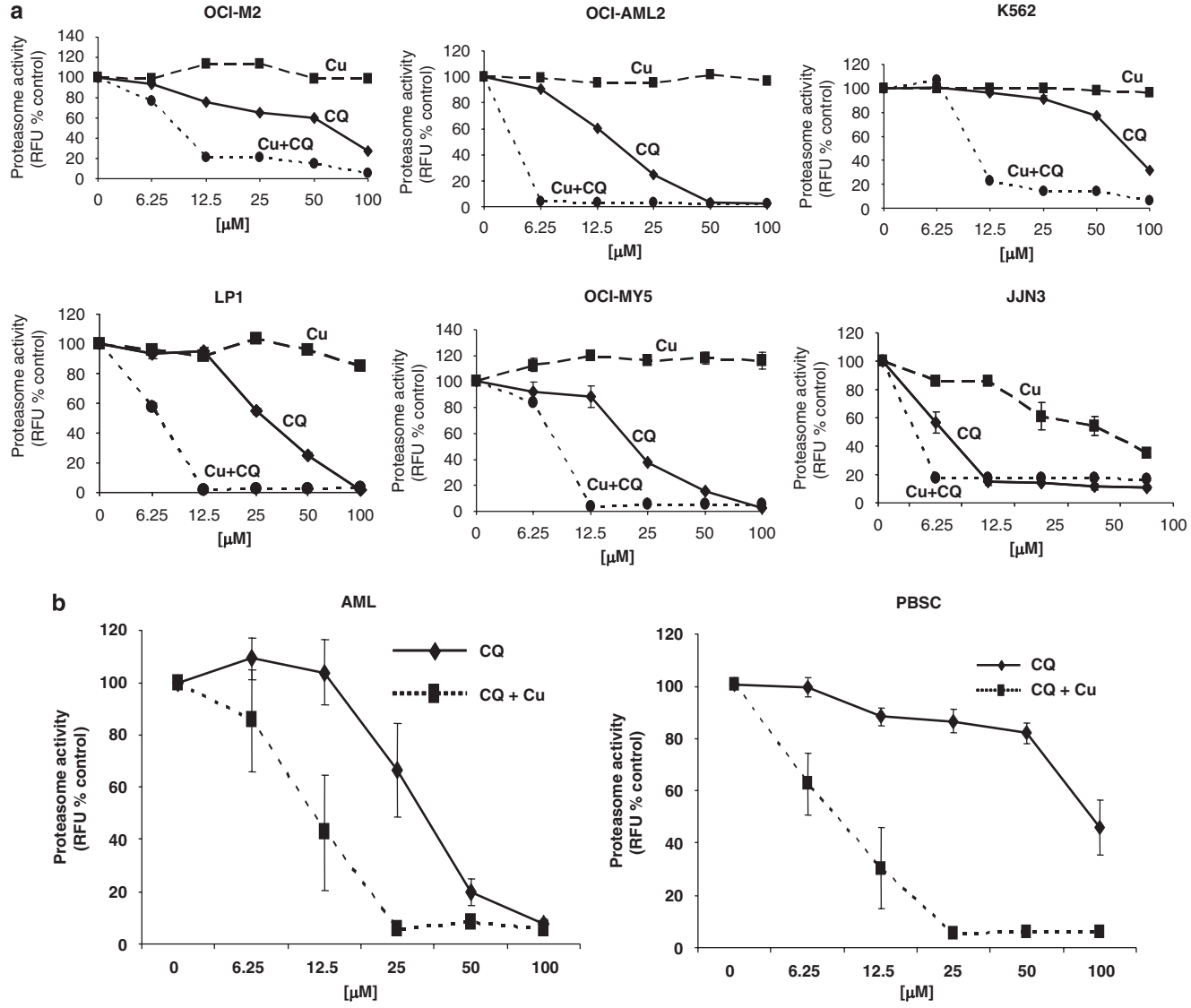

Figure 2 Clioquinol inhibits the proteasome through a copper-dependent mechanism. (**a**) MDAY-D2, OCI-M2, AML2, K562 leukemia and LP-1, OCI-MY5 and JJN3 myeloma cells were treated with increasing concentrations of clioquinol (CQ) (diamond), $CuCl_2$ (Cu) (square) or an equimolar concentration of CQ and Cu (circle). Twenty hours after treatment, cells were lysed and the chymotrypsin-like enzymatic activity was measured as described in Figure 1b) Primary acute myeloid leukemia (AML) blasts ($n=7$) or normal peripheral blood stem cells (PBSCs) ($n=3$) were obtained from the peripheral blood of consenting patients with AML or donors of PBSC for allotransplantation, respectively. Mononuclear cells were isolated by Ficol separation and treated with increasing concentrations of clioquinol (CQ) with or without an equimolar concentration of $CuCl_2$ (Cu). Twenty hours after treatment, cells were lysed and the chymotrypsin-like proteasomal activity was measured.

responsible for the observed inhibition. Therefore, we tested the ability of clioquinol to inhibit the enzymatic activity of isolated *Thermoplasma Acidophilium* proteasome complexes in buffers devoid of copper or other heavy metals. Even in the absence of heavy metals, clioquinol continued to inhibit the proteasome, supporting a copper-independent mechanism of action at higher concentrations of the drug (Figure 3).

To further assess a copper-independent mechanism of proteasome inhibition, we evaluated the effects of a non-copper binding analog of clioquinol on the enzymatic function of the proteasome. For these studies, we evaluated the antimalarial drug chloroquine, as it shares a common quinoline structure with clioquinol but does not bind copper by spectrophotometric titration studies (Supplementary Figure 2). Specifically, by spectrophotometric titration, clioquinol formed a 2:1 stoichiometry ligand-to-copper(II) complex with a logβ′ value of 11.53, but chloroquine did not bind copper. When added to intact cells, cell extract, or isolated proteasome from *T. Acidophilium*, chloroquine inhibited the chymotrypsin-like enzyme activity (Figure 3 and data not shown). Furthermore, consistent with a copper-independent inhibition, supplementing chloroquine with copper did not enhance its ability to inhibit the proteasome in intact cells (data not shown). Thus, these results support a copper-independent effect of clioquinol on the proteasome at higher concentrations. The mechanism by which clioquinol exerts this copper-independent effect is uncertain. However, we recently demonstrated that the structurally related molecule chloroquine binds the α-subunits of the antechamber of the 20S proteasome by NMR.[6] Consistent with binding the proteasome outside of the active site, enzymatic assays demonstrated that chloroquine inhibited the proteasome in a non-competitive manner.[6] As clioquinol and chloroquine are structurally related, clioquinol may also bind the α-subunits of the proteasome similar to chloroquine. To address this question, we attempted to investigate the binding of clioquinol to the proteasome by NMR, but the compound was not adequately water soluble to permit the NMR analysis. Although the copper-independent effects of clioquinol on the proteasome are best appreciated at higher concentrations, they still merit further study as they may represent a new mechanism to inhibit the proteasome. As such, clioquinol may be a lead to develop a molecule that binds and inhibits the α-subunits with greater potency.

The ability of clioquinol to directly inhibit the proteasome in leukemia and myeloma cells and its preferential inhibition of the proteasome in malignant cells over normal cells suggests that clioquinol could be repurposed as a novel antileukemia and myeloma agent. To support the development of clioquinol for this anticancer indication, we evaluated its effects on the viability of acute leukemia, myeloma and solid tumor cell lines as well as primary AML samples and primary normal hematopoietic cells. Cells were incubated with increasing concentrations of clioquinol. Forty-eight hours after incubation, viability was measured by the MTS assay and annexin V staining. Clioquinol induced cell death in AML and myeloma but not in solid tumor cell lines, and clioquinol preferentially induced cell death in primary AML patient samples over normal hematopoietic cells with an IC_{50} $<20\,\mu M$ (Figure 4a–d and data not shown). Clioquinol-induced cell death matched its ability to inhibit the proteasome, as no cell death or proteasomal inhibition was detected in the solid tumor cell lines and the normal hematopoietic cells. Interestingly, these clioquinol-resistant solid tumor cells were sensitive to bortezomib-induced cell death (data not shown), suggesting that clioquinol acts through a mechanism distinct from Bortezomib.

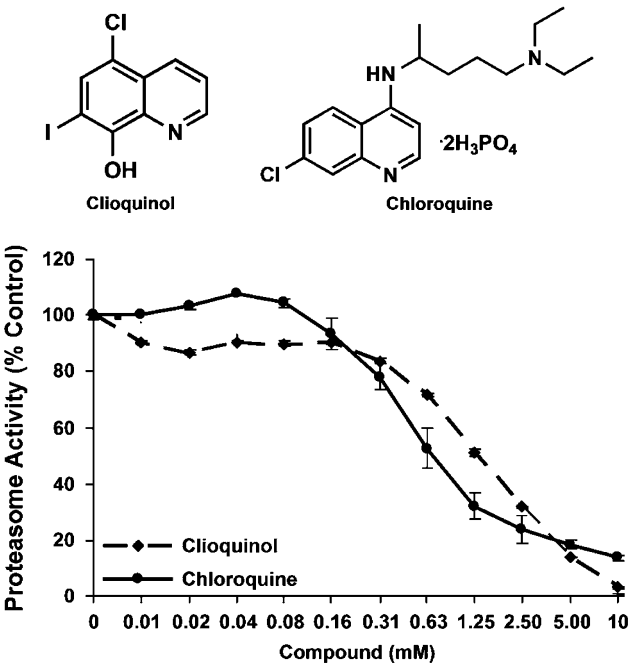

Figure 3 Clioquinol and chloroquine inhibit the proteasome through a copper-independent mechanism. Proteasomes isolated from *T. Acidophilum* were treated with increasing concentrations of clioquinol and chloroquine. Three hours after treatment, the preferential chymotrypsin-like substrate Suc-LLVY-AMC was added and the rate of free AMC was measured over time as described in Figure 1.

Next, we determined whether clioquinol-induced cell death was copper dependent. In cell lines and patient samples, supplementing copper in the medium enhanced the toxicity of clioquinol. In contrast, cell death induced by $20\,\mu M$ of clioquinol was inhibited by adding the strong copper chelator tetrathiomolybdate (Supplementary Figure 3 and data not shown). We also evaluated the effects of adding copper to Clioquinol in primary AML and normal hematopoietic cells. Here, supplementing the media with copper increased the cytotoxicity of clioquinol in both the primary AML and normal cells and negated the preferential toxicity toward malignant cells (Supplementary Figure 3). Therefore, when repurposing clioquinol as an anticancer agent, supplementing patients with copper may not be advisable.

Given the selective cytotoxicity for malignant cells in culture, we evaluated clioquinol in three leukemia mouse models. U937 and K562 human leukemia cells were implanted subcutaneously in the flanks of sublethally irradiated NOD/SCID mice. Likewise, MDAY-D2 murine leukemia cells were implanted intraperitoneally into sublethally irradiated NOD/SCID mice. After tumor implantation, mice were treated with twice daily oral clioquinol (25–50 mg/kg) or vehicle control. In all three leukemia models, oral clioquinol decreased the weight and volume of the tumor compared to mice treated with vehicle alone. Reductions in tumor growth were obtained without reductions in body weight or gross organ toxicity (Figure 4e and data not shown). Thus, clioquinol may be an effective therapy for hematologic malignancies.

Given the prior clinical experience with clioquinol as an antiparasitic and the preclinical efficacy of clioquinol as a proteasome in inhibitor in malignant cell lines and animal models, this drug could be repurposed for the treatment of leukemia and myeloma. When used in the 1950s–1970s as an

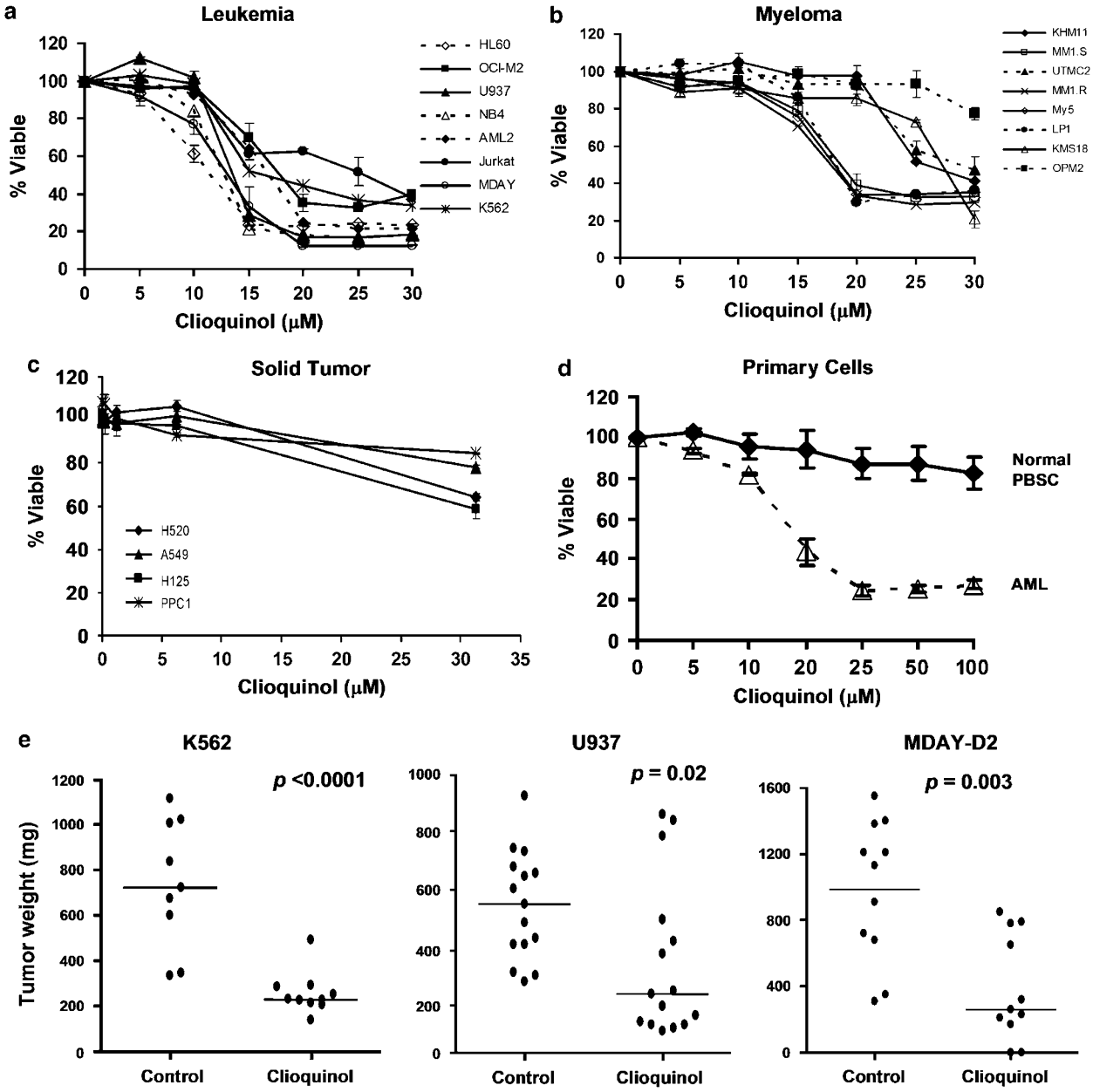

Figure 4 Clioquinol displays preclinical activity in leukemia and myeloma. (**a**) Leukemia, (**b**) myeloma and (**c**) solid tumor cell lines were treated with increasing concentrations of clioquinol (CQ). Forty-eight hours after treatment, cell viability was measured by the MTS assay. Data represent the mean ± s.d. percent viability compared to buffer-treated cells. (**d**) Primary acute myeloid leukemia (AML) blasts ($n = 7$) (triangle) or normal peripheral blood stem cells (PBSCs) (diamond) ($n = 3$) were obtained from the peripheral blood of consenting patients with AML or donors of PBSC for allotransplantation, respectively. Mononuclear cells were isolated by Ficol separation and treated with increasing concentrations of CQ. Twenty-four hours after incubation, cell viability and apoptosis were measured by annexin V and propidium iodide staining and flow cytometry. Data represent the mean ± s.d. percentage of viable annexin V negative cells compared with buffer-treated cells. (**e**) Sublethally irradiated NOD/SCID mice were injected subcutaneously with U937 and K562 leukemia cells or intraperitoneally with MDAY-D2 leukemia cells. Mice were then treated twice daily by oral gavage with buffer or clioquinol (50 mg/kg for MDAY-D2 and K562 or 25 mg/kg for U937). Eight (MDAY-D2) or ten (U937 and K562) days after tumor inoculation, mice were killed, and the subcutaneous or intraperitoneal tumor was excised and the weight of the tumors measured. The bars represent the median of the population. *P*-values reflect the Mann–Whitney nonparametric test.

oral antiparasitic agent, the drug was routinely administered orally at doses exceeding 2 g per day over a period of weeks. Furthermore, after oral administration, serum levels of clioquinol ranged from 13–25 μM[7] and this concentration is within the range required to exert an antitumor effect in culture.

However, efforts to repurpose clioquinol must proceed with caution, as the drug was withdrawn from the market in the 1970s owing to an association with subacute myelo-optic neuropathy (SMON) in Japanese patients. In the Japanese patients with SMON, the side effects emerged after prolonged treatment, as the average cumulative dose of clioquinol in the cases was 136 g.[8] Notably, these side effects occurred almost exclusively in Japanese patients, and cases of SMON in patients outside of Japan are exceedingly rare and are essentially at the case report level. In fact, before the withdrawal of clioquinol from the market, there were 10 000 cases in Japan and only 220

cases in the rest of the world despite its use for over 500 million patient days. The explanation for the neurological side effects among the Japanese is unknown, but many of these cases may have been related to concomitant vitamin B12 deficiency. Alternatively, the neurological side effects may be related to the different formulation of clioquinol used in Japan or a genetic susceptibility to these side effects among the Japanese. Finally, it is noteworthy that neuropathy is a side effect of bortezomib.[2,3]

In summary, we have investigated clioquinol in leukemia and myeloma cell lines and patient samples. We have determined that it inhibits the proteasome and induces cell death primarily through a copper-dependent mechanism. In addition, there is a copper-independent effect that merits further investigation. As the antileukemia/myeloma effects of clioquinol appear obtainable at clinically relevant concentrations, this drug could be repurposed for the treatment of these diseases.

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References

1 Lowe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R. Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 A resolution. *Science* 1995; **268**: 533–539.
2 Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T *et al.* Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 2005; **352**: 2487–2498.
3 Fisher RI, Bernstein SH, Kahl BS, Djulbegovic B, Robertson MJ, de Vos S *et al.* Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. *J Clin Oncol* 2006; **24**: 4867–4874.
4 Groll M, Berkers CR, Ploegh HL, Ovaa H. Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. *Structure* 2006; **14**: 451–456.
5 Chen D, Cui QC, Yang H, Barrea RA, Sarkar FH, Sheng S *et al.* Clioquinol, a therapeutic agent for Alzheimer's disease, has proteasome-inhibitory, androgen receptor-suppressing, apoptosis-inducing, and antitumor activities in human prostate cancer cells and xenografts. *Cancer Res* 2007; **67**: 1636–1644.
6 Sprangers R, Li X, Mao X, Rubinstein JL, Schimmer AD, Kay LE. TROSY-based NMR evidence for a novel class of 20S proteasome inhibitors. *Biochemistry* 2008; **112**: 760–769.
7 Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L *et al.* Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch Neurol* 2003; **60**: 1685–1691.
8 Tateishi J. Subacute myelo-optico-neuropathy: clioquinol intoxication in humans and animals. *Neuropathology* 2000; **20** (Suppl): S20–S24.

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Dasatinib is a potent inhibitor of tumour-associated macrophages, osteoclasts and the FMS receptor

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There is a growing realisation that the non-cancerous cell types present in the microenvironment of tumours, lymphomas and leukaemias are important auxiliary targets for drug development.[1,2] In particular, most tumours contain substantial numbers of tumour-associated macrophages (TAMs), and these appear to be polarised towards an M2 macrophage phenotype, which is normally associated with the promotion of angiogenesis and the stimulation of tissue growth.[1,2] M2 macrophages are important for wound healing but in a tumour these same macrophage properties are now thought to promote tumour progression and metastasis.[1,2] Another property of TAMs is that they may also down modulate the activity of T cells against tumourigenic cells.[3]

The FMS receptor for macrophage colony-stimulating factor (M-CSF or CSF-1) is closely related to the KIT and platelet-derived growth factor (PDGF) receptors and is important for the production of macrophages, osteoclasts and microglial cells.[4,5] In addition to tumourigenesis, these cell types are also implicated in various inflammatory, bone and neurological diseases; consequently the FMS receptor is a highly promising therapeutic target.[6–11]

Dasatinib is an adenosine triphosphate (ATP)-based competitor that was developed as a dual inhibitor of the Src family and Abl tyrosine kinases.[12,13] It is also an effective inhibitor of many of the ephrin receptor family members and of the KIT and PDGF receptors.[12,14] Dasatinib is reasonably well tolerated by patients and has Food and Drug Administration approval for the treatment of chronic myeloid leukaemia that is resistant to imatinib.[15] Here we report that dasatinib is a potent inhibitor of the FMS receptor and inhibits the production of normal and