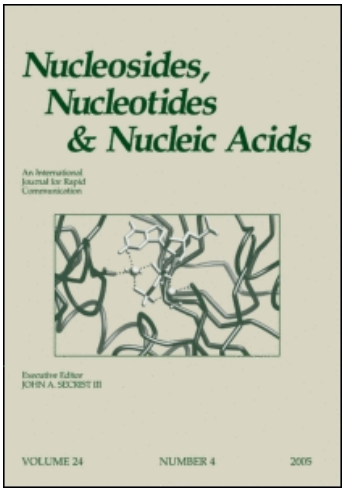
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

Comparison of mRNA Expression Levels Determined with TAQ-Man and Competitive Template RT-PCR

R. Mauritz[a]; W. Schwabe[b]; P. Haeusler[b]; P. Noordhuis[a]; K. Smid[a]; H. L. McLeod[c]; D. Behnke[b]; C. J. van Groeningen[a]; G. J. Peters[a]
[a] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands [b] Oncoscreen Research Institute, Jena, Germany [c] Division of Oncology, Washington University School of Medicine, St. Louis, Missouri, USA

Online publication date: 27 October 2004

To cite this Article Mauritz, R. , Schwabe, W. , Haeusler, P. , Noordhuis, P. , Smid, K. , McLeod, H. L. , Behnke, D. , van Groeningen, C. J. and Peters, G. J.(2004) 'Comparison of mRNA Expression Levels Determined with TAQ-Man and Competitive Template RT-PCR', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1471 — 1474
To link to this Article: DOI: 10.1081/NCN-200027689
URL: http://dx.doi.org/10.1081/NCN-200027689

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1471–1474, 2004

Comparison of mRNA Expression Levels Determined with TAQ–Man and Competitive Template RT–PCR

R. Mauritz,[1,*] W. Schwabe,[2] P. Haeusler,[2] P. Noordhuis,[1] K. Smid,[1] H. L. McLeod,[3] D. Behnke,[2] C. J. van Groeningen,[1] and G. J. Peters[1]

[1]Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
[2]Oncoscreen Research Institute, Jena, Germany
[3]Division of Oncology, Washington University School of Medicine, St. Louis, Missouri, USA

Downloaded At: 10:44 26 January 2011

ABSTRACT

Two methods for measurement of thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) mRNA expression were compared. Although the relative mRNA levels compared to β-actin measured with competitive template RT–PCR were different from the data obtained with a TaqMan based PCR, a significant correlation between the two assays was found.

Key Words: 5-Fluorouracil; Colorectal carcinoma; Thymidylate synthase; Dihydropyrimidine dehydrogenase; Gene expression; RT-PCR.

INTRODUCTION

Response to treatment with 5-fluorouracil (5FU) is associated with mRNA expression levels of its target enzyme TS and the rate-limiting catabolic enzyme

*Correspondence: R. Mauritz, Department of Medical Oncology, VU University Medical Center, 1081 HV Amsterdam, The Netherlands.

DOI: 10.1081/NCN-200027689
1525-7770 (Print); 1532-2335 (Online)
Copyright © 2004 by Marcel Dekker, Inc.
www.dekker.com

Request Permissions / Order Reprints
powered by RIGHTSLINK

DPD.[1] The expression of mRNA can be measured with several methods. Traditionally this was done with Northern blot analysis, but this method is rather insensitive and not quantitative. In the last decade various PCR methods have been developed, which are much more sensitive and (semi)-quantitative. We compared a competitive template RT–PCR method with a real-time PCR (TaqMan PCR) assay to measure the mRNA expression of TS and DPD. Using the TaqMan method, Salonga et al. proposed cut-off levels to determine whether patients would respond to 5-FU based chemotherapy or not.[1]

Historical samples from patients with colorectal cancer were used for this comparison. We have previously described that in this group of patients the TS enzyme activity levels and TS immunohistochemistry were related to response to 5FU treatment,[2–5] while a relation with survival was also reported.[6] TS mRNA levels were also related to enzyme levels.[7]

MATERIALS AND METHODS

Randomly selected tumor samples taken from colorectal cancer patients were evaluated retrospectively for levels of enzyme activity and mRNA expression levels. The samples were taken from both primary tumors and liver metastases as described previously.[2–5,7,8] TS levels were measured with standard radiochemical methods, which estimated the number of binding sites for FdUMP, the active metabolite of 5FU.[3] TS and DPD–mRNA levels were measured by Oncoscreen (Jena, Germany) using a TaqMan assay as reported by Salonga et al.[1] and in our department by using a CT–RT–PCR developed by us[9] or in collaboration with H.L. McLeod.[10] Evaluation of the data was performed using SPSS with a parametric test (Pearson correlation coefficient) and a non-parametric test (Spearman's rho). Since both the enzyme assay and the TS–mRNA with the TaqMan PCR and CT–RT–PCR were performed in the same tissue samples a one-tailed test was used.

RESULTS AND DISCUSSION

The mRNA expression of TS and DPD was measured in historical samples collected at our department. In most of these samples we also measured TS enzymatically. The absolute mRNA levels, normalized to β-actin for both TS and DPD, were different when comparing the assays. The TS/β-actin ratio in the TaqMan assay varied from 0.33 to 9.22 ($\times 10^{-3}$) and that for DPD/β-actin from 0.48 to 6.12 ($\times 10^{-3}$). However, in the CT–RT–PCR assay these values varied from 1.03 to 68.1 ($\times 10^{-3}$) and from 0 (not detectable) to 668 ($\times 10^{-3}$), respectively. The TaqMan data were in the same range as reported by Salonga et al.,[1] who used the same assay. The larger range for the CT–RT–PCR data is possibly related to the use of different primers and differences in amplification efficiency. Despite differences in absolute mRNA expression levels of TS and DPD, both the Pearson and Spearman analysis showed a quite good, significant correlation between the data obtained with TaqMan PCR and CT–RT–PCR (Table 1). Both methods generated reproducible results. Moreover, it has been shown that the CT–RT–PCR method is reproducible among multiple laboratories.[11]

Downloaded At: 10:44 26 January 2011

Table 1. Correlation between mRNA expression levels and enzyme activities.

PCR assay				Pearson correlation		Spearman	
	n		*n*	R	*p*	Rho	*p*
TaqMan TS	16	CT–RT–PCR TS	16	0.767	0.0005	0.581	0.009
TaqMan TS	16	TS levels[a]	13	0.600	0.015	0.670	0.006
TaqMan DPD	16	CT–RT–PCR DPD	16	0.798	< 0.0001	0.837	< 0.0001

[a]TS levels were measured with the FdUMP-binding assay.
(From Ref. [3].)

Downloaded At: 10:44 26 January 2011

We also found a significant correlation between TS levels and mRNA expression determined with either the TaqMan or CT–RT–PCR method (Table 1). This correlation is rather satisfactory considering the fact that TS can undergo a number of posttranslational modifications, especially after treatment with 5FU.[7,12] The correlation with enzyme levels demonstrates that the PCR assay can also predict levels with a functional assay for which we previously reported a correlation with response.[3,5]

These correlations were used to determine cut-off points for the CT–RT–PCR assay. The initial cut-off points were defined by Salonga et al.[1] for the TaqMan PCR assay to determine whether patients would respond to 5FU based chemotherapy. The cut-off point for TS in the TaqMan assay was 3.5×10^{-3}, which corresponded to 5×10^{-3} in the CT–RT–PCR assay. For DPD the TaqMan cut-off point was 2.5×10^{-3}, which corresponded to 45×10^{-3} for the CT–RT–PCR assay.

Several retrospective studies have shown that the tumoral levels of TS and DPD might be related to response to treatment with 5-FU.[1] Therefore we are currently undertaking a prospective clinical trial in order to determine whether TS and DPD expression can be used to tailor therapy for patients with advanced colorectal cancer. A core biopsy of the metastatic lesion is performed and this tissue is analyzed with the CT–RT–PCR assay. Patients with low mRNA levels of both TS and DPD (based on the above mentioned cut-off levels) are treated with 5-FU and leucovorin, whereas patients with high TS and/or high DPD mRNA expression are given a first-line combination treatment with oxaliplatin and irinotecan. A superior survival of patients in the 5-FU arm would provide support for clinical application of this approach of pretreatment measurement of tumoral TS and DPD levels.

REFERENCES

1. Salonga, D.; Danenberg, K.D.; Johnson, M.; Metzger, R.; Groshen, S.; Tsao, D.D.; Lenz, H.G.; Leichman, C.G.; Leichman, L.; Diasio, R.B.; Danenberg, P. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase and thymidine phosphorylase. Clin. Cancer Res. **2000**, *6*, 1322–1327.
2. Backus, H.H.J.; Van Riel, J.M.G.H.; Van Groeningen, C.J.; Vos, W.; Dukers, D.F.; Bloemena, E.; Wouters, D.; Pinedo, H.M.; Peters, G.J. Rb, mcl-1 and p53

expression correlate with clinical outcome in patients with liver metastases from colorectal cancer. Ann. Oncol. **2001**, *12*, 779–785.
3. Peters, G.J.; Van der Wilt, C.L.; Van Groeningen, C.J.; Meijer, S.; Smid, K.; Pinedo, H.M. Thymidylate synthase inhibition after administration of 5-fluorouracil with or without leucovorin; implications for treatment with 5-fluorouracil. J. Clin. Oncol. **1994**, *12*, 2035–2042.
4. Peters, G.J.; Van der Wilt, C.L.; Van Triest, B.; Codacci-Pisanelli, G.; Johnston, P.G.; Van Groeningen, C.J.; Pinedo, H.M. Thymidylate synthase and drug resistance. Eur. J. Cancer **1995**, *31A*, 1299–1305.
5. Peters, G.J.; Van der Wilt, C.L.; Van Groeningen, C.J.; Priest, D.G.; Schmitz, J.; Smid, K.; Meijer, S.; Noordhuis, P.; Hoekman, K.; Pinedo, H.M. Effect of different leucovorin formulations on 5-fluorouracil induced thymidylate synthase inhibition in colon tumors and normal tissues from patients in relation to response to 5-fluorouracil. In *Chemistry and Biology of Pteridines and Folates (Proc. 11th International Symposium)*; Pfleiderer, W., Rokos, H., Eds.; Blackwell Science: Berlin, 1997; 145–150.
6. Van Triest, B.; Pinedo, H.M.; Blauwgeers, J.L.G.; Van Diest, P.J.; Schoenmakers, P.S.; Voorn, D.A.; Smid, K.; Hoekman, K.; Hoitsma, H.F.W.; Peters, G.J. Prognostic role of thymidylate synthase, thymidine phosphorylase/platelet derived endothelial cell growth factor, and proliferation markers in colorectal cancer. Clin. Cancer Res. **2000**, *6*, 1063–1072.
7. Peters, G.J.; Backus, H.H.; Freemantle, S.; van Triest, B.; Codacci-Pisanelli, G.; van der Wilt, C.L.; Smid, K.; Lunec, J.; Calvert, A.H.; Marsh, S.; McLeod, H.L.; Bloemena, E.; Meijer, S.; Jansen, G.; van Groeningen, C.J.; Pinedo, H.M. Induction of thymidylate synthase as a 5-fluorouracil resistance mechanism. Biochim. Biophys. Acta **2002**, *1587*, 194–205.
8. Backus, H.H.J.; Dukers, D.J.; Van Groeningen, C.J.; Vos, W.; Bloemena, E.; Wouters, D.; Van Riel, J.M.G.H.; Smid, K.; Giaccone, G.; Pinedo, H.M.; Peters, G.J. 5-Fluorouracil induced Fas upregulation associated with apoptosis in liver metastases of colorectal cancer patients. Ann. Oncol. **2001**, *12*, 209–216.
9. Rots, M.G.; Willey, J.C.; Jansen, G.; Van Zantwijk, C.H.; Noordhuis, P.; DeMuth, J.P.; Kuiper, E.; Veerman, A.J.P.; Pieters, R.; Peters, G.J. MRNA expression levels of methotrexate resistance-related proteins in childhood leukemia as determined by a standardized competitive template-based RT–PCR method. Leukemia **2000**, *14*, 2166–2175.
10. Johnston, S.J.; Ridge, S.A.; Cassidy, J.; Mcleod, H.L. Regulation of dihydropyrimidine dehydrogenase in colorectal cancer. Clin. Cancer Res. **1999**, *5*, 2566–2570.
11. Crawford, E.L.; Peters, G.J.; Noordhuis, P.; Rots, M.G.; Vondracek, M.; Grafstrom, R.C.; Lieuallen, K.; Lennon, G.; Zahorchak, R.J.; Georgeson, M.J.; Wali, A.; Lechner, J.F.; Fan, P.S.; Kahaleh, M.B.; Khuder, S.A.; Warner, K.A.; Weaver, D.A.; Willey, J.C. Reproducible gene expression measurement among multiple laboratories obtained in a blinded study using standardized RT (StaRT)–PCR. Mol. Diagn. **2001**, *4*, 217–225.
12. Chu, E.; Koeller, D.M.; Johnston, P.G.; Zinn, S.; Allegra, C.J. Regulation of thymidylate synthase in human colon cancer cells treated with 5-fluorouracil and interferon-gamma. Mol. Pharmacol. **1993**, *4*, 527–533.

Downloaded At: 10:44 26 January 2011