Human equilibrative nucleoside transporter 1 is not predictive for gemcitabine efficacy in advanced pancreatic cancer: Translational results from the AIO-PK0104 phase III study with the clone SP120 rabbit antibody

Steffen Ormanns a, Volker Heinemann b,c,d, Mitch Raponi e, Jeff Isaacson e, Rüdiger P. Laubender c,d,f, Michael Haas b, Stephan Kruger b, Axel Kleespies g, Elaina Mann e, Mike Bartosiewicz e, Thomas Kirchner a,c,d, Stefan Boeck b,*

a Department of Pathology, Ludwig-Maximilians-University of Munich, Germany
b Department of Internal Medicine III and Comprehensive Cancer Center, Klinikum Grosshadern, Ludwig-Maximilians-University of Munich, Germany
c German Cancer Consortium (DKTK), Heidelberg, Germany
d German Cancer Research Center (DKFZ), Heidelberg, Germany
e Clovis Oncology Inc., San Francisco, CA, USA
f Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University of Munich, Germany
g Department of General, Visceral, Transplantation, Vascular and Thoracic Surgery, Klinikum Grosshadern, Ludwig-Maximilians-University of Munich, Germany

Received 23 February 2014; received in revised form 13 April 2014; accepted 28 April 2014
Available online 20 May 2014

KEYWORDS
Gemcitabine
hENT1
Pancreatic cancer

Abstract  Background: The role of human equilibrative nucleoside transporter 1 (hENT1) as a predictive biomarker for gemcitabine efficacy in advanced pancreatic cancer remains unclear to date.

Patients and methods: AIO-PK0104 was a German multicenter phase III trial comparing gemcitabine/erlotinib followed by capecitabine (GEC) with capecitabine/erlotinib followed by gemcitabine (CEG) in advanced pancreatic cancer. Archival tumour tissue from 169 of the 274 eligible study patients was available for a central and standardised immunohistochemistry staining for hENT1 expression using the SP120 rabbit monoclonal anti-hENT1 antibody. Within a retrospective translational subgroup analysis, biomarker data were correlated with efficacy end-points.

* Previous presentation: 48th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, USA; June 1–June 5, 2012.
* Corresponding author: Address: Department of Internal Medicine III and Comprehensive Cancer Center, Klinikum Grosshadern, Ludwig-Maximilians-University of Munich, Marchioninistr. 15, D-81377 Munich, Germany. Tel.: +49 89 7095 2208; fax: +49 89 7095 5256. E-mail address: stefan.boeck@med.uni-muenchen.de (S. Boeck).

http://dx.doi.org/10.1016/j.ejca.2014.04.023
0959-8049/© 2014 Elsevier Ltd. All rights reserved.
**Results:** Thirty-nine out of 130 fresh-cut slides were scored as hENT1\textsuperscript{low} (70%). For the 62 patients randomised to CEG median overall survival was estimated with 6.4 months in the hENT1\textsuperscript{low} compared to 6.9 months in the hENT1\textsuperscript{high} subgroup (Hazard Ratio (HR) 0.88, 95% confidence interval (CI) 0.48–1.61, \( p = 0.67 \)). For the 68 patients randomised to GEC survival was 5.7 months in the hENT1\textsuperscript{low} compared to 4.4 months in the hENT1\textsuperscript{high} subgroup (HR 1.16, 95% CI 0.69–1.96, \( p = 0.57 \)). In 101 patients receiving gemcitabine at any time during study treatment (either within the 1st- or 2nd-line setting) hENT1\textsuperscript{low} cases had a median overall survival of 7.5 months and hENT1\textsuperscript{high} patients an overall survival of 4.4 months (HR 1.30, 95% CI 0.84–2.03, \( p = 0.24 \)), respectively.

**Conclusion:** Within this subgroup analysis from Arbeitsgemeinschaft Internistische Onkologie-pancreatic cancer (AIO-PK0104), no evidence supporting the use of hENT1 as a predictive biomarker for gemcitabine efficacy in patients with advanced pancreatic cancer was found.

© 2014 Elsevier Ltd. All rights reserved.

---

1. Introduction

Advanced pancreatic adenocarcinoma remains a disease with a dismal prognosis: the current median survival time in this patient population is in the range of about 6–10 months [1]. However, significant advances have been achieved during the last years: (1) in patients with resectable disease, the introduction of adjuvant chemotherapy with gemcitabine, 5-fluorouracil/folinic acid or, more recently, with S-1 in Asian patients resulted in an improvement of overall survival (OS) [2–4]; (2) the development of novel combination chemotherapy regimens like FOLFIRINOX or gemcitabine + nab-paclitaxel resulted in a clinically meaningful prolongation of OS also for patients with metastatic disease [5–7]. However, up to now, no predictive biomarker has been defined for the above named drugs or treatment regimens [5].

The human equilibrative nucleoside transporter 1 (hENT1) is a transmembrane protein that is thought to be responsible for the intracellular uptake of the prodrug gemcitabine into tumour cells. In 2004, Spratlin and co-workers were among the first to report a potential association between hENT1 expression status and OS in gemcitabine-treated patients with pancreatic cancer [8]. During the following decade, several pre-clinical models and clinical investigations (mainly conducted retrospectively in resected pancreatic cancer) confirmed the hypothesis that hENT1 overexpression might serve as a predictive biomarker for the efficacy of gemcitabine [9–15]. More recently, Jordheim and Dumontet summarised the currently available data on hENT1 as a biomarker in pancreatic cancer in their comprehensive review published in 2013 [16]. However, during the last few years, some investigators reported that hENT1 may potentially act as a prognostic rather than as a predictive marker in resected pancreatic cancer [17]; one group also found no evidence for hENT1 as a predictive biomarker in their patient population treated with gemcitabine-based neoadjuvant chemoradiotherapy [18]. Data on hENT1 in patients with unresectable disease however still are very limited: a recent Asian study with 71 gemcitabine-treated patients reported a significant association of high hENT1 expression levels with a longer time to progression, but found no association with a prolongation in OS [19].

Up to now, translational hENT1 data have been reported from three controlled clinical trials in pancreatic cancer: Radiation Therapy Oncology Group (RTOG) 97-04 and European Study Group for Pancreatic Cancer (ESPAC-3) were prospective clinical trials conducted in the adjuvant setting [12,20], and Low hENT1 and Adenocarcinoma of the Pancreas (LEAP) was a prospective phase II study conducted in metastatic disease [21]. While RTOG 97-04 and ESPAC-3 assessed the hENT1 expression status within retrospective subgroup analyses, LEAP prospectively pre-defined a standardised and central hENT1 assessment for all included patients based on biopsies from tumour metastases. RTOG 97-04 and ESPAC-3 both found significant evidence for hENT1 as a predictive biomarker for adjuvant gemcitabine treatment after surgical resection of pancreatic adenocarcinoma, whereas LEAP was not able to prospectively confirm this hypothesis in gemcitabine-treated metastatic patients [21].

AIO-PK0104 was a prospective German multicenter phase III trial conducted in patients with advanced pancreatic cancer: patients were randomised between a treatment sequence of gemcitabine + erlotinib followed by capecitabine versus capecitabine + erlotinib followed by gemcitabine. Detailed clinical and translational results of this study have already been published [22–24]. The current retrospective hENT1 analysis based on archival formalin-fixed paraffin-embedded (FFPE) tissue from AIO-PK0104 was conducted in order to further define the role of hENT1 as a biomarker in patients with non-resectable, advanced pancreatic cancer receiving gemcitabine-based chemotherapy in palliative intent.

2. Materials and methods

2.1. Patient population

Adult patients (18–75 years) with a confirmed diagnosis of treatment-naïve, advanced, exocrine pancreatic
2.3. Statistical analyses

All statistical analyses for this translational study from AIO-PK0104 trial were performed by RPL and JI. Translational biomarker data were correlated with the pre-defined secondary study end-points OS and time-to-treatment failure after 1st-line therapy (TTF1) from AIO-PK0104 using univariate analyses [22]. Biomarker results were thereby handled as dichotomous variables (hENT1\textsuperscript{low} versus hENT1\textsuperscript{high}). Time-to-event end-points were analysed using the Kaplan-Meier method; differences were compared using the log-rank test with a 2-sided p-value of $\leq 0.05$ being regarded as statistically significant.

3. Results

3.1. Patient characteristics and hENT1 distribution

For the hENT1 analysis of AIO-PK0104, FFPE tumour blocks were available from 169 of the 274 eligible study patients. Overall 39 out of the 130 fresh-cut slides were scored as hENT1\textsuperscript{high} (30%), whereas 91 samples were classified as hENT1\textsuperscript{low} (70%). For the additionally scored 39 pre-cut archival slides, a significantly lower rate of hENT1\textsuperscript{high} samples (3 out of 39, 8%) was defined. The investigators thus decided to perform all subsequent analyses for the “fresh-cut” set only, also in order to avoid a pre-analytical sampling error based on a potentially inferior tissue quality in the older, pre-cut slides. 

Table 1 summarises baseline patient characteristics from the intention-to-treat study population (ITT; $n = 274$) and from the translational study population consisting of 130 patients. No significant imbalances in important baseline parameters (e.g. age, gender, stage of disease, performance status) were apparent between the ITT population and the translational study population. 

Table 2 summarises the hENT1 distribution in AIO-PK0104 primary and metastatic tumour samples: independent of tissue origin (primary versus metastatic versus unknown) a hENT1\textsuperscript{high} rate of about 30% was found in all analysed tissue samples.

3.2. Correlation of hENT1 status with overall survival

Of the 130 patients analysed for hENT1 status, 75 patients received the assigned 1st-line regimen only (gemcitabine + erlotinib: $n = 46$; capecitabine + erlotinib: $n = 29$) and 55 study participants received the full pre-defined treatment sequence of 1st- and 2nd-line therapy (gemcitabine + erlotinib followed by capecitabine: $n = 22$; capecitabine + erlotinib followed by gemcitabine: $n = 33$; see Table 3). Thus, 101 study patients received gemcitabine in any line of the pre-defined treatment strategy. Table 3 summarises the results on the hENT1 status within these 4 different treatment groups.
OS results based on hENT1 status for the randomly assigned treatment groups are shown in Fig. 1: for the 62 patients randomised to capecitabine + erlotinib followed by gemcitabine median OS was estimated with 6.4 months in the hENT1\textsuperscript{low} compared to 6.9 months in the hENT1\textsuperscript{high} subgroup (HR 0.88, 95% confidence interval (CI) 0.48–1.61, \(p = 0.67\); Fig. 1 A). For the 68 patients randomised to gemcitabine + erlotinib followed by capecitabine median OS was estimated with 5.7 months in the hENT1\textsuperscript{low} compared to 4.4 months in the hENT1\textsuperscript{high} subgroup (HR 1.16, 95% CI 0.69–1.96, \(p = 0.57\); Fig. 1 B). When exploratively analyzing all 101 patients that received gemcitabine at any time during study treatment (either within the 1st- or 2nd-line setting), patients with a hENT1\textsuperscript{low} status had a median OS of 7.5 months, and patients with a hENT1\textsuperscript{high} status had a median OS of 4.4 months (HR 1.30, 95% CI 0.84–2.03, \(p = 0.24\); Fig. 1 C), respectively.

### 3.3. Correlation of hENT1 status with time-to-treatment failure after 1st-line therapy (TTF1)

TTF1 was a pre-defined secondary study end-point of AIO-PK0104 \cite{22}; TTF is a composite end-point including not only disease progression and death due to any cause as event, but also treatment termination e.g. due to toxicity or patient withdrawal of consent. However, in a majority of study patients the event relevant for a TTF1 event was disease progression or death (76%), whereas 5% of the patients terminated 1st-line treatment due to toxicity and 2% were lost to follow-up.

TTF1 results based on hENT1 status for the randomly assigned treatment groups are shown in Fig. 2: for the 62 patients randomised to 1st-line capecitabine + erlotinib median TTF1 was estimated with 2.2 months in the hENT1\textsuperscript{low} compared to 2.5 months in the hENT1\textsuperscript{high} subgroup (HR 0.81, 95% CI 0.44–1.47, \(p = 0.48\); Fig. 2 A). For the 68 patients randomised to gemcitabine + erlotinib followed by capecitabine median TTF1 was estimated with 2.5 months in the hENT1\textsuperscript{low} compared to 3.1 months in the hENT1\textsuperscript{high} subgroup (HR 0.83, 95% CI 0.45–1.52, \(p = 0.56\); Fig. 2 B). When exploratively analyzing all 101 patients that received gemcitabine at any time during study treatment (either within the 1st- or 2nd-line setting), patients with a hENT1\textsuperscript{low} status had a median TTF1 of 2.4 months, and patients with a hENT1\textsuperscript{high} status had a median TTF1 of 3.0 months (HR 0.80, 95% CI 0.44–1.47, \(p = 0.56\); Fig. 2 C), respectively.
Fig. 1. (A) Overall survival (OS) in patients treated in the capecitabine + erlotinib followed by gemcitabine arm based on the human equilibrative nucleoside transporter 1 (hENT1) expression level (n = 62). (B) Overall survival (OS) in patients treated in the gemcitabine + erlotinib followed by capecitabine arm based on the hENT1 expression level (n = 68). (C) Overall survival (OS) in patients treated with gemcitabine at any time on study protocol (either 1st- or 2nd-line) based on the hENT1 expression level (n = 101).
randomised to 1st-line gemcitabine + erlotinib median TTF1 was estimated with 3.7 months in the hENT1 low compared to 1.7 months in the hENT1 high subgroup (HR 2.01, 95% CI 1.19–3.38, \( p = 0.007 \); Fig. 2B).

4. Discussion

No prognostic or predictive tissue biomarker has yet been defined in advanced pancreatic cancer based on translational data from controlled prospective clinical trials [25]. Regarding hENT1, increasing evidence for an important role as a predictive biomarker for the efficacy of gemcitabine in the adjuvant setting became available during the last years; however, it remains unclear if these observations also hold promise in the palliative treatment context [12,16,20].

Based on the presented OS and TTF1 data from AIO-PK0104 we were not able to find evidence for hENT1 as a prognostic or predictive biomarker in our patient population with advanced disease: in patients randomly assigned to the gemcitabine + erlotinib followed by capecitabine study arm there was no significant difference in OS based on the hENT1 expression status (Fig. 1B). When we exploratively looked at all study participants that received gemcitabine at any time during study treatment (either within the 1st- or 2nd-line setting), we also found no significant difference in OS with regard to hENT1 status (Fig. 1C). In fact, there was a non-significant trend for an inferior OS for hENT1 high patients that were treated with gemcitabine in any line. This observation was supported by the TTF1 data that only took the 1st-line treatment regimens into account: hENT1 high patients treated with gemcitabine + erlotinib had an inferior TTF1 compared to hENT1 low patients receiving the same regimen (HR 2.01, \( p = 0.007 \); Fig. 2B). In contrast, such a difference was not observed in patients randomised to 1st-line capecitabine + erlotinib (HR 0.81, \( p = 0.48 \); Fig. 2A).
These data from our subgroup analysis of the AIO-PK0104 phase III study thus support the hENT1 data generated within the randomised phase II LEAP study; within LEAP, 367 patients with metastatic pancreatic adenocarcinoma were randomly assigned between standard gemcitabine and CP-4126, a novel lipid-drug conjugate of gemcitabine (which was designed to enter cells independently of hENT1) [21]. In 358 of the 367 study participants the hENT1 status was assessed in prospectively collected biopsy specimens from metastatic tumour tissue and 232 patients (65%) were found to have a hENT1low expressing tumour. The rate of hENT1low tumours in LEAP is comparable to the data from AIO-PK0104 (hENT1low: 70%, Table 2). Within the gemcitabine reference arm in LEAP, there was no difference in OS between the hENT1high and hENT1low subgroups (HR 1.14, \( p = 0.45 \)).

It remains unclear what reasons for these unexpected negative findings for hENT1 in the metastatic setting can be found. One might hypothesise that the trial design (prospective hENT1 assessment in LEAP versus retrospective assessment in RTOG 97-04 and ESPAC-3) may have contributed to the differing results; additionally, the different scoring algorithms and monoclonal antibodies used for determining the hENT1 status by immunohistochemistry may have had a significant impact on the contradictory results. The SP120 antibody assay used in LEAP (and AIO-PK0104) was previously validated in the RTOG 97-04 cohort and yielded comparable results like the 10D7G2 anti-hENT1 mouse antibody used in the original RTOG 97-04 publication by Farrell and co-workers [12,21]. The hENT1 data from ESPAC-3 were also generated using the 10D7G2 anti-hENT1 antibody; however, the scoring algorithm used by the ESAPC group (H score) was a different one compared to the RTOG study (no hENT1 versus low hENT1 versus high hENT1 staining) [12,20]. Thus, a cross-study comparison of the above named results should only be performed with caution since different histopathological methods were used. At least in our opinion, a prospective and standardised confirmation of the hENT1 hypothesis in gemcitabine-treated patients after surgical removal of the primary tumour still needs to be performed, ideally with the used of both antibodies (10D7G2 and SP 120) within the same patient cohort.

The current hENT1 analysis based on archival FFPE tissue from AIO-PK0104 clearly has several limitations that should be addressed and taken into account when interpreting the data: (1) AIO-PK0104 included a protocol pre-defined 2nd-line treatment, thus a gemcitabine-"only" or gemcitabine-"free" control arm unfortunately was not available [22]. However, this also could be regarded as an advantage as we know the applied subsequent treatment regimens in all study patients – e.g. in contrast to adjuvant trials where we do not always know which treatment was applied upon relapse (e.g. gemcitabine in palliative intent in patients initially not treated with adjuvant gemcitabine). Of note, preliminary data from a human pancreatic tumour xenograft study in nude mice suggested that a significant increase in hENT1 expression and gemcitabine cellular uptake may be observed after treatment with S-1, a novel oral fluoropyrimidine [26]. As all patients in AIO-PK0104 were chemotherapy-naïve at study entry, such a potential ‘molecular’ bias can be excluded for the current analysis. (2) Up to now, we do not know for sure if the hENT1 expression status is the same in the primary tumour and in metastatic tissue, or – even more important – if the hENT1 status may change over time in the course of the disease (e.g. hENT1low primary pancreatic tumour and hENT1high subsequent metachronous pancreatic cancer liver metastasis or vice versa). Nevertheless, a hENT1low rate of about 60–70% has been reported consistently from previous pancreatic cancer studies, involving both primary and metastatic tissue. (3) The current analysis from AIO-PK0104 was conducted as an unplanned retrospective subgroup analysis, thus potentially including a relevant selection bias. Due to the limitation in tumour tissue (we successfully analysed 130 of 274 study patients, representing 47% of the eligible participants) we were not able to analyse a higher number of patients; this, however, is a well known hurdle in many translational studies from recent pancreatic cancer trials [25].

It additionally should be kept in mind that hENT1 may not be the exclusive mediator for gemcitabine sensitivity as several other potential predictive biomarkers for gemcitabine efficacy exist: besides hENT1, other nucleoside transporter proteins are involved in the intracellular uptake of gemcitabine, e.g. hENT3 and concentrative nucleoside transporters (CNT) [11,27]. Several enzymes involved into the complex metabolism of gemcitabine may also contribute to the efficacy of the drug: ribonucleotide reductase subunit 1 (RRM1, an intracellular target of gemcitabine) and deoxycytidine kinase (dCK, the enzyme that is responsible for the initial phosphorylation of gemcitabine into its active forms) both could serve as additional predictive biomarkers based on preliminary evidence from several translational studies [14–17,27].

In conclusion, based on the current hENT1 subgroup analysis from AIO-PK0104 we were not able to find evidence for a prominent role of hENT1 as a predictive biomarker for gemcitabine in advanced pancreatic cancer. Further well designed prospective and standardised investigations are clearly necessary in both the adjuvant and the palliative setting in order to define the role of hENT1 as a selection method for the use of gemcitabine in pancreatic adenocarcinoma. Until these results are generated, hENT1 should not be used as a molecular biomarker for gemcitabine efficacy in advanced pancreatic cancer in daily clinical practice.
Conflict of interest statement

Steffen Ormanns received a travel grant from Clovis Oncology. Volker Heinemann received research funding from Clovis Oncology, Roche and Celgene. He additionally received honoraria for scientific presentations from Roche and serves as a consultant for Roche. Mitch Raponi, Jeff Isaacson, Elaina Mann and Mike Bartosiewicz are employees of Clovis Oncology. Michael Haas received honoraria for scientific presentations and a travel grant from Celgene. Thomas Kirchner received honoraria from Merck Serono, Amgen, Astra Zeneca and Roche. He served as a consultant for Amgen, Roche, Pfizer and Novartis and received research funding from Amgen and Merck. Stefan Boeck received research funding from Clovis Oncology, Roche and Celgene. He additionally received honoraria for scientific presentations from Roche and Celgene and serves as a consultant for Celgene.

All remaining authors have declared no potential conflicts of interest.

Acknowledgements

The authors wish to thank all patients and their families, nurses, study coordinators and investigators for their participation in the AIO-PK0104 study. Additionally, the active commitment of the pathologists providing FFPE archival tumour tissue is gratefully acknowledged because they enabled this translational study.

AIO-PK 0104 was supported by Roche Pharma AG, Germany and the translational hENT1 project was supported by an unrestricted research grant from Clovis Oncology Inc., San Francisco, CA, USA.

References


