Cell viability assays
Cell growth and viability was was assessed by the MTS assay (Promega, Madison, WI) according to the manufacturer's instructions and as previously described.[1] Cell death was measured by Annexin V-fluoroscein isothiocyanate (FITC) and Propidium Iodide (PI) (Biovision Research Products, Mountain View, CA) staining using flow cytometry according to the manufacturer's instructions and as previously described.[2] To identify $CD34^+$ cells, a normal PBSC sample was co-stained with PE–anti-$CD34^+$ (Beckman Coulter, Marseille France), and APC–anti-CD45 (Becton Dickenson, San Jose CA).

To assess clonogenic growth, primary AML cells or granulocyte colony-stimulating factor (G-CSF) mobilized PBSCs (4×10^5/mL) were treated with ivermectin or buffer control for 24 hours. After treatment, cells were washed and 10^5cells/mL were plated by equal volume in duplicate in MethoCult GF H4434 medium (StemCell Technologies, Vancouver, BC) containing 1% methycellulose in IMDM, 30% FCS, 1% bovine serum albumin, 3 U/mL of recombinant human erythropoietin, 10^{-4} M of 2-mercaptoethanol, 2 mM of L-glutamine, 50 ng/mL of recombinant human stem cell factor, 10 ng/mL of GM-CSF, and 10 ng/mL of rh IL-3. Alternatively, primary cells were plated directly into MethoCult GF H4434 medium with ivermectin. Seven days (AML samples) or 14 days (normal PBCS) after plating, the number of colonies containing 10 or more cells for AML or over 100 cells for normal samples was counted as previously described.[3]

Determination of plasma and mitochondrial membrane potential
To measure plasma membrane potential cells were treated with ivermectin or buffer control in RPMI, chloride complete medium (140 mM sodium chloride, 5mM potassium chloride, 1 mM magnesium sulfate, 1.8 mM calcium acetate, 10 mM glucose, 10 mM HEPES and 0.1% (wt/v) BSA), or chloride-free media where equimolar gluconate salts of sodium and potassium replaced the sodium chloride in the chloride replete medium. After incubation, cells were stained with $DiBAC_4(3)$ (final concentration 30 nM) and fluorescence determined by flow cytometry (BD FACS Canto, Becton Dickinson, San Jose, CA) (excitation = 488 nm, emission = 516 nm) Analysis was conducted using FACSDiva Software (BD Biosciences). Calibration curves were prepared using phosphate buffers with varying potassium ion concentrations as previously described.[4]

To obtain a relative mitochondrial membrane potential, cells were treated with ivermectin similarly as described above and then washed twice with PBS and incubated with 2 μM of 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl benzimidazolylcarbocyanine iodide (JC-1, Invitrogen) for 20 minutes at 37°C. Each sample was then washed twice with 1 mL PBS and resuspended in 300 μL PBS prior to being read on a BD FACS Canto. Samples were excited at 488nm and emission was collected at 526nm (green) and 595nm (red). Analysis was conducted using FACSDiva Software (BD Biosciences).

Detection of reactive oxygen species
Intracellular Reactive oxygen species (ROS) were detected by staining cells with Carboxy-H_2DCFDA (10 μM) and analysing with flow cytometry. Cells were treated with ivermectin, cytarabine and daunorubicin overnight and stained with Carboxy-H_2DCFDA and PI in PBS buffer at 37°C for 30 minutes to identify viable cells and assess their reactive oxygen intermediate levels. Data were analyzed with FlowJo version 8.8 (TreeStar, Ashland, OR).

Gene expression studies

OCI-AML2 leukemia cells were treated with buffer control or ivermectin (3 μM) for 30 and 40 hours. After treatment, cells were harvested, total RNA was isolated. Total RNA (10 μg) was used for cRNA amplification using the Invitrogen SuperScript kit (Life Technologies, Inc., Burlington, ON, Canada). Amplification and biotin labeling of antisense cRNA was performed using the Enzo® BioArray™ High Yield™ RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY, USA), according to the manufacturer's instructions. RNA was then hybridized to Affymetrix HG U133 Plus 2.0 gene expression oligonucleotide arrays (Affymetrix, Santa Clara, CA, USA). Microarray slides were scanned using the GeneArray 2500 scanner (Agilent Technologies). Microarray data were analyzed using GeneSpring GX v10.0 (Agilent), and lists of genes deregulated > 2-fold after 30 and 40 hr ivermectin treatment were derived (Table S1). Pathways and gene ontology analyses were carried out using Ingenuity Pathways Analysis; and the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov). Data have been deposited into Array Express (E-MEXP-2528).

Gene expression changes were validated in OCI AML2, U937, HL60, DU145 and PPC-1 cells by real-time Q-PCR, as described previously.[5,6] Primer sequences for *STAT1A, STAT1B, TRIM22, OAS1* and *IFIT3* are provided in Table S2.

REFERENCES

1. Simpson CD, Mawji IA, Anyiwe K, et al. Inhibition of the sodium potassium adenosine triphosphatase pump sensitizes cancer cells to anoikis and prevents distant tumor formation. *Cancer Res*. 2009;69:2739–2747.
2. Mawji IA, Simpson CD, Hurren R, et al. Critical role for Fas-associated death domain-like interleukin-1–converting enzyme-like inhibitory protein in anoikis resistance and distant tumor formation. *J Natl Cancer Inst*. 2007;99:811–822.
3. Buick RN, Till JE, McCulloch EA. Colony assay for proliferative blast cells circulating in myeloblastic leukaemia. *Lancet*. 1977;1:862–863.
4. Rothbard JB, Jessop TC, Lewis RS, Murray BA, Wender PA. Role of membrane potential and hydrogen bonding in the mechanism of translocation of guanidinium-rich peptides into cells. *J Am Chem Soc*. 2004;126:9506–9507.
5. Eberhard Y, McDermott S, Wang X, et al. Chelation of intracellular iron with the anti-fungal agent ciclopirox olamine induces cell death in leukemia and myeloma cells. *Blood*. 2009.
6. Wood TE, Dalili S, Simpson CD, et al. A novel inhibitor of glucose uptake sensitizes cells to FAS-induced cell death. *Mol Cancer Ther*. 2008;7:3546–3555.