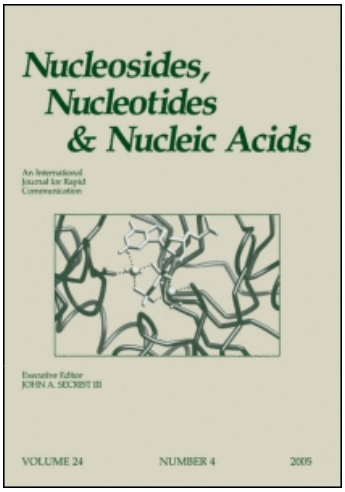

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Correlation Between Cytidine Deaminase Genotype and Gemcitabine Deamination in Blood Samples

E. Giovannetti[ab]; A. C. Laan[a]; E. Vasile[c]; C. Tibaldi[c]; S. Nannizzi[b]; S. Ricciardi[b]; A. Falcone[c]; R. Danesi[b]; G. J. Peters[a]
[a] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands [b] Division of Pharmacology, Department of Internal Medicine, University of Pisa, Pisa [c] Division of Oncology, Department of. Oncology, Azienda USL-6 of Livorno, Livorno, Italy

To cite this Article Giovannetti, E. , Laan, A. C. , Vasile, E. , Tibaldi, C. , Nannizzi, S. , Ricciardi, S. , Falcone, A. , Danesi, R. and Peters, G. J.(2008) 'Correlation Between Cytidine Deaminase Genotype and Gemcitabine Deamination in Blood Samples', Nucleosides, Nucleotides and Nucleic Acids, 27: 6, 720 — 725
To link to this Article: DOI: 10.1080/15257770802145447
URL: http://dx.doi.org/10.1080/15257770802145447

Nucleosides, Nucleotides, and Nucleic Acids, 27:720–725, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802145447

Taylor & Francis
Taylor & Francis Group

CORRELATION BETWEEN CYTIDINE DEAMINASE GENOTYPE AND GEMCITABINE DEAMINATION IN BLOOD SAMPLES

E. Giovannetti,[1,2] A. C. Laan,[1] E. Vasile,[3] C. Tibaldi,[3] S. Nannizzi,[2] S. Ricciardi,[2] A. Falcone,[3] R. Danesi,[2] and G. J. Peters[1]

[1]*Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands*
[2]*Division of Pharmacology, Department of Internal Medicine, University of Pisa, Pisa*
[3]*Division of Oncology, Department of. Oncology, Azienda USL-6 of Livorno, Livorno, Italy*

□ *Cytidine deaminase (CDA) is the major enzyme of gemcitabine inactivation. The aim of this study was to determine whether the CDA Lys27Gln polymorphism influenced gemcitabine deamination in blood samples from 90 lung cancer patients. The polymorphism was studied with Taqman probes-based assay; CDA activity was evaluated by HPLC in cytoplasmic extracts from red blood cells. Mean enzymatic activity was significantly lower in patients carrying the CDA Lys27Lys than in patients with the Lys27Gln or Gln27Gln protein ($P < 0.05$). CDA genotyping may be useful in screening patients before gemcitabine treatment, in order to identify subjects with lower CDA activity and potentially better clinical outcomes after gemcitabine-based chemotherapy.*

Keywords Gemcitabine; cytidine deaminase; CDA polymorphism; CDA activity

INTRODUCTION

Gemcitabine is a cytotoxic drug commonly used, alone or in combination with platinum compounds, in the treatment of pancreas and non-small cell lung cancer (NSCLC).[1,2] Cytidine deaminase (CDA) is the major enzyme involved in gemcitabine inactivation, and its overexpression in transfected cells reduced gemcitabine sensitivity.[3,4] Recent clinical studies showed significant correlations between CDA mRNA expression in peripheral blood mononuclear cells and clinical outcome in pancreatic cancer patients treated with gemcitabine[5] and between CDA gene expression in bone marrow mononuclear cells and haematological toxicity from gemcitabine.[6]

Address correspondence to G. J. Peters, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands. E-mail: gj.peters@vumc.nl

Other studies have focused on CDA polymorphisms. In particular, Fitzgerald et al.[7] described several functional SNPs in the CDA promoter, which might affect CDA expression and activity. A significant decrease in deamination activity of the wild-type CDA was also reported in a variant characterized by the common nonsynonymous polymorphism of lysine 27 glutamine (Lys27Gln), resulting from an $A \rightarrow C$ substitution in exon 79.[8] However, no differences were found in expression levels,[9] and sensitivity to cytarabine was not changed by introducing the polymorphic Gln27Gln CDA into yeast CDA-null mutants.[10] Furthermore, several in vitro studies did not show any relationship between CDA activity and sensitivity to gemcitabine or cytarabine,[11,12] suggesting that CDA might only play a role in gemcitabine pharmacokinetics and its disposition. However, a recent study in 256 Japanese patients treated with gemcitabine showed a clear relationship between pharmacokinetic parameters and the CDA haplotype harbouring the Ala208Thr polymorphic variant.[13] This polymorphism has a frequency of 3.7% in the Japanese population, but has not yet been detected in Caucasians.[14] In particular, the patients with the Ala208Ala genotype had AUC and clearance values 5-fold and one-fifth, respectively, of the median levels reported in the Thr208Thr patients. Moreover, although without a statistical significance, the Ala208Thr haplotype increased the incidence of severe neutropenia in patients co-treated with platinum-containing regimens or 5-fluorouracil. In the Japanese patients the CDA Lys27Gln haplotype showed no significant effects on gemcitabine pharmacokinetics, but data from a Caucasian population showed that the CDA Lys27Lys polymorphism was a predictive marker of response, toxicity, time to progression and overall survival in advanced NSCLC patients treated with cisplatin and gemcitabine.[15]

No previous studies have evaluated the relationship between CDA polymorphisms and gemcitabine deamination in patient-derived samples. Therefore, the present study was undertaken to evaluate whether the CDA Lys27Gln polymorphism influenced gemcitabine deamination in blood samples from 90 NSCLC patients.

MATERIALS AND METHODS

Genomic DNA was extracted from blood samples (5 ml) of 90 NSCLC patients, using the QIAamp DNA mini Kit (Qiagen, Hilden, Germany). DNA yields and integrity were checked by optical density at 260 nm with an Uvikon-940 spectrophotometer (Kontron, Milano, Italy). The CDA Lys27Gln polymorphism was studied with Taqman probes-based assays using the ABI PRISM 7900HT instrument (Applied Biosystems, Foster City, CA, USA). The PCR reactions were performed using 10 ng of DNA diluted in 11.875 μl DNAse-RNAse free water, 12.5 μl of TaqMan Universal PCR

Master Mix, with AmpliTaq Gold, and 0.625 μl of the assay mix, in a total volume of 25 μl, as described previously.[15]

CDA activity was measured by a previously described reverse-phase high performance liquid chromatography with ultraviolet detection (HPLC-UV) method [11], optimized for the present study. Red blood cells were snap-frozen and stored at -20°C. For measurement of CDA activity red blood cells were lysed by mixing 10 μl thawed red blood cell suspension with 100 μl double deionised water, left on ice for 20 minutes, and centrifuged for 10 minutes at 20000 g, 4°C. Red blood cells suspension (10 μl) were lysed by mixing with 100 μl of deionised water, left on ice for 20 minutes, snap frozen in liquid nitrogen and centrifuged for 10 minutes at 20000 g, 4°C. The supernatant layer was used as a crude cytoplasmic extract containing the CDA enzyme. 20 μl of this extract was mixed with 170 μl buffer (50 mM β-mercapto-ethanol in 0.1 M Tris/HCl, pH 8.0) and the substrate gemcitabine (final concentration, 250 μM). The reaction mixture was incubated at 37°C for 30 minutes and then terminated with 50 μl 40% trichloroacetic acid. After centrifugation the supernatant layer was neutralized with 400 μl trioctylamine/1,1,2-trichloro-trifluoroethane (1:4) and used for HPLC analysis.[11] The formation of the gemcitabine catabolite dFdU was normalized for protein concentration, measured with the Bradford assay (Sigma, St Louis, MO, USA).

RESULTS

The distribution of the studied Lys/Lys, Lys/Gln and Gln/Gln CDA polymorphisms and the allelic frequencies are shown in Table 1. In the studied population the CDA Lys27Gln polymorphism followed the Hardy-Weinberg equilibrium ($P > 0.05$) and genotype frequencies were comparable with those reported in a Caucasian population.[14]

The mean CDA enzymatic activity in patients with the CDA Lys27Lys protein was 8933 ± 1081 pmol/hr/mg protein; in contrast, in patients carrying the Lys27Gln and the Gln27Gln polymorphism mean CDA activity was 12090 ± 1220 pmol/hr/mg protein. Statistical analysis with the two-tailed unpaired Student's t-test revealed a significant difference ($P=0.028$)

TABLE 1 Distribution of CDA Lys27Gln polymorphism

Polymorphism	No. Patients	%	Allelic frequencies
CDA			A(Lys) 0.62
Lys27Gln			C(Gln) 0.38
Lys/Lys	32	35.6	
Lys/Gln	47	52.2	
Gln/Gln	11	12.7	

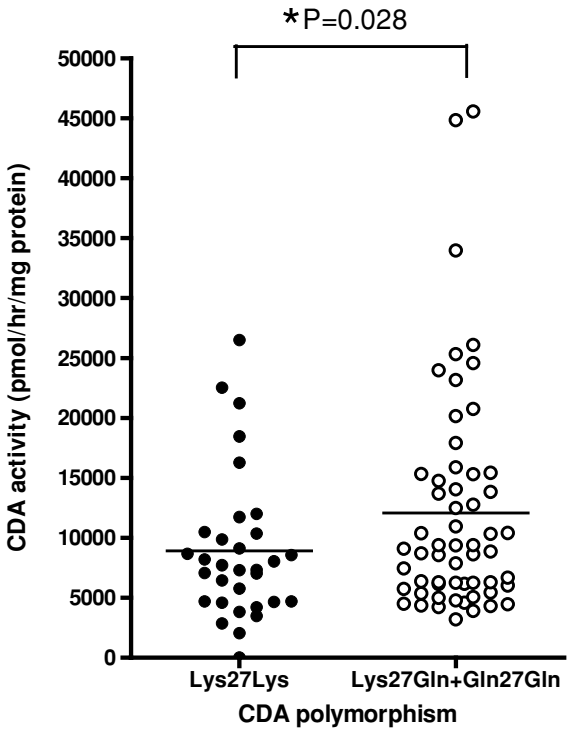

FIGURE 1 Evaluation of the variability of CDA activity observed across the cohort of 90 patients enrolled in the study. Values of CDA activity are presented as pmol/hr/mg protein. Patients were categorized according to CDA polymorphism Lys27Lys (black circle) and Lys27Gln+Gln27Gln (white circle). The significant difference ($P < 0.05$) between CDA activity in patients categorized according to CDA polymorphisms was calculated with the two-tailed unpaired Student's t-test.

between patients harbouring the wild-type and carriers of CA and CC genotypes (Figure 1).

DISCUSSION

The present study demonstrates for the first time a significant relationship between a polymorphism occurring in the CDA gene and gemcitabine deamination by CDA in blood samples. Previous studies have shown that pharmacogenetic variability in drug metabolizing enzyme systems is a major determinant of variations in predicting the outcome of therapy, both in terms of tumour response and host toxicity. Depending on whether the germline polymorphism is causing increased or decreased function of the metabolizing enzyme, the patients receiving chemotherapy may be undertreated, with no toxicity, or overtreated, with excessive toxicity.[16]

A number of promising polymorphisms candidates for predicting chemotherapeutic efficacy or toxicity have been identified, but, few stud-

ies have evaluated gemcitabine catabolism.[13,15] Furthermore, most studies on the relationship between the CDA Lys27Gln polymorphism and CDA activity have been performed *in vitro* or using alternative CDA substrates.[8–10] Our ex vivo analysis showed a significantly lower CDA activity in cells from patients with the Lys27Lys CDA protein. These results provide the biological basis of the results observed in a previous clinical study reporting a strong correlation of this polymorphism with response rate, time to progression and overall survival in 52 advanced NSCLC patients treated with gemcitabine plus cisplatin.[15] Evaluation of CDA genotype may be helpful in screening patients before treatment, in order to identify subjects with slower CDA-mediated gemcitabine metabolism, who might therefore derive an improved clinical outcome from gemcitabine-based chemotherapy.

REFERENCES

1. Li, D.; Xie, K.; Wolff, R.; Abbruzzese, J.L. Pancreatic cancer.*Lancet* **2004**, 363, 1049–1057.
2. Schiller, J.H.; Harrington, D.; Belani, C.P.; et al. Comparison of four chemotherapy regimens for advanced non-small cell lung cancer. *N. Engl. J. Med.* **2002**, 346, 92–98.
3. Eliopoulos, N.; Cournoyer, D.; Momparler, R.L. Drug resistance to 5-za-2′-deoxycytidine, 2′,2′-difluorodeoxycytidine, and cytosine arabinoside conferred by retroviral-mediated transfer of human cytidine deaminase cDNA into murine cells. *Cancer Chemother. Pharmacol.* **1998**, 42, 373–378.
4. Neff, T.; Blau, C.A. Forced expression of cytidine deaminase confers resistance to cytosine arabinoside and gemcitabine. *Exp. Hematol.* **1996**, 24, 1340–1346.
5. Bengala, C.. Guarneri, V.; Giovannetti, E.; Lencioni, M.; Fontana, E.; Mey, V.; Fontana, A.; Boggi, U.; Del Chiaro, M.; Danesi, R.; Ricci, S.; Mosca, F.; Del Tacca, M.; Conte, P.F. Prolonged fixed dose rate infusion of gemcitabine with autologous haemopoietic support in advanced pancreatic adenocarcinoma. *Br. J. Cancer* **2005**, 93, 35–40.
6. Ganti, A.K.; Parr, A.; Nguyen, D.; Ganti, A.K.; Parr, A.; Nguyen, D.; Grem, J.L. Gene expression profile of enzymes involved in gemcitabine (Gem) metabolism in bone marrow mononuclear cells as predictors of myelosuppression. *J. Clin. Oncol.* **2006**, 24(18S), 2007.
7. Fitzgerald, S.M.; Goyal, R.K.; Osborne, W.R.; Roy, J.D., Wilson, J.W., Ferrell, R.E. Identification of functional single polymorphism haplotypes in the cytidine deaminase promoter. *Hum. Genet.* **2006**, 119, 276–283.
8. Gilbert, J.A.; Salavaggione, O.E.; Yuan, J.; Pelleymounter, L.L.; Eckloff, B.W.; Wieben, E.D.; Ames, M.M.; Weinshilboum, R.M. Gemcitabine pharmacogenomics: cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. *Clin. Cancer Res.* **2006**, 12, 1794–1803.
9. Kirch, H.-C.; Schroder, J.; Hoppe, H.; Esche, H.; Seeber, S.; Schütte, J. Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine in vitro. *Exp. Hematol.* **1998**, 26, 421–425.
10. Yue, L.; Saikawa, Y.; Ota, K.; Tanaka, M.; Nishimura, R.; Uehara, T.; Maeba, H.; Ito, T.; Sasaki, T.; Koizumi, S. A functional single-nucleotide polymorphism in human cytidine deaminase gene contributing to ara-C sensitivity. *Pharmacogenetics* **2003**, 13, 29–38.
11. Ruiz van Haperen, V.W.; Veerman, G.; Vermorken, J.B.; Pinedo, H.M.; Peters, G.J. Regulation of phosphorylation of deoxycytidine and 2′,2′-difluorodeoxycytidine (gemcitabine); effects of cytidine 5′-triphosphate and uridine 5′-triphosphate in relation to chemosensitivity for 2′,2′-difluorodeoxycytidine. *Biochem. Pharmacol.* **1996**, 51, 911–981.
12. Bergman, A.M.; Pinedo, H.M.; Jongsma, A.P.; Brouwer, M.; Ruiz van Haperen, V.W.; Veerman, G.; Leyva, A.; Eriksson, S.; Peters G.J. Decreased resistance to gemcitabine (2′,2′-difluorodeoxycitidine) of cytosine arabinoside-resistant myeloblastic murine and rat leukemia cell lines: role of altered activity and substrate specificity of deoxycytidine kinase. *Biochem. Pharmacol.* **1999**, 57, 397–406.
13. Sugiyama, E.; Kaniwa, N.; Kim, S.R.; Kikura-Hanajiri, R.; Hasegawa, R.; Maekawa, K.; Saito, Y.; Ozawa, S.; Sawada, J.; Kamatani, N.; Furuse, J.; Ishii, H.; Yoshida, T.; Ueno, H.; Okusaka, T.; Saijo, N.

Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. *J. Clin. Oncol.* **2007**, 25, 32–41.
14. Fukunaga, A.K.; Marsh, S.; Murry, D.J.; Hurley, T.D.; McLeod, H.L. Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. *Pharmacogenomics. J.* **2004**, 4, 307–314.
15. Tibaldi, C.; Giovannetti, E.; Vasile, E.; Mey, V.; Laan, A.C.; Nannizzi, S.; Di Marsico, R.; Antonuzzo, A.; Orlandini, C.; Ricciardi, S.; Del Tacca, M.; Peters, G.J.; Falcone, A.; Danesi, R. Correlation of cytidine deaminase, ERCC1 and XPD polymorphisms with response and survival in gemcitabine/cisplatin-treated advanced non-small-cell lung cancer patients. *Clin. Cancer Res.* **2008** (in press).
16. Evans, W.E.; Relling, M.V. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* **1999**, 286, 487–491.