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Human equilibrative nucleoside transporter 1 expression analysed by the clone SP 120 rabbit antibody is not predictive in patients with pancreatic cancer treated with adjuvant gemcitabine – Results from the CONKO-001 trial ☆

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Abstract ***Background:*** High expression of human equilibrative nucleoside transporter 1 (hENT1) is considered to predict survival in patients treated with adjuvant gemcitabine for pancreatic cancer. A standard evaluation system for immunohistochemical analysis (antibody, scoring system) has not yet been established.
Methods: CONKO-001, a prospective randomised phase III study investigated the role of adjuvant gemcitabine (gem) as compared to observation (obs). Tumour samples of 156 patients were analysed by immunohistochemistry with the rabbit monoclonal antibody SP120 (Ventana Medical Systems) for expression of hENT1. Kaplan–Meier analyses for median disease-free survival (DFS) and overall survival (OS) were performed in dependence of hENT1 expression measured analogously to Farrell et al. 2009 and Poplin et al. 2013.
Results: For the 88 gem and 68 obs patients, median DFS/OS was 12.9/22.7 months and 6.2/19.1 months. High hENT1 expression was not associated with improved median DFS (Farrell: no hENT1 22.2 months, low hENT1 13.7 months, high hENT1 12.1 months,

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$p = 0.248$; Poplin: low hENT1 13.2 months versus high hENT1 11.5 months, $p = 0.5$) or median OS (Farrell: no hENT1 21.7 months, low hENT1 24.7 months, high hENT1 19.5, $p = 0.571$; Poplin: low hENT1 24.4 months versus high hENT1 19.7 months, $p = 0.92$;) in the gem group or in the obs group (median DFS Farrell: no hENT1 5.1 months, low hENT1 6.2 months, high hENT1 7.5 months, $p = 0.375$; Poplin: low hENT1 6.2 months versus high hENT1 5.9 months, $p = 0.83$; median OS Farrell: no hENT1 20.2 months, low hENT1 17.7 months, high HENT1 19.1 months, $p = 0.738$; Poplin: low hENT1 17.7 months versus high hENT1 20.4 months, $p = 0.65$) measured by the Farrell or Poplin Score.

Conclusions: We cannot confirm a predictive role of hENT1 measured by the clone SP120 rabbit antibody in our study population. Reproducible standard procedures are urgently needed prior to the implementation or exclusion of hENT1 as a predictive biomarker in the treatment of pancreatic cancer.

Trial registration: ISRCTN34802808.

1. Introduction

Pancreatic cancer is predicted to become the second cause of cancer-related death in the United States [1] and to be one of the few oncological diseases with a still rising mortality [2].

Fortunately, some progress has been made in recent years by improvements in adjuvant and palliative chemotherapy raising hopes in this still devastating disease [3]. In the adjuvant situation the nucleoside analogue gemcitabine and the antimetabolite 5-FU are possible therapeutic options (CONKO-001, ESPAC 3) which can improve disease-free and overall survival [4,5]. Until now, the fact that gemcitabine had a better toxicity profile might influence physicians' choices, but so far no biomarker is available to predict which patient will profit more from gemcitabine or 5-FU in daily clinical practice. The human equilibrative nucleoside transporter 1 (hENT1), a transmembrane glycoprotein which mediates gemcitabine uptake into the (tumour) cells [6], is considered a promising predictor for this clinically relevant decision.

For almost 20 years, gemcitabine was the best evidence-based treatment option in palliative therapy for patients with advanced pancreatic cancer. In 2011, the so-called FOLFIRNOX regimen, a combination of 5-FU, folinic acid, oxaliplatin and irinotecan, became established for patients with a very good performance state [7], and in 2013 with the approval of nab-paclitaxel and gemcitabine, a second effective combination therapy [8] became available. As a result, a predictive biomarker to aid decision making during palliative treatment by identifying whether patients will profit from a gemcitabine or 5-FU based combination regimen is urgently needed too.

CONKO-001, a prospective phase III trial, was the first to investigate the role of adjuvant gemcitabine in R0 or R1 resected pancreatic cancer patients [9]. The study can be considered an ideal tool for biomarker analyses in pancreatic cancer, especially with regard to the efficacy of gemcitabine, as it provides not only prospectively collected clinical data and a follow-up of more than 5 years, but two randomised groups of patients: one treated for 6 months postoperatively with gemcitabine compared with one that was only observed.

The objective of the investigation we present here was to analyse hENT1, considered to be a predictive biomarker for adjuvant gemcitabine in pancreatic cancer [10]. We expected to find high hENT1 expression as a positive predictive factor in the gemcitabine group and to see no difference between high and low hENT1 expression in the observation group.

In addition, we tried to reproduce two different scoring systems: (1) a score used by Farrell et al. in postoperative pancreatic cancer patients with the monoclonal mouse antibody 10D7G2 (RTOG 9704 study) [11]; and (2) a score investigated by Poplin et al. in the palliative situation using the monoclonal rabbit antibody SP120 (CO-101 study) [12].

2. Methods

2.1. CONKO-001: Baseline data

A total of 368 patients were recruited between July 1998 and December 2004 who had undergone complete resection of pancreatic cancer (R0 and R1). Adenocarcinoma was histologically verified by the local pathologist prior to randomisation. Adjuvant treatment with gemcitabine (1000 mg/m^2 d1, 8, 15, q29) was continued for 6 months and compared with observation only, which was performed in an outpatient setting. Gemcitabine significantly improved median disease free survival (13.4 versus 6.9 months, $p < 0.001$)), overall survival (22.8 versus 20.2 months, $p = 0.005$) and 5-year-survival (20.7 versus 10.4%) [9,4].

The study was approved by the institutional review committee (trial registration isrctn.org Identifier: ISRCTN34802808).