**British Journal of Cancer (2011) 104,** 543 – 544

**www.bjcancer.com**

**Letter to the Editor**

# Reply: Modulation of plasma complement by the initial dose of epirubicin/docetaxel therapy in breast cancer and its predictive value

**A Michlmayr[1], T Bachleitner-Hofmann[1], M Bergmann[1] and R Oehler[*,1]**

[1]*Department of Surgery, Medical University of Vienna, Anna Spiegel Forschungsgebäude 25.05.003, Waehringer Guertel 18-20, Vienna A-1090, Austria*

*British Journal of Cancer* (2011) **104,** 543 – 544. doi:10.1038/sj.bjc.6606069 www.bjcancer.com
Published online 11 January 2011


**Sir,**

We appreciate the comments provided by Garantziotis (2011) on our recent publication in *British Journal of Cancer* (Michlmayr *et al*, 2010). In this study, we investigated whether the protein expression profile in plasma samples from breast cancer patients changes within a few days in response to the initial dose of epirubicin/docetaxel therapy. The expression of several plasma proteins was found to be modulated by the therapy, including inter-α-trypsin inhibitor (IαI) and different members of the complement cascade. We focused our attention on the complement components and might have underestimated the potential importance of increased IαI levels.

IαI proteins are a family of structurally related serine protease inhibitors with hyaluronan-binding capacities, assembled from a light chain and one of five homologous heavy chains (H1 – H5). In correspondence, separation of IαI by two-dimensional gel electrophoresis results in different spots with a molecular weight of ~80, 125 and 250 kDa (Josic *et al*, 2006). In our study, we found that the abundance of several 125 kDa IαI spots (388, 393, 397, 405, 406 and 407) is influenced by the epirubicin/docetaxel therapy (see Figure 1A). All these spots reacted nearly identical to the treatment. Figure 1B shows the expression level of the IαI spot 405 before and after the initial dose of epirubicin/docetaxel (time a and b, respectively). The abundance of this spot increased in nearly all patients ($n = 22$; increase = 32 ± 28%). Garantziotis referred in his letter to a recently found interaction of IαI with the complement system. In an experimental study, he could show that IαI attenuates *in vitro* complement activation and reduces *in vivo* complement-induced lung injury (Garantziotis *et al*, 2007). This is of special interest with respect to our findings. Our study revealed that the plasma level of total C3 decreases in response to epirubicin/docetaxel therapy (Figure 1C). Comparing the abundances of IαI (spot 405) with the total plasma C3 revealed that both parameters correlate negatively with each other (Spearman's Rho $r = -0.49$, $P < 0.01$, $n = 44$). The total plasma

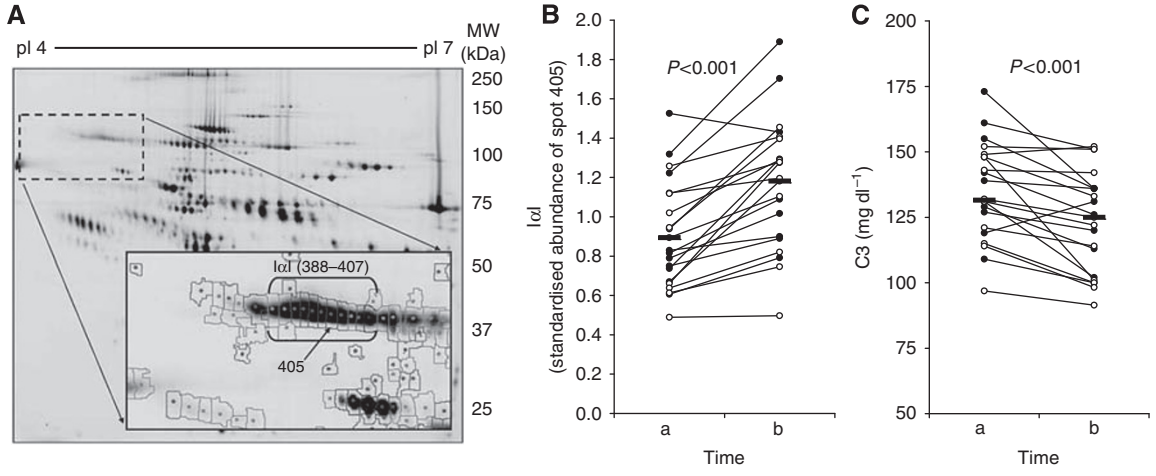


**Figure 1** Inter-α-trypsin inhibitor (IαI) in plasma samples. (**A**) Two-dimensional gel electrophoresis of plasma proteins indicating the 125 kDa IαI spots. (**B**) Standardised abundance of spot 405 before (time a) and after (time b) the initial dose of epirubicin/docetaxel given to 22 breast cancer patients. (**C**) Plasma total levels of complement component C3, as determined by immunonephelometry.

*Correspondence: Dr R Oehler; E-mail: rudolf.oehler@meduniwien.ac.at
Published online 11 January 2011

C3 was measured by a routine nephelometric immunoassay that cannot distinguish between the different C3 isoforms. In our study, we also investigated the single isoforms of C3 by two-dimensional gel electrophoresis. They responded unequally to the therapy and some of them correlated with the tumour size at the end of treatment. However, none of these single isoforms showed a correlation with the IαI spots, and no 125 kDa IαI spot showed a correlation with the success of therapy.

Although these findings corroborate the interaction of IαI and the complement system, several questions remain. Is the therapy-induced increase in plasma IαI the causative factor for C3 reduction? Is the decrease in C3 associated with reduced complement reactivity? How do the other IαI isoforms (80 and 250 kDa) react in response to therapy (they were not identified on our 2D gels)? Further studies are needed to find satisfactory answers.

## REFERENCES

Garantziotis S (2011) Modulation of plasma complement by the initial dose of epirubicin/docetaxel therapy in breast cancer and its predictive value. *Br J Cancer* **104**(3)**:** 542

Garantziotis S, Hollingsworth JW, Ghanayem RB, Timberlake S, Zhuo L, Kimata K, Schwartz DA (2007) Inter-alpha-trypsin inhibitor attenuates complement activation and complement-induced lung injury. *J Immunol* **179**(6)**:** 4187 – 4192

Josic D, Brown MK, Huang F, Lim YP, Rucevic M, Clifton JG, Hixson DC (2006) Proteomic characterization of inter-alpha inhibitor proteins from human plasma. *Proteomics* **6**(9)**:** 2874 – 2885

Michlmayr A, Bachleitner-Hofmann T, Baumann S, Marchetti-Deschmann M, Rech-Weichselbraun I, Burghuber C, Pluschnig U, Bartsch R, Graf A, Greil R, Allmaier G, Steger G, Gnant M, Bergmann M, Oehler R (2010) Modulation of plasma complement by the initial dose of epirubicin/docetaxel therapy in breast cancer and its predictive value. *Br J Cancer* **103**(8)**:** 1201 – 1208