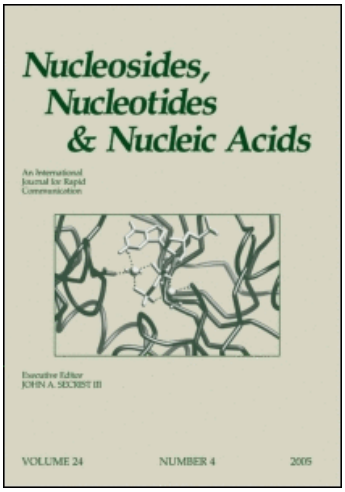


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# BACTERIAL DEOXYRIBONUCLEOSIDE KINASES ARE POOR SUICIDE GENES IN MAMMALIAN CELLS

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□ *Transfer of deoxyribonucleoside kinases (dNKs) into cancer cells increases the activity of cytotoxic nucleoside analogues. It has been shown that bacterial dNKs, when introduced into* Escherichia coli*, sensitize this bacterium toward nucleoside analogues. We studied the possibility of using bacterial dNKs, for example deoxyadenosine kinases (dAKs), to sensitize human cancer cells to gemcitabine. Stable and transient transfections of bacterial dNKs into human cells showed that these were much less active than human and fruitfly dNKs. The fusion of dAK from* Bacillus cereus *to the green fluorescent protein induced a modest sensitization. Apparently, bacterial dNKs did not get properly expressed or are unstable in the mammalian cell.*

**Keywords** Deoxyribonucleoside kinases; gemcitabine; gene therapy; suicide gene; bacteria; cancer; resistance

## INTRODUCTION

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine, dFdC) is a nucleoside analogue used in cancer treatments. To exert its activity, gemcitabine is phosphorylated inside cancer cells by deoxyribonucleoside kinases (dNKs) to dFdC-monophosphate (dFdCMP), and subsequently by nucleotide kinases to its active di- and tri-phosphorylated forms (dFdCDP and dFdCTP). Earlier studies have shown that human deoxycytidine kinase (HsdCK)

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phosphorylates gemcitabine, and that a deficiency in this kinase induces resistance to the nucleoside analogue.[1] The importance of this step in the activating metabolism of gemcitabine, and the presence of kinase-deficient resistant cells, has lead to the research on and the development of suicide-gene based therapy in association with nucleoside analogues.[2] It has thus been shown that the introduction of HsdCK or the highly efficient dNK from *Drosophila melanogaster* (DmdNK) in dNK-proficient or -deficient cancer cells, increases the activity of gemcitabine and other nucleoside analogues.[3–6] Further studies have reported attempts to improve the activity of dNKs in order to increase their possible use in gene therapy.[7–10]

The recent discovery of bacterial dNKs activating gemcitabine as well as other nucleoside analogues, indicates that there might exist dNKs more suitable for gene therapy than HsdCK or DmdNK.[11,12] Some of the identified bacterial kinases, when introduced into the *Escherichia coli* model cell, greatly sensitize this bacterium toward gemcitabine. It has also been shown that these dNKs phosphorylate gemcitabine in vitro. We, therefore, selected a few of these dNKs for a study on the sensitization toward gemcitabine in human cancer cells transfected with these kinases. Here, we present results from the first study using bacterial dNKs to sensitize dCK-deficient human cancer cells to the cytotoxic activity of gemcitabine. The studied kinases were deoxyadenosine kinases from *Clostridium perfringens* (CpdAK), *Bacillus cereus* (BcdAK), *Staphyllococcus aureus* (SadAK), and *Streptococcus pyogenes* (SpdAK), in addition to HsdCK and DmdNK used as positive controls.



## MATERIAL AND METHODS

### Cell Culture

The cell lines used in our study were the gemcitabine-resistant and dCK-deficient cells derived from human ovarian sarcoma Messa (Messa 10K), the human lung carcinoma SW-1573 (SWg) and the human ovarian carcinoma A2780 (AG6000). The development and the characterization of these gemcitabine resistant variants has been described elsewhere.[4,13,14] All cells were grown at 37°C in presence of $CO_2$ 5% in either RPMI medium (Messa and SW-1573; Invitrogen, Cergy Pontoise, France) or DMEM medium (A2780; Invitrogen, Cergy Pontoise, France) containing l-glutamine, penicilline (200 UI/ml, Gibco, Cergy Pontoise, France), streptomycine (200 $\mu$g/ml, Gibco, Cergy Pontoise, France), and fetal bovine serum (10%, PAN Biotech GmBH, Aidenbach, Germany).

### Cloning

cDNA for BcdAK (accession number DQ384596), CpdAK (accession number DQ384599), SadAK (accession number DQ384604), and SpdAK

(accession number EF061223) were amplified from pGEX-2T plasmids containing the corresponding genes and cloned into pcDNA3 vector with KpnI and BamHI. HsdCK was amplified from cDNA from human mammalian cancer cells (MCF7) cells and cloned into pcDNA3 using KpnI and BamHI. The preparation of pcDNA3-DmdNK has been described earlier.[4] For GFP-containing plasmids, GFP was amplified from pAcGFP1-tubulin (Clontech Laboratories, Saint-Germain-en-Laye, France) and cloned into empty or kinase-containing pcDNA3 vector with HindIII and KpnI.

## Transfections and Cell Survival Studies

For stable transfections, 150,000 Messa 10K, AG6000 and SWg cells were transfected with 1.5 $\mu$g plasmid, 5 $\mu$l Lipofectine (Invitrogen, Cergy Pontoise, France) and 500 $\mu$l OptiMEM (Invitrogen, Cergy Pontoise, France), and selected with 400, 600, and 800 $\mu$g/ml geneticin (Invitrogen, Cergy Pontoise, France) starting 72 hours after the transfection. Expression of transgenes was verified by RT-PCR.



For cell survival analysis, transfected cells were seeded in 96-well plates (3000 cell per well), and incubated for 24 hours before different concentrations of gemcitabine were added. After another 72 hours of incubation, methylthiazoletetrazolium (MTT, 100 $\mu$g per well; Sigma Aldrich, Saint-Quentin, France) was added, and after 2 hours, the supernatant was replaced with 100 $\mu$l isopropanol/$H_2O$/HCl (90:9:1, v/v/v). Optical density was determined with a microplate reader and the $IC_{50}$ (concentration inhibiting 50% of cell proliferation) was determined with Microsoft Excel.

For transient transfections, we used the protocol described earlier.[4] Briefly, cells were seeded in 6-well plates (50,000 cells per well), and transfected with 1.5 $\mu$g plasmide, 5 $\mu$l Lipofectin and 500 $\mu$l OptiMEM. Complete culture medium containing different concentrations of gemcitabine was added 4 hours after the transfection and cells were incubated for 72 hours. MTT (500 $\mu$g/well) was added and the determination of $IC_{50}$ was done as for stably transfected cells.

Ratio of sensitization (RS) is the ratio between the $IC_{50}$ of empty pcDNA3-transfected cells and the $IC_{50}$ of kinase-containing pcDNA3-transfected cells.

## Deoxyribonucleoside Kinase Activity Measurements

Cells were pelleted and the pellets lysed in lysis-buffer (1XPBS, 5mM DTT, 0.1% Triton X-100, 2 mM ATP, 2 mM $MnCl_2$, 2 mM $MnCl_2$, 10% glycerol), ultrasonicated twice for 2 seconds, and centrifuged 5 minutes at 13,000 rpm. The supernatant was removed and 3 mM NaF and 0.2 mM tetrahydrouridine were added to the reaction mix. The reaction contained 2 mM ATP as the phosphate donor and 100 $\mu$M 3H labelled deoxynucleoside

**TABLE 1** Sensitivity to gemcitabine (IC50 in $\mu$M) and ratio of sensitization (RS) of dCK-deficient cancer cells stably transfected with deoxyribonucleside kinases

| | SWg | | AG6000 | | Messa 10K | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ ($\mu$M) | RS | $IC_{50}$ ($\mu$M) | RS | $IC_{50}$ ($\mu$M) | RS |
| **pcDNA3** | $43.0 \pm 6.5$ | | $17.2 \pm 1.2$ | | $10.7 \pm 3.9$ | |
| **HsdCK** | $0.018 \pm 0.005$* | 2389 | $<0.1$ | $>172$ | $17.7 \pm 3.8$ | 0.6 |
| **DmdNK** | $0.013 \pm 0.001$* | 3308 | $<0.1$ | $>172$ | $6.2 \pm 1.1$ | 1.7 |
| **CpdAK** | $33.3 \pm 15.8$ | 1.29 | $16.8 \pm 2.2$ | 1.0 | $10.1 \pm 2.4$ | 1.1 |
| **SpdAK** | $25.0 \pm 7.8$ | 1.72 | $29.3 \pm 3.8$ | 0.6 | $7.9 \pm 1.1$ | 1.4 |
| **SadAK** | $16.4 \pm 4.2$* | 2.6 | $79.3 \pm 16.1$* | 0.2 | $12.9 \pm 1.6$ | 0.8 |
| **BcdAK** | $19.7 \pm 4.2$ | 2.2 | $32.8 \pm 7.1$ | 0.5 | $29.5 \pm 10.9$ | 0.4 |

Data are mean values $\pm$ standard error from at least three experiments.
*$p < 0.05$ using Student's $t$ test and comparing with pcDNA3.

from Moravek as the phosphate acceptor. The reaction was terminated by a transfer to an anion-exchange paper disk and non-phosphorylated substrates were subsequently washed off the paper. The phosphorylated products were eluted from the paper disk and the quantity of the labeled monophosphate product determined in a scintillation counter.[8,15]



## RESULTS

### Stably Transfected Cells

Cell survival of stably transfected SWg, AG6000 and Messa 10K cells in presence of gemcitabine was determined. As shown in Table 1, HsdCK and DmdNK kinases were able to restore sensitivity to gemcitabine in the SWg cell line, with a ratio of sensitization (RS) of 2389 and 3308, respectively. We also observed a statistically significant 2.6-fold sensitization in SWg cells transfected with SadAK, whereas the 1.3- to 2.2-fold sensitizations observed with the other bacterial kinases were not statistically significant. Similar results were observed in AG6000 cells transfected with HsdCK and DmdNK (RS $>172$), whereas no sensitization was observed with the bacterial kinases. In Messa 10K cells, we did not obtain any sensitization upon transfection of deoxyribonucleoside kinases (Table 1). This result confirms our previous results based on the stable transfection of DmdNK in these cells.[4]

### Transiently Transfected Cells

As results for bacterial dNK from the stably transfected cells were not promising (SWg and AG6000 cells) or difficult to interpret (Messa 10K cells), we performed cell survival analysis after transient transfection of the dCK-deficient cells. Here, using SWg cells, we only obtained weak and non significant sensitization with DmdNK and no effect with HsdCK or bacterial

**TABLE 2** Sensitivity to gemcitabine (IC50 in $\mu$M) and ratio of sensitization (RS) of dCK-deficient cancer cells transiently transfected with deoxyribonucleside kinases

| | SWg | | AG6000 | | Messa 10K | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ ($\mu$M) | RS | $IC_{50}$ ($\mu$M) | RS | $IC_{50}$ ($\mu$M) | RS |
| **pcDNA3** | $14.8 \pm 5.5$ | | $60.0 \pm 9.6$ | | $5.9 \pm 0.5$ | |
| **HsdCK** | $15.8 \pm 4.5$ | 0.9 | $2.2 \pm 0.7$* | 27.3 | $2.4 \pm 0.7$* | 2.5 |
| **DmdNK** | $2.7 \pm 0.8$ | 5.5 | $1.7 \pm 1.1$* | 35.3 | $1.2 \pm 0.6$* | 4.9 |
| **CpdAK** | $13.4 \pm 3.0$ | 1.1 | $47.0 \pm 10.8$ | 1.3 | $8.7 \pm 2.2$ | 0.7 |
| **SpdAK** | $18.4 \pm 7.0$ | 0.8 | $40.0 \pm 5.9$ | 1.5 | $6.4 \pm 0.5$ | 0.9 |
| **SadAK** | $17.8 \pm 6.7$ | 0.8 | $49.0 \pm 5.9$ | 1.2 | $6.4 \pm 0.7$ | 0.9 |
| **BcdAK** | $17.4 \pm 5.6$ | 0.9 | $42.7 \pm 2.2$ | 1.4 | $5.5 \pm 0.4$ | 1.1 |
| **pcDNA3-GFP** | $10.0 \pm 1.5$ | | $50.5 \pm 9.1$ | | $5.3 \pm 0.6$ | |
| **GFP-DmdNK** | $11.7 \pm 3.9$ | 0.9 | $1.6 \pm 0.5$* | 31.6 | $5.6 \pm 0.3$ | 0.9 |
| **GFP-BcdAK** | $13.1 \pm 3.7$ | 0.8 | $10.0 \pm 2.6$* | 5.1 | $5.2 \pm 0.2$ | 1.0 |

Data are mean values $\pm$ standard error from at least three experiments.
*$p < 0.05$ using Student's $t$ test and comparing with pcDNA3 or pcDNA3-GFP.



dNKs (Table 2). The negative results with HsdCK and DmdNK could be due to weak transfection rate under these conditions, as we observed low fluorescence in SWg cells transiently transfected with GFP-containing plasmid using the same conditions (data not shown). However, in Messa 10K cells, we obtained statistically significant 2.5- and 4.9-fold sensitizations in cells transfected with HsdCK and DmdNK, respectively. The result obtained with DmdNK confirmed our earlier results, and indicated good transfection rate in these cells.[4] No sensitization was observed for bacterial dNKs under the same conditions. We also performed the transient transfections on AG6000 cells. As shown in Table 2, we obtained similar results as for Messa 10K cells, *i.e.* a sensitization with HsdCK (27.3-fold) and DmdNK (35.3-fold), and no sensitization with bacterial dNKs.

The absence of sensitization observed with bacterial dNKs could be due to insufficient stability of the corresponding transcript or protein. Therefore, we constructed and transfected GFP-coupled kinases with DmdNK and BcdAK. This is expected to stabilize the transcript and the corresponding GFP-coupled kinase. Transient tranfection of GFP-DmdNK and GFP-BcdAK in SWg cells did not sensitize these cells to gemcitabine as compared to GFP-transfected cells, strengthening the hypothesis of bad transfection rate in these cells (Table 2). More surprisingly, these constructs did not sensitize Messa 10K cells either, although under the same conditions, these cells were sensitized with only DmdNK. Finally, in AG6000 cells, we observed a 31.6-fold sensitization with GFP-DmdNK similar to the uncoupled kinase from the fruitfly, indicating that the coupled protein retained kinase activity. In addition, GFP-BcdAK induced a 5.1-fold sensitization in these cells indicating that transcript and/or protein instability can explain some of the negative results obtained with uncoupled kinases.

**TABLE 3** Phosphorylation activity (nmol/min/mg) in AG6000 cells stably transfected with pcDNA3, DmdNK, BcdAK, and SpdAK, determined with 100 $\mu$M dC and gemcitabine

| Substrate | pcDNA3 | DmdNK | BcdAK | SpdAK |
|---|---|---|---|---|
| **dC** | 0.18 | 1.6 | 0.13 | 0.09 |
| **dFdC** | 0.007 ± 0.002 | 1.9 ± 0.3* | 0.009 ± 0.001 | 0.007 ± 0.001 |

Data for dFdC are mean values ± standard error from at least three experiments.

Only one experiment was done with dC.

*$p < 0.05$ using Student's $t$ test and comparing with pcDNA3.

## Deoxyribonucleoside Kinase Activity in Transfected Cells

To complete the characterization of some of our transfected cell lines, we determined the phosphorylation of dC and gemcitabine in extracts from AG6000 cells transfected with pcDNA3, DmdNK, BcdAK, and SpdAK. Extracts from DmdNK-transfected cells phosphorylated efficiently both substrates, whereas phosphorylation by extracts from BcdAK and SpdAK transfected cells was similar to controls (Table 3).



## DISCUSSION

In the mammalian cells, dCK-deficiency results in resistance to cytotoxic nucleoside analogues. This has been shown on resistant cancer cells cultivated in vitro, but decreased expression of dCK is also associated with lower response to nucleoside analogue-based cancer treatment.[16] In addition to the use of monophosphorylated prodrugs, this resistance can be overcome with dNK-based gene therapy.[1] The recent characterization of bacterial dNKs phosphorylating gemcitabine enlarged the panel of possible kinases to be used in gene therapy. We have previously shown that these dNKs could efficiently sensitize the *E. coli* host.[11,12]

We selected four bacterial dNKs and transfected them in gemcitabine-resistant human cancer cells with a well described dCK-deficiency. We used kinases from human and fruit fly as positive controls. We obtained only weak sensitizations with bacterial kinases in stably transfected lung adenocarcinoma cells as compared to important changes in $IC_{50}$ in cells with HsdCK and DmdNK (Table 1). We hypothesize that this result could be due to weak expression of bacterial kinases, production of inactive bacterial kinases in mammalian cells (differences in post-translational modifications), or weaker activity toward gemcitabine. When comparing data from the literature on affinity of these kinases toward dC and gemcitabine, we found higher $K_m$-values for bacterial kinases and gemcitabine that could at least partially explain the observed differences.[11,12,17,18]

The results from stably transfected Messa 10K suggest that these cells are not suitable for sensitization studies, as no positive result was obtained with HsdCK or DmdNK. This could be due to the pcDNA3 plasmid used, even though we observed some transgene-specific expression of these genes in Messa 10K cells. We had the same problems with stably transfected Messa 10K in an earlier study with DmdNK.[4]

Sensitization of cancer cells with DmdNK differs between stable and transient transfection. Therefore, we performed transient transfection of our cell lines with the bacterial dNKs as well. For Messa 10K and AG6000 cells, we observed increased activity of gemcitabine when using HsdCK and DmdNK, but no differences in cells transfected with BcdAK, CpdAK, SadAK or SpdAK (Table 2). These results thus confirm those obtained with the stably transfected cells, but allowed us to enlarge the panel of cancer cells. The absence of sensitization in SWg cells is probably due to a weak transfection rate of these cells under our conditions.

We further used plasmids coding GFP-fusionned DmdNK or BcdAK to study whether the instability of BcdAK transcript or protein could be responsible for the low activity of gemcitabine in BcdAK-transfected cells. As shown in Table 2, GFP-fusionned BcdAK sensitized much better than BcdAK in AG6000 cells and induced a 5-fold sensitization to gemcitabine as compared to GFP-transfected cells. In these cells, GFP-fused DmdNK sensitized as well as DmdNK, whereas this construct was inactive in Messa 10K cells. The results from AG6000 cells indicated that in addition to the lower affinity toward gemcitabine, transcript- or protein-instability could explain the lower result obtained with bacterial kinases than with HsdCK or DmdNK.

We also performed kinase phosphorylation assay using protein extracts from the transfected AG6000 cells. These cells have, because the native dCK gene is deleted, low background activity on dC and dFdC. The transfection of bacterial dNKs in these cells did not enhance the phosphorylation of gemcitabine or dC as compared to controls, thus showing that kinase activity was not present in these cells. As these kinases efficiently phosphorylate gemcitabine *in vitro*, it is likely that the kinases are not present in the cells, either because of low production or because of high degradation. It is also possible that these bacterial kinases are submitted to different post-translational modifications in mammalian cells than in bacteria, giving inactive kinases.

Our results show that the bacterial dNKs studied here are less suitable, in our conditions, than DmdNK and HsdCK for the development of gene therapy strategies in association with gemcitabine. However, these kinases can be used to increase the knowledge on dNK structure-activity relationship and help in the development of highly efficient “super-kinases” to be used in gene therapy.

## REFERENCES


1. Jordheim, L.P.; Dumontet, C. Review of recent studies on resistance to cytotoxic deoxynucleoside analogues. *Biochim. Biophys. Acta* **2007**, 1776, 138–159.
2. Hebrard, C.; Dumontet, C.; Jordheim, L.P. Development of gene therapy in association with clinically used cytotoxic deoxynucleoside analogues. *Cancer Gene Ther.* **2009**, 16, 541–550.
3. Hapke, D.M.; Stegmann, A.P.; Mitchell, B.S. Retroviral transfer of deoxycytidine kinase into tumor cell lines enhances nucleoside toxicity. *Cancer Res.* **1996**, 56, 2343–2347.
4. Jordheim, L.P.; Galmarini, C.M.; Dumontet, C. Gemcitabine resistance due to deoxycytidine kinase deficiency can be reverted by fruitfly deoxynucleoside kinase, DmdNK, in human uterine sarcoma cells. *Cancer Chemother. Pharmacol.* **2006**; 58, 547–554.
5. Stegmann, A.P.; Honders, W.H.; Willemze, R.; Ruiz van Haperen, V.W.; Landegent, J.E. Transfection of wild-type deoxycytidine kinase (dck) cDNA into an AraC- and DAC-resistant rat leukemic cell line of clonal origin fully restores drug sensitivity. *Blood* **1995**, 85, 1188–1194.
6. Zheng, X.; Johansson, M.; Karlsson, A. Retroviral transduction of cancer cell lines with the gene encoding Drosophila melanogaster multisubstrate deoxyribonucleoside kinase. *J. Biol. Chem.* **2000**, 275, 39125–39129.
7. Egeblad-Welin, L.; Sonntag, Y.; Eklund, H.; Munch-Petersen, B. Functional studies of active-site mutants from Drosophila melanogaster deoxyribonucleoside kinase. Investigations of the putative catalytic glutamate-arginine pair and of residues responsible for substrate specificity. *FEBS J.* **2007**, 274, 1542–1551.
8. Knecht, W.; Mikkelsen, N.E.; Clausen, A.R.; Willer, M.; Eklund, H.; Gojkovic, Z.; Piskur, J. Drosophila melanogaster deoxyribonucleoside kinase activates gemcitabine. *Biochem. Biophys. Res. Commun.* **2009**; 382, 430–433.
9. Sabini, E.; Ort, S.; Monnerjahn, C.; Konrad, M.; Lavie, A. Structure of human dCK suggests strategies to improve anticancer and antiviral therapy. *Nat. Struct. Biol.* **2003**; 10, 513–519.
10. Solaroli, N.; Bjerke, M.; Amiri, M.H.; Johansson, M.; Karlsson, A. Active site mutants of Drosophila melanogaster multisubstrate deoxyribonucleoside kinase. *Eur. J. Biochem.* **2003**, 270, 2879–2884.
11. Sandrini, M.P.; Clausen, A.R.; On, S.L.; Aarestrup, F.M.; Munch-Petersen, B.; Piskur, J. Nucleoside analogues are activated by bacterial deoxyribonucleoside kinases in a species-specific manner. *J. Antimicrob. Chemother.* **2007**, 60, 510–520.
12. Sandrini, M.P.; Shannon, O.; Clausen, A.R.; Bjorck, L.; Piskur, J. Deoxyribonucleoside kinases activate nucleoside antibiotics in severely pathogenic bacteria. *Antimicrob. Agents Chemother.* **2007**, 51, 2726–2732.
13. Ruiz van Haperen, V.W.; Veerman, G.; Eriksson, S.; Boven, E.; Stegmann, A.P.; Hermsen, M.; Vermorken, J.B.; Pinedo, H.M.; Peters, G.J. Development and molecular characterization of a 2′,2′-difluorodeoxycytidine-resistant variant of the human ovarian carcinoma cell line A2780. *Cancer Res.* **1994**; 54, 4138–4143.
14. van Bree, C.; Castro Kreder, N.; Loves, W.J.; Franken, N.A.; Peters, G.J.; Haveman, J. Sensitivity to ionizing radiation and chemotherapeutic agents in gemcitabine-resistant human tumor cell lines. *Int. J. Radiat. Oncol. Biol. Phys.* **2002**, 54, 237–244.
15. Knecht, W.; Rozpedowska, E.; Le Breton, C.; Willer, M.; Gojkovic, Z.; Sandrini, M.P.; Joergensen, T.; Hasholt, L.; Munch-Petersen, B.; Piskur, J. Drosophila deoxyribonucleoside kinase mutants with enhanced ability to phosphorylate purine analogs. *Gene Ther.* **2007**, 14, 1278–1286.
16. Galmarini, C.M.; Thomas, X.; Graham, K.; El Jafaari, A.; Cros, E.; Jordheim, L.; Mackey, J.R.; Dumontet, C. Deoxycytidine kinase and cN-II nucleotidase expression in blast cells predict survival in acute myeloid leukaemia patients treated with cytarabine. *Br. J. Haematol.* **2003**, 122, 53–60.
17. McSorley, T.; Ort, S.; Hazra, S.; Lavie, A.; Konrad, M. Mimicking phosphorylation of Ser-74 on human deoxycytidine kinase selectively increases catalytic activity for dC and dC analogues. *FEBS Lett.* **2008**, 582, 720–724.
18. Munch-Petersen, B.; Knecht, W.; Lenz, C.; Sondergaard, L.; Piskur, J. Functional expression of a multisubstrate deoxyribonucleoside kinase from Drosophila melanogaster and its C-terminal deletion mutants. *J. Biol. Chem.* **2000**, 275, 6673–6679.