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Predictive role of plasma vascular endothelial growth factor for the effect of celecoxib in advanced non-small cell lung cancer treated with chemotherapy

Sverre Sörenson [a,b,*,n], Helena Fohlin [c,d,n], Andrea Lindgren [d,e,n], Magnus Lindskog [f,g,n], Bengt Bergman [h,i,n], Christer Sederholm [b,n], Lars Ek [j,n], Kristina Lamberg [k,n], Birgitta Clinchy [l,m,n]

[a] *Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Sweden*
[b] *Department of Pulmonary Medicine UHL, County Council of Östergötland, Linköping, Sweden*
[c] *Regional Cancer Centre, County Council of Östergötland, Linköping, Sweden*
[d] *Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Sweden*
[e] *Allergy Centre UHL, County Council of Östergötland, Linköping, Sweden*
[f] *Department of Radiology, Oncology and Radiation Sciences, Uppsala University, Uppsala, Sweden*
[g] *Department of Medical Sciences, Uppsala University, Uppsala, Sweden*
[h] *Department of Respiratory Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden*
[i] *Institute of Medicine, University of Gothenburg, Sweden*
[j] *Department of Respiratory Medicine and Allergology, Skåne University Hospital, Lund, Sweden*
[k] *Department of Pulmonary Medicine, Uppsala University Hospital, Uppsala, Sweden*
[l] *Division of Clinical Immunology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Sweden*
[m] *Department of Clinical immunology and Transfusion Medicine, County Council of Östergötland, Linköping, Sweden*

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Abstract ***Aim of the study:*** The primary purpose of this study is to investigate if pretreatment plasma levels of vascular endothelial growth factor (VEGF) are predictive of the effect of celecoxib on survival in advanced non-small cell lung cancer (NSCLC) treated with palliative chemotherapy. A secondary objective is to describe the course of plasma VEGF levels during and after treatment with cytotoxic chemotherapy combined with celecoxib or placebo.
Methods: In a previously published double-blind multicenter phase III trial, 316 patients with NSCLC stage IIIB or IV and World Health Organisation (WHO) performance status 0–2 were randomised to receive celecoxib 400 mg b.i.d. or placebo in combination with two-drug platinum-based chemotherapy. Chemotherapy cycle length was three weeks and planned duration of chemotherapy was four cycles. Celecoxib was given for a maximum of one year but was stopped earlier in case of disease progression or prohibitive toxicity. In a subset of

* *Corresponding author:* Address: Bronsåldersgatan 15, Lgh 1202, SE-589 51 Linköping, Sweden. Tel.: +46 13 161766.
E-mail address: sverre.sorenson@liu.se (S. Sörenson).
[n] On behalf of the Swedish Lung Cancer Study Group.

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patients, plasma VEGF levels were examined at onset of treatment and at 6, 12 and 20 weeks.
Results: VEGF levels at start of treatment were obtained in 107 patients at four study sites. The median value was 70 pg/ml. Mean values declined during the first 12 weeks and then increased at 20 weeks. A subpopulation treatment effect pattern plot (STEPP) analysis showed an inverse relationship between initial plasma VEGF and the impact of celecoxib on survival with zero effect at 200 pg/ml. The effect on survival by celecoxib in the whole subset of patients was positive (hazard ratio (HR) = 0.64 [confidence interval (CI) 0.43–0.95], $p = 0.028$).
Conclusion: Low pretreatment plasma levels of VEGF appear to be predictive of a positive effect of celecoxib on survival.

1. Introduction

The COX-2 inhibitor celecoxib has shown activity against non-small-cell lung cancer (NSCLC) in experimental systems.[1,2] Two phase III studies investigating the effect on overall survival by adding celecoxib to chemotherapy in advanced NSCLC were, however, negative.[3,4] The negative overall results do not, however, exclude a clinical role of the compound in certain subpopulations. Since one of the mechanisms involved in the antineoplastic effect of COX-2 inhibitors appears to be inhibition of angiogenesis,[5–7] biomarkers related to angiogenesis might be useful as predictors of a positive clinical effect on survival by celecoxib. Plasma levels of the angiogenic factor vascular endothelial growth factor (VEGF) have previously been shown to correlate to microvessel density in patients with NSCLC.[8]

There has been much debate as to which specimen, serum or plasma, is best suited for analysis of VEGF as a biomarker in cancer.[9,10] Large amounts of VEGF can be released from platelets and leukocytes during clotting of serum and the amount released is dependent on clotting time and temperature.[10,11] Hence, serum VEGF may represent blood platelet count rather than VEGF produced by other tissue. In this study we therefore chose to follow the levels of VEGF in plasma as this would be more likely to reflect tumour associated production of VEGF and be less sensitive to variations in sample handling than serum VEGF.

In the Swedish CYCLUS trial, comparing celecoxib with placebo in patients with advanced NSCLC receiving two-drug platinum-based chemotherapy,[3] a subpopulation was examined with respect to plasma levels of VEGF before, during and after primary chemotherapy.

2. Patients and methods

Between May 2006 and May 2009, 316 patients with NSCLC stage IIIB–IV from 13 Swedish hospitals were included in a prospective randomised double-blind phase III trial comparing celecoxib at a dose of 400 mg b.i.d. with placebo in combination with platinum-based chemotherapy. The details of the trial have been presented elsewhere.[3] The drug regimens used by the participating hospitals were carboplatin + gemcitabine or carboplatin + vinorelbine. The cycle duration was three weeks and the planned number of cycles was four. Celecoxib or placebo was given from the first day of chemotherapy for a maximum of 1 year. Treatment with celecoxib/placebo was stopped earlier in case of disease progression, prohibitive toxicity related to the study drug or at the patient's wish.

At four hospitals that had accepted to do so, specimens for examination of plasma levels of VEGF were obtained before start of treatment, at 6, 12 and 20 weeks. The VEGF analyses were approved by the regional ethics committee of the University of Linköping. All participating patients had given oral and written consent to the procedure.

2.1. Laboratory methods

2.1.1. Blood samples

Venous blood was collected in sterile vacutainer tubes using sodium EDTA as anticoagulant. The samples were kept on ice and centrifuged (1500*g*, 10 min, 4 °C) within one hour of sampling. The plasma was aspirated into a centrifuge tube and centrifuged again (3000*g*, 10 min, 4 °C) to remove residual cells. The plasma was then transferred into polypropylene microcentrifuge tubes (Costar, Corning Incorporated Life Sciences, Lowell, MA) and stored at −70 °C to −80 °C until analysis.

2.1.2. VEGF immunoassay

Samples from sites outside Linköping were shipped over night on dry ice to the Laboratory of Clinical Immunology at the University Hospital in Linköping where all VEGF analyses were performed. The plasma was thawed at room temperature immediately before assay. The plasma levels of VEGF were analysed using a commercially available quantitative sandwich ELISA (Quantikine Human VEGF Immunoassay, R&D Systems Europe Ltd., Abingdon, United Kingdom (UK)) according to the manufacturer's instructions. The assay measures both VEGF 165 and VEGF 121. The limit of sensitivity of the assay was 9.0 pg/ml. Quantitation of VEGF in the samples was based on comparison to a linear standard curve ranging from 31.2 to 2000 pg/ml