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Cancer Diagnostics

A Bioluminescent Microbial Biosensor for In Vitro Pretreatment Assessment of Cytarabine Efficacy in Leukemia

Habib M. Alloush,[1,2] Elizabeth Anderson[1], Ashley D. Martin,[3] Mark W. Ruddock,[3] Johanna E. Angell,[1] Phil J. Hill,[4] Priyanka Mehta,[5] M. Ann Smith,[6] J. Graham Smith,[7] and Vyv C. Salisbury[1*]

BACKGROUND: The nucleoside analog cytarabine (Ara-C [cytosine arabinoside]) is the key agent for treating acute myeloid leukemia (AML); however, up to 30% of patients fail to respond to treatment. Screening of patient blood samples to determine drug response before commencement of treatment is needed. This project aimed to construct and evaluate a self-bioluminescent reporter strain of *Escherichia coli* for use as an Ara-C biosensor and to design an in vitro assay to predict Ara-C response in clinical samples.

METHODS: We used transposition mutagenesis to create a cytidine deaminase (*cdd*)-deficient mutant of *E. coli* MG1655 that responded to Ara-C. The strain was transformed with the *luxCDABE* operon and used as a whole-cell biosensor for development an 8-h assay to determine Ara-C uptake and phosphorylation by leukemic cells.

RESULTS: Intracellular concentrations of 0.025 μmol/L phosphorylated Ara-C were detected by significantly increased light output ($P < 0.05$) from the bacterial biosensor. Results using AML cell lines with known response to Ara-C showed close correlation between the 8-h assay and a 3-day cytotoxicity test for Ara-C cell killing. In retrospective tests with 24 clinical samples of bone marrow or peripheral blood, the biosensor-based assay predicted leukemic cell response to Ara-C within 8 h.

CONCLUSIONS: The biosensor-based assay may offer a predictor for evaluating the sensitivity of leukemic cells to Ara-C before patients undergo chemotherapy and allow customized treatment of drug-sensitive patients with reduced Ara-C dose levels. The 8-h assay monitors intracellular Ara-CTP (cytosine arabinoside triphosphate) levels and, if fully validated, may be suitable for use in clinical settings.

Although cytotoxic chemotherapy is widely used for treatment of many forms of cancer, failure to respond to anticancer drugs and resistance to treatment are major obstacles to satisfactory clinical outcome. Over 30% of acute myeloid leukemia (AML)[8] patients fail to respond to the nucleoside analog cytarabine (Ara-C [cytosine arabinoside]), the main drug used for AML treatment, and a substantial proportion fail to achieve long-term remission, whereas others respond to reduced levels of the drug *(1)*. Currently, patients undergo no prescreening for any of the courses of cytotoxic therapy to determine potential response *(1)*. For nonresponding patients, this lack of screening can result in a delay in commencement of effective treatment and unnecessary exposure to cytotoxic drugs with associated morbidity and mortality due to side effects and potential drug-induced secondary malignancies *(2)*. There is a need for rapid screening of patient samples to determine level of drug response before commencement of treatment that not only will give increased patient benefits, but also reduce healthcare costs *(1)*. It is anticipated that such a test could be analogous to the existing antibiotic sensitivity tests routinely used in clinical settings before antibiotic treatment for infection.

The nucleoside analog Ara-C is one of the most active single anticancer agents and is the main drug of chemotherapy for AML *(3, 4)*. In vivo, Ara-C is trans-

[1] Faculty of Health and Life Sciences, University of the West of England, Bristol, UK; [2] Faculty of Sciences, Division of Natural Sciences, Haigazian University, Beirut, Lebanon; [3] Randox Laboratories, Antrim, UK; [4] Division of Food Sciences, Nottingham University, Nottingham, UK; [5] Bristol Haematology and Oncology Centre, University Hospitals Bristol NHS Foundation Trust, Bristol, UK; [6] Stem Cell Transplant Laboratory, Royal Marsden NHS Foundation Trust, Sutton, Surrey, UK; [7] Department of Haematology, Frimley Park Hospital NHS Foundation Trust, Camberley, Surrey, UK.
* Address correspondence to this author at: Faculty of Health and Life Sciences, University of the West of England, Bristol BS16 1QY, UK. Fax +44-117-328-2904; e-mail vyv.salisbury@uwe.ac.uk.
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[8] Nonstandard abbreviations: AML, acute myeloid leukemia; Ara-C, cytarabine (cytosine arabinoside); dCK, deoxycytidine kinase; Ara-CMP, cytosine arabinoside monophosphate; Ara-CTP, cytosine arabinoside triphosphate; $FMNH_2$, reduced flavin mononucleotide; IPTG, isopropyl β-D-1-thiogalactopyranoside; *A*, absorbance; AP, alkaline phosphatase.

ported into the cell via the human equilibrium nucleoside transporter (hENT1) and is rapidly phosphorylated by deoxycytidine kinase (dCK) to its monophosphate form, cytosine arabinoside monophosphate (Ara-CMP), which is further phosphorylated by nucleoside kinases and finally to the triphosphorylated and active form, cytosine arabinoside triphosphate (Ara-CTP). Drug inactivation can result from Ara-CMP conversion into Ara-uracil by cytidine deaminase (*cdd*), or from dephosphorylation of Ara-CMP by cytoplasmic nucleotidase *(3)*. The antiproliferative and cytotoxic effects of Ara-CTP are largely due to its ability to interfere with DNA polymerase and its incorporation into DNA strands leading to chain termination and DNA synthesis arrest *(4)*.

Chemoresistance to Ara-C can arise from a number of factors influencing the rate of Ara-CTP formation and incorporation into DNA *(5)*, including low drug uptake, conversion into Ara-uracil by cytidine deaminase, or dephosphorylation of active metabolite by cytoplasmic nucleotidase *(6)*. Intracellular concentrations of Ara-CTP are reported to correlate with response to Ara-C therapy *(4)*. In vitro assessment of Ara-C efficacy has previously involved measurement of cell death, assessed by colony forming units–blast clonogenic assays *(7)*. This methodology involves primary cell culture over ≥3 days *(7)* and is not suitable for routine screening. In clinical practice, patients are treated with regimens including Ara-C regardless of their possible nonresponse to the drug and, as a result, suffer from debilitating side effects *(2, 8)*. Patients are given induction therapy over a period of weeks, before sensitivity or resistance is determined by leukemic cell count *(4)*.

Recombinant cell-based bioluminescence technology is an approach that has received increasing attention for biomedical applications *(9)* and has previously been used for determination of androgen bioactivity in clinical samples *(10)*. This technology has the potential to detect anticancer drug activity in malignant cells from clinical samples, within a period of hours. Light emission from bioluminescent bacteria is due to the activity of bacterial luciferase, which catalyzes the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain aliphatic aldehyde in the presence of O_2 to produce FMN and acid with the emission of blue-green light. The genes coding for the luciferase [alkanal monooxygenase subunit alpha (*luxA*)[9] and alkanal monooxygenase subunit beta (*luxB*)] are linked to those coding for the fatty acid reductase complex [fatty acid reductase (*luxC*); acyl transferase (*luxD*); and acyl-protein synthetase (long-chain-fatty-acid-luciferin-component ligase) (*luxE*)], which catalyzes the production of luciferin from the fatty acid pool *(11)*. Because the production of $FMNH_2$ directly depends on a functional electron transport system, only metabolically active cells emit light. Hence, any alteration of cellular metabolism is reflected by a change in light emission. Bioluminescent bacterial biosensors have been used to assess the effect of various chemical, biological, and physical signals, providing an accurate, nondestructive, and real-time assay technology *(9)*. Bioluminescence phenotype can be conferred upon most bacteria by introducing and expressing the *luxCDABE* operon, isolated from *Photorhabdus luminescens*, under the control of constitutive promoters. These whole-cell bacterial biosensors do not require exogenous substrate and give out light as a direct indicator of the physiological status of the bacteria in real time *(12)*. The high metabolic rate of bacterial cells, compared with mammalian cells, means that these reporters are ideal for fast, accurate, real-time in situ toxicity testing. Light output from these biosensors can be accurately measured, with no background interference, by using either luminometers or low-light cameras. *Lux* gene–based reporter technology in bacterial biosensors has been successfully used to monitor pharmacodynamics of antimicrobial agents in real time *(13, 14)*.

Ara-C has no effect on *Escherichia coli*, since the bacteria lack dCK that phosphorylates Ara-C into its active form and they actively deaminate Ara-C into Ara-uracil through the activity of cytidine/deoxycytidine deaminase (*cdd*). Wang et al. *(15)* constructed a *cdd*-deficient *E. coli* strain (SØ5218) that, upon the expression of the human deoxycytidine kinase (*DCK*) gene, exhibited reduced relative growth in the presence of Ara-C in minimal growth medium. Therefore, this indicates that expression of human dCK in SØ5218 leads to the incorporation of Ara-CTP into bacterial DNA. However, the growth inhibitory effect of Ara-C was completely abolished when assayed in rich growth medium due to incorporation of cytosine triphosphate instead of the toxic analog Ara-CTP into bacterial DNA *(15)*.

The main aim of this study was the construction of a pyrimidine-requiring, *cdd*-deficient mutant of *E. coli* MG1655 strain with enhanced sensitivity toward Ara-C in rich medium and its use as a bioluminescent biosensor in an 8-h assay to determine Ara-C uptake as well as its phosphorylation by human leukemic cells. The assay provides information on the phenotype of leukemic cells in clinical samples of peripheral blood or bone marrow.

[9] Genes: *luxA*, alkanal monooxygenase subunit alpha; *luxB*, alkanal monooxygenase subunit beta; *luxC*, fatty acid reductase; *luxD*, acyl transferase; *luxE*, acyl-protein synthetase (long-chain-fatty-acid-luciferin-component ligase); *cdd*, cytidine/deoxycytidine deaminase; *pyrE*, orotate phosphoribosyltransferase; *DCK*, deoxycytidine kinase.

Materials and Methods

BACTERIAL STRAINS, PLASMIDS, AND GROWTH MEDIA

E. coli SØ5218 (a gift from S. Eriksson, University of Copenhagen) is a *cdd*-deficient strain expressing the human *DCK* cDNA on the isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible pTrc99-A plasmid *(14)*. *E. coli* MG1655 (a gift from K.J. Jensen, University of Copenhagen, Denmark) is a derivative of the wild-type *E. coli* K-12 that requires pyrimidine in minimal growth medium, due to suboptimal expression of the enzyme orotate phospho-ribosyltransferase coded for by the orotate phosphoribosyltransferase (*pyrE*) gene *(16)*. Both strains were made self-bioluminescent by transformation, using standard methods previously described *(9)*, with the broad-host vector pBBR1MCS-2 *(17)* carrying the *luxCDABE* cassette from *Photorhabdus luminescens* as an *Eco*RI PCR fragment. All chemicals and growth media were supplied by Sigma-Aldrich (www.sigmaaldrich. com) unless otherwise stated. Luria-Bertani growth medium was used for routine culturing of bacteria. The antibiotics tetracycline, ampicillin, and kanamycin were added at a final concentration of 10, 100, and 10 mg/L, respectively. Growth inhibition experiments were performed in Luria-Bertani growth medium, RPMI 1640 (Invitrogen, www.invitrogen.com), or AB minimal medium *(16)* plus 2.0 g/L glucose and 2.0 g/L vitamin-free casamino acids and supplemented with thiamine and leucine at final concentrations of 1 and 20 mg/L, respectively.

CELL LINES, BONE MARROW, AND BLOOD SAMPLES

The monocytic AML cell lines KG-1a and THP-1 (DSMZ, www.dsmz.de) were grown and maintained as a cell suspension in RPMI 1640 medium supplemented with 2 mmol/L glutamine (Invitrogen) and 10% fetal calf serum (Biosera, www.biosera.com) at 37 °C and 5% CO_2. Cultures were maintained at a density of $1–4 \times 10^6$ cells/mL and were monitored by vital staining using trypan blue. Cryopreserved bone marrow samples, previously separated by density gradient centrifugation to yield the mononuclear cell fraction, were thawed and resuspended in RPMI 1640 medium before incubation with Ara-C. Mononuclear cells from fresh AML peripheral blood samples (collected from patients at Frimley Park Hospital, Surrey, UK, and Bristol Haematology and Oncology Centre, Bristol, UK, after obtaining informed consent) were also isolated by density gradient centrifugation and resuspended in RPMI 1640 medium. In all cases, samples were obtained from patients at diagnosis, before the start of treatment, and with blood blast burdens >80% and were analyzed blind to avoid bias. Subsequent clinical outcome after induction chemotherapy with Ara-C–containing treatment cycles was available in all cases. Ara-C was added at a final concentration of 25 μmol/L, which represents the equivalent of the standard in vivo dose of 200 $mg \cdot m^{-2} \cdot day^{-1}$ (based on an 80-kg individual (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue12) *(18)*.

CONSTRUCTION OF A *cdd*-DEFICIENT MUTANT OF *E. coli* MG1655

A library of *E. coli* MG1655 mutants was constructed by using random transposition mutagenesis with P1 phage carrying Tn10 transposon, which can transpose from the phage into the *E. coli* chromosome, according to Miller *(19)* with slight modification. Briefly, *E. coli* MG1655 mutants were grown in Luria-Bertani broth until the midexponential phase and were infected with P1 phage at a multiplicity of infection of approximately 5. The infected cells were plated on Luria-Bertani agar plates containing tetracycline to select for mutants carrying the transposon. The *cdd* mutants were selected by growth after 24-h incubation at 37 °C on plates containing 10 mg/L of the analog 5-fluoro-2′deoxycytidine, which is toxic to *E. coli*–containing *cdd* activity. The phenotype of the *cdd* mutant (*E. coli* HA1) was confirmed by the absence of cytidine deaminase activity.

WESTERN BLOT ANALYSIS FOR *DCK* EXPRESSION

Lyophilized HA1 cells were reconstituted with 1 mL water per vial and incubated at 37 °C for 1 h. Cells were diluted 1/10 in prewarmed RPMI 1640 containing tetracycline, ampicillin, and kanamycin, added at a final concentration of 10, 100, and 10 mg/L, respectively. A 1-mL $t = 0$ sample was removed, and 1 mmol/L IPTG was added to the remaining culture. Cells were incubated at 37 °C with shaking at 220 rpm. Samples of 1 mL were removed every 30 min for 3 h. The samples collected at different time points were centrifuged, and the supernatant was removed. Pellets were lysed with 100 μL CelLyticB containing 2 mmol/L phenylmethanesulfonyl fluoride and 10 mg/L DNase I, to extract soluble protein. Protein concentrations were determined by Bradford Assay (A595 nm) using BSA as a standard, and 100 ng protein for each sample was then separated on a 12% SDS-PAGE gel for 1 h at 300 V. The separated proteins were transferred to a nitrocellulose membrane for 1 h at 100 V. Membranes were blocked for 1 h at room temperature with 3% BSA in Tris-buffered saline with Tween. Detection of protein was carried out by using the anti-dCK primary antibody (rabbit polyclonal to dCK; Abcam AB38012, Abcam, www.abcam.com) at 1:500 dilution and goat anti-rabbit horse radish peroxidase–conjugated polyclonal as secondary conjugate (Sigma A0545) at 1:5000 dilution. Presence of bound horse radish peroxidase conjugate was detected by using the BM chemiluminescent

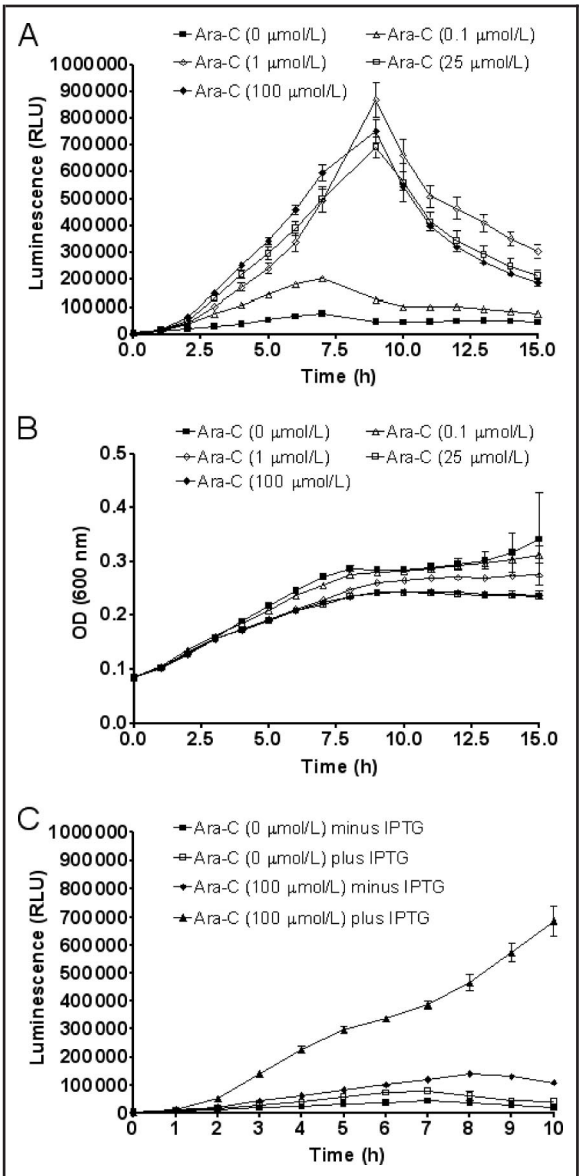

Fig. 1. Biosensor response.

The effect of Ara-C concentrations (0.1, 1, 25, and 100 μmol/L) on luminescence (A) and growth (B) of *E. coli* HA1 in the presence of IPTG; the effect of IPTG induction of dCK in the presence of Ara-C (C) is also shown. Growth was monitored by measuring 600 *A*; luminescence is shown as relative light units (RLU); n = 10 and error bars represent SD.

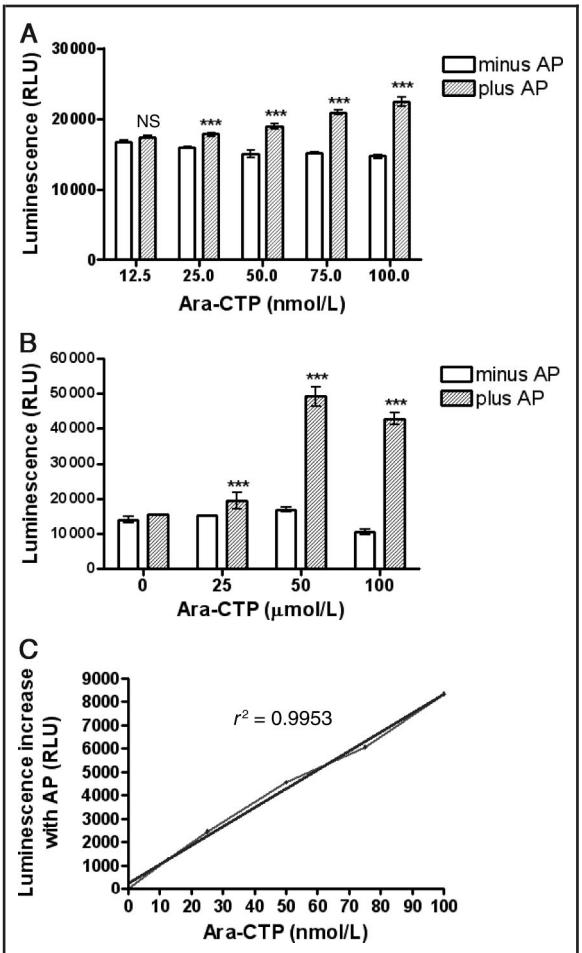

Fig. 2. The effect of Ara-CTP on the biosensor.

Peak light emission, measured as relative light units (RLU), from *E. coli* HA1 in RPMI at 37 °C treated with Ara-CTP at 12.5, 25, 50, 75, and 100 nmol/L (A) and 0, 25, 50, and 100 μmol/L (B) in the absence and presence of AP (mean of n = 3 and error bars show range). (C), correlation between Ara-CTP and light emission +/− AP (significance with 1-way ANOVA and Bonferroni adjusted post hoc tests, shown in Table 3 in the online Data Supplement; ***$P <$ 0.001). NS, not significant.

detection system (Roche, www.roche.com) and standard x-ray film (Kodak, www.kodak.co.uk).

BIOSENSOR DOSE RESPONSE ANALYSIS

An overnight culture of *E. coli* HA1 was diluted to an absorbance (*A*) at 600 nm (600 *A*) of 0.1 in RPMI 1640 medium and incubated for 30 min at 37 °C with shaking at 220 rpm. Samples of 200 μL with either Ara-C or fludarabine (F-ara-A) concentrations of 0–100 μmol/L and 1 mmol/L IPTG were loaded into a black-walled microtiter plate (Invitrogen). Bioluminescence and growth (600 *A*) were monitored every 15 min for 15 h in a multimode microplate reader (Tecan, www. tecan.com).

CELL LYSIS ASSAY

Cell lines were harvested by centrifuging at 300*g* for 5 min at room temperature and resuspended in

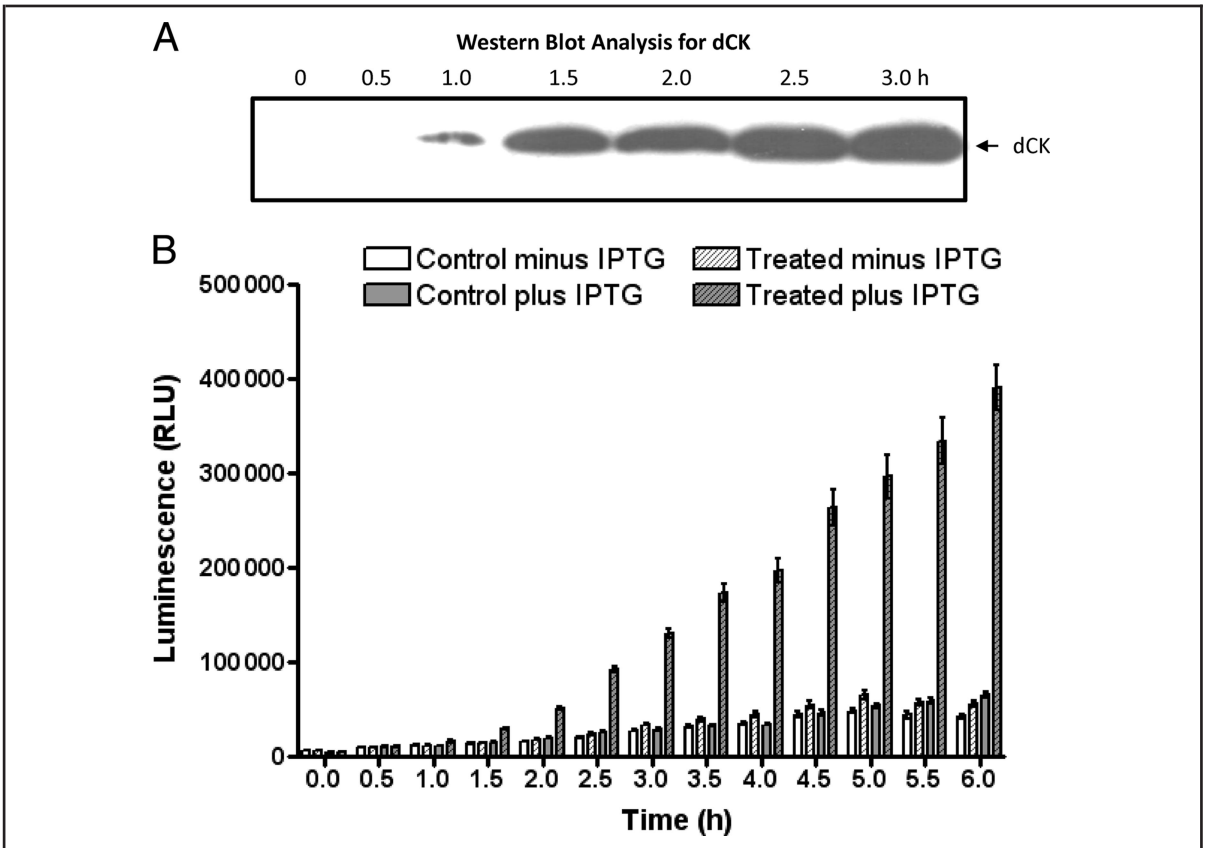

Fig. 3. IPTG induction of dCK expression in HA1 cells.

(A), Representative Western blot for time course of IPTG-induced expression of dCK protein between 0 and 3 h. (B), Effect of IPTG-induced expression of dCK on light emission by HA1 with (treated) and without (control) 25 μmol/L Ara-C (mean of n = 10 and error bars show SD). Expression is maximal at t = 3 h.

RPMI 1640 with no additives at a concentration of 2×10^6 cells/mL. Cell lines, peripheral blood, and bone marrow blasts were treated with 25 μmol/L Ara-C for 30 min at 37 °C and 5% CO_2. After exposure to Ara-C, cultures were centrifuged as previously described and the supernatant was removed. Harvested cells were washed and resuspended in fresh RPMI 1640 medium. EDTA and saponin were added to cell suspensions at final concentrations of 1.5 mmol/L and 1.0 g/L, respectively. Samples were vortex-mixed for 30 s, and cell debris was removed by centrifugation at 2800*g* for 5 min at room temperature. Lysate samples were each mixed with an overnight culture of the reporter bacteria, freshly diluted to a 600 *A* of 0.1 in a black microtiter plate (Invitrogen).

A total of 1 mmol/L IPTG and 10 U alkaline phosphatase (AP) was added to the relevant samples. Bioluminescence and growth (600 *A*) were monitored in a Tecan multimode microplate reader. Ara-C toxicity was also assayed with a 3-day cytotoxicity assay using the Cell Titer-Glo® Luminescent Cell Viability Assay kit (Promega, www.promega. com). Cells were treated as above with 25 μmol/L Ara-C for 72 h at 37 °C and 5% CO_2, to allow cells to enter the S phase and hence be sensitized to Ara-CTP, before measuring intracellular levels of ATP according to the manufacturer's instructions (Promega G7570 Cell Titer-Glo Luminescent Cell Viability Assay).

STATISTICAL ANALYSIS

Statistical significance was determined by using one-way ANOVA with Bonferroni corrected post hoc tests (GraphPad Prism 4). Number of repeat treatments was 3 or more throughout; standard deviation was calculated for n = 10 repeats, and mean and range is shown for n = 3 repeats.

Results

E. coli SØ5218, the *cdd*-deficient strain constructed by Wang et al. *(15)*, expressing the human *dCK* gene, ex-

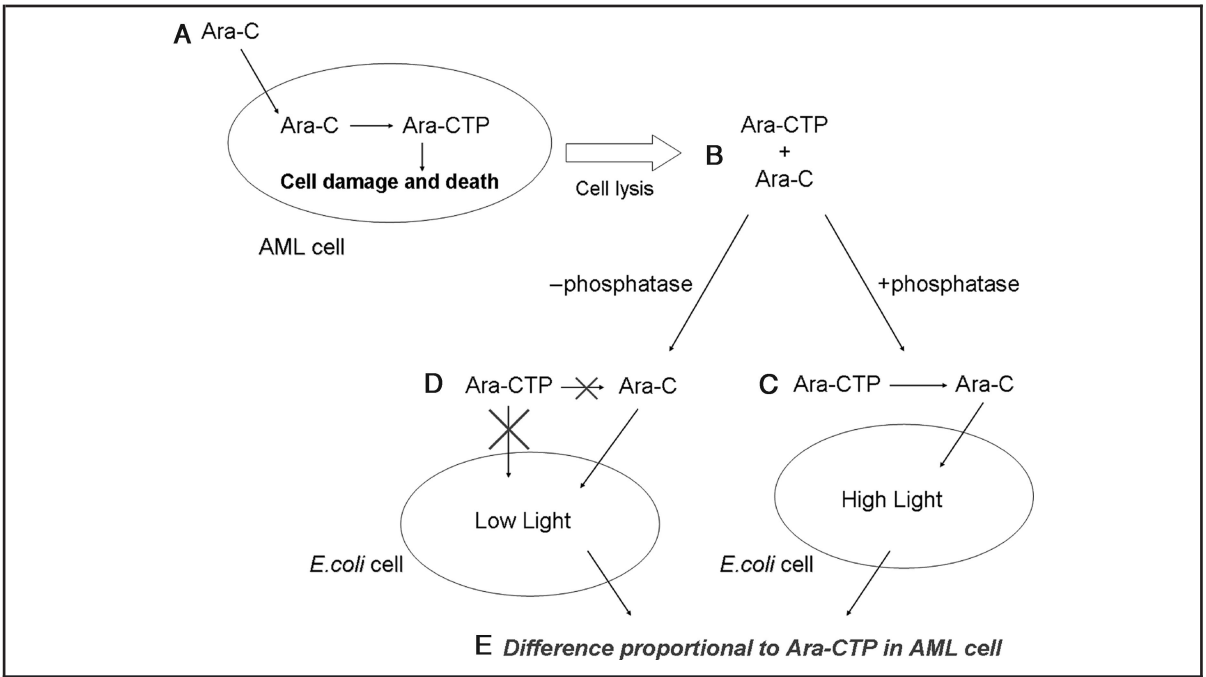

Fig. 4. Schematic of the cell lysis assay using *E. coli* HA1 to show intracellular response to Ara-C.

(A), Mononuclear cells from patients with AML incubated with Ara-C were washed and lysed in the presence of EDTA (1.5 mmol/L) and saponin (1.0 g/L). (B), Subsequent cell lysate (containing both Ara-C and Ara-CTP) was exposed to the bacterial biosensor in the presence or absence of AP. (C), In the presence of AP, Ara-CTP is converted to Ara-C, which on entering the bacterium allows the generation of bioluminescence. (D), In the absence of AP, Ara-CTP remains intact and cannot enter the bacterium. (E), The ratio between +/− AP is directly proportional to the concentration of Ara-CTP in the patient blasts, which is representative of the patient's ability to convert Ara-C to Ara-CTP.

hibited reduced relative growth in the presence of Ara-C in minimal medium but not in a rich growth medium, due to incorporation of cytosine triphosphate instead of the toxic analog Ara-CTP into bacterial DNA.

To measure Ara-C response in a nutrient-rich intracellular environment, an alternative *cdd*-deficient mutant of a bioluminescent *E. coli* MG1655 strain, requiring exogenous pyrimidine due to suboptimal expression of the enzyme orotate phosphoribosyltransferase coded for by the *pyrE* gene *(16)*, was constructed by using transposon mutagenesis. This mutant was further transformed with pTrcHUMdCK *(15)* to give IPTG-inducible expression of dCK. The newly constructed bacterial biosensor, *E. coli* HA1, showed reduced growth in the presence of Ara-C, even when assayed in nutrient-rich medium (Fig. 1B). Ara-C caused significantly ($P < 0.001$; see Table 2 in the online Data Supplement) increased light output from the biosensor (Fig. 1A) with concentrations as low as 0.1 μmol/L. This significantly increased light output was only observed during treatment with the pyrimidine analog Ara-C and only in the presence of IPTG-activated dCK (Fig. 1C). The specificity of the biosensor to Ara-C was indicated by a lack of biosensor response in a control assay with the purine analog fludarabine (Fig. 1 in the online Data Supplement shows that IPTG activation of dCK had no effect on *E. coli* HA1 treated with fludarabine).

To increase the specificity of the assay, direct effects of the active intracellular drug derivative, Ara-CTP, on the bacterial biosensor were monitored (Fig. 2). The results indicate that Ara-CTP does not enter the reporter bacteria, showing no increase in light output compared with the untreated control, unless AP is added at the start of the assay. The increase in peak light output in the AP-treated samples (Fig. 2, A and B) is similar to that observed with Ara-C (Fig. 1A) and supports our reasoning. By measuring peak bioluminescence increase in the presence and absence of AP (+/−), it is possible to quantify the intracellular conversion of Ara-C to Ara-CTP, within leukemic cells. Fig. 2, A and C, show that there is good correlation between light output +/− AP and nanomolar concentrations of Ara-CTP and also that concentrations as low as 25 nmol/L

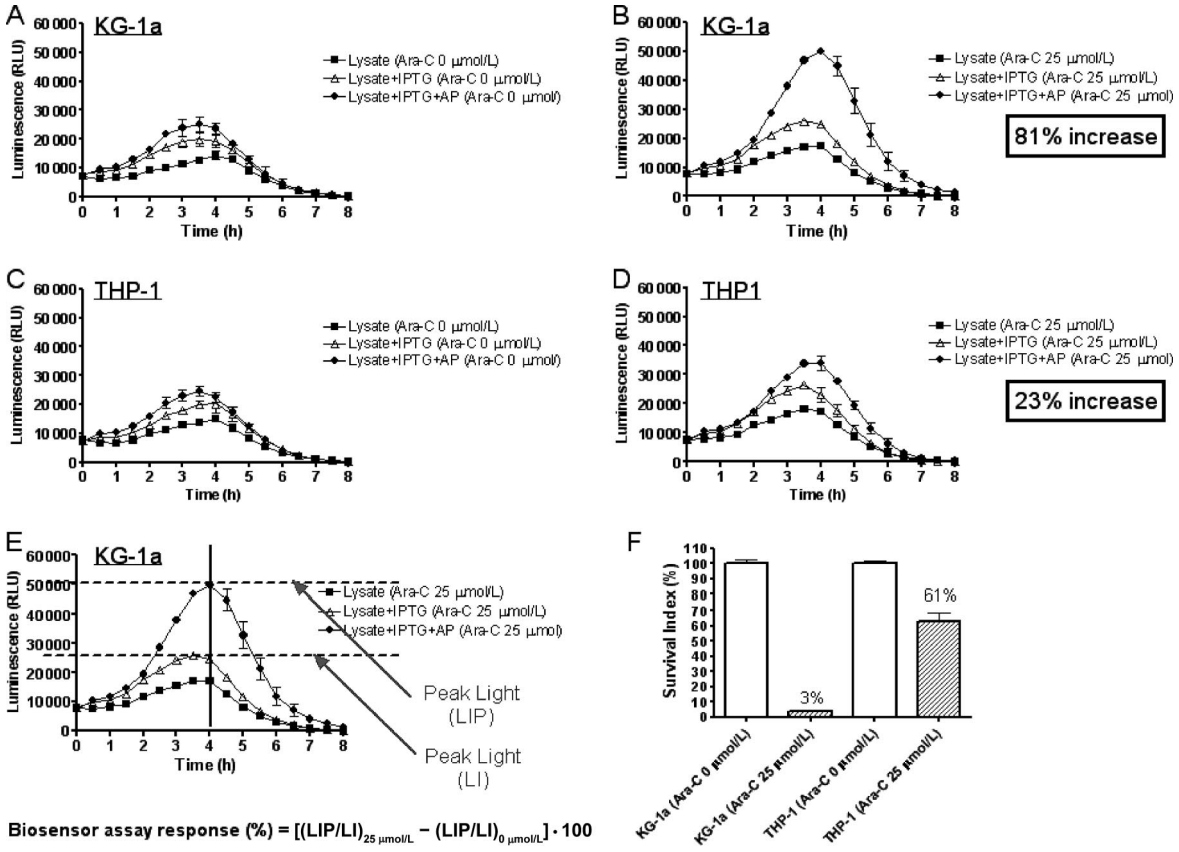

Fig. 5. Effect of 30-min treatment with Ara-C (25 μmol/L) on KG-1a (A and B) and THP-1 (C and D) cell lines by using the bioluminescent bacterial reporter *E. coli* HA1.

Calculation of biosensor assay response is shown in (E). Equivalent results using the Cell Titer-Glo Luminescent Cell Viability assay kit (survival index) are shown in (F). n = 10, and error bars are SD.

can be detected by a significant increase in light output ($P < 0.05$; see Table 3 in the online Data Supplement).

A Western blot for IPTG-induced expression of dCK in the biosensor showed a high level of expression after 3 h (Fig. 3A), leading to the increase in bioluminescence (Fig. 3B) only in Ara-C–treated cells plus IPTG.

AML cell lines and leukemic cell samples from AML patients with known clinical outcome to Ara-C treatment were used to evaluate the assay outlined by the schematic in Fig. 4. A 3-day cytotoxicity test confirmed that KG-1a cells were sensitive to Ara-C, whereas the THP-1 cell line was partially resistant (Fig. 5F). Running the assay with and without AP in parallel enabled the determination of the relative proportion of Ara-C that had been converted within AML cells into Ara-CTP. THP-1 cells that had not converted Ara-C to Ara-CTP generated similar output whether or not AP was present in the assay (Fig. 5, C and D). KG1-a cells generated the greatest difference ($P < 0.001$) in light output, in the presence of AP, due to conversion of Ara-C to Ara-CTP, indicating high drug response (Fig. 5, A and B). The diagnostic discriminator, assuming that generation of Ara-CTP in AML cells is the major cause of cell death *(4, 5)*, is the maximal difference in light output between the IPTG (LI) and IPTG + AP (LIP) curves that are shown in Fig. 5E.

Data were analyzed by single point measurement at peak light output, comparing treated and untreated samples +/− AP, as shown in Fig. 5E. The calculation of the percentage response uses a simple formula:

$$\text{Biosensor assay response (\%)} = [(\text{LIP/LI})_{25\ \mu\text{mol/L}} - (\text{LIP/LI})_{0\ \mu\text{mol/L}}] \times 100$$

Preliminary assay of stored blood and bone marrow samples taken at presentation from 12 known responding and 12 known nonresponding Ara-C–treated pa-

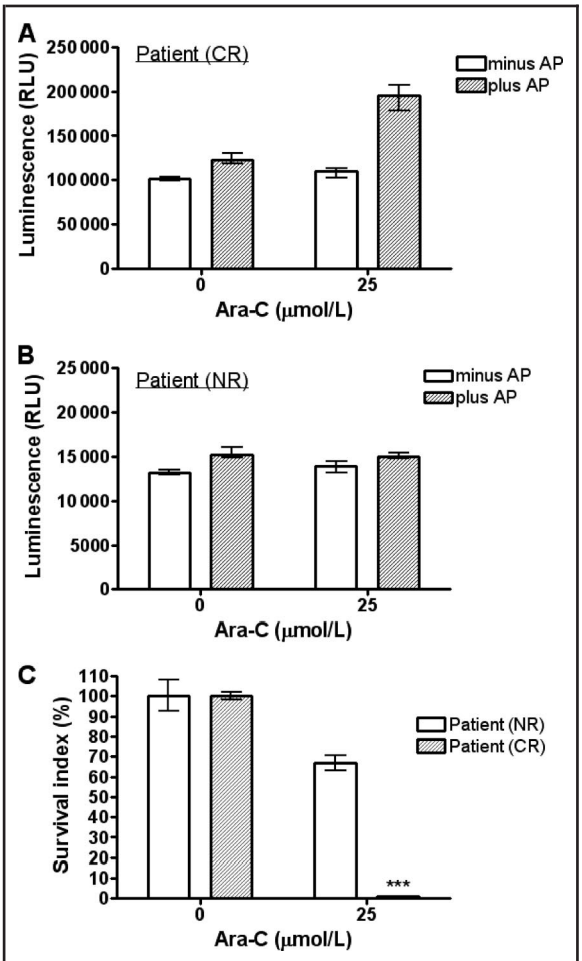

Fig. 6. Effect of 30 min treatment with Ara-C (25 μmol/L) on clinical samples from 2 patients by use of the bioluminescent bacterial reporter *E. coli* HA1.

Results from a clinically sensitive patient (A) and a clinically resistant patient (B) are shown. Equivalent results using the Cell Titer-Glo Luminescent Cell Viability assay kit (survival index) are shown (C); significance was determined with 1-way ANOVA and Bonferroni adjusted post hoc tests. ***$P < 0.001$. Mean of n = 3 and error bars show range.

tients gave biosensor assay responses between 21 and 128% (median 55%) for responding patients and between −7 and 6% (median 0%) for nonresponding patients (see Table 4 in the online Supplemental Data). A typical example of each type is shown in Fig. 6; patient sample CR (with clinical outcome of complete remission) showed a significant difference in the peak light output ($P < 0.001$) in the presence and absence of AP, indicating response to a drug (Fig. 6A), whereas sample NR (nonresponsive clinical outcome) exhibited no significant difference ($P > 0.05$) in light output between the (+/−) curves, indicating a low concentration of Ara-CTP in the cells (Fig. 6B). The 3-day cytotoxicity test also showed that cells from patient sample CR were significantly more sensitive to Ara-C compared with cells from patient sample NR (Fig. 6C).

Discussion

The cell lysis assay, using the bioluminescent bacterial biosensor as a reporter of Ara-C activity, relies on the response of bacteria to Ara-C. Because of the increased metabolic rate and shorter cell cycle time of bacteria, results can be achieved within a period of hours. In contrast, mammalian cells undergo slower cell division, which is the reason for the incubation times of 3 days or more needed to measure Ara-C cytotoxicity. The increase in light output from the biosensor, which closely correlates with the concentration of an active drug under assay conditions in clinical samples, gives a measure of mononuclear cell phenotype with regard to intracellular Ara-CTP production during the time interval that the cells are exposed to Ara-C.

The bioluminescence increase brought about by Ara-C in the biosensor is similar to previous reports of enhanced light emission in luciferase-based biosensors brought about by impairment of the bacterial intracellular equilibrium, leading to NADPH accumulation *(20)*, or by DNA damage *(21)*. It is reported that bioluminescence stimulates DNA repair in bacteria, possibly by providing photons for bacterial photolyase activity *(22)* and that *lux* genes are regulated by the bacterial SOS stress response *(23)*, which may explain the increase in bioluminescence in the presence of Ara-C.

The biosensor assay demonstrates Ara-C uptake and activity in mononuclear cells isolated from AML patient bone marrow and peripheral blood samples within 8 h. The assay is the first of its kind, showing phenotypic drug response within 8 h and here has been used in a retrospective study of 24 clinical samples. This is a preliminary validation study, and further work will include prospective, controlled randomized trials to establish the usefulness of the assay for AML and acute lymphoblastic leukemia, which account for 2% of cancers in the UK *(24)*. The assay has the potential to detect response to Ara-C of myeloid blasts, irrespective of whether they are obtained at diagnosis or at relapse/nonresponse. This will be fully evaluated in prospective clinical trials. The assay demonstrates that Ara-C sensitivity of AML cells can be simply and accurately determined within 8 h, by using the bioluminescent bacterial reporter construct. Currently, we are extending our studies to include samples from a larger cohort of AML patients to investigate correlation with clinical outcome. If full clinical trials demonstrate that the as-

say has predictive value, it may have the potential for development as a diagnostic kit suitable for use in a routine clinical laboratory, enabling rapid determination of the degree of response before patients undergo chemotherapy.

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Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

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