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hENT1 expression is predictive of gemcitabine outcome in pancreatic cancer: A systematic review

Nordh S *et al*. hENT1 expression in pancreatic cancer

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**Abstract**

High hENT1-expression has shown a survival benefit in pancreatic cancer patients treated with gemcitabine in several studies. The aim of this systematic review was to summarise the results and try to assess the predictive value of hENT1 for determining gemcitabine outcome in pancreatic cancer. Relevant articles were obtained from the PubMed, Embase and Cochrane databases. Studies evaluating hENT1-expression in pancreatic tumour cells from patients treated with gemcitabine were selected. Outcome measures were overall survival, disease free survival, toxicity and response rate. The database searches identified ten studies that met the eligibility criteria, and a total of 855 patients were included. Nine of ten studies showed statistically significant longer overall survival (OS) in univariate analyses in patients with high hENT1-expression compared to those with low expression. In the seven studies that reported disease-free survival (DFS) as an outcome measure, six had statistically longer DFS in the high hENT1 groups. Both toxicity and response rate were only reported in two articles and it was therefore hard to draw any major conclusions. This review provides evidence that hENT1 is a predictive marker for pancreatic cancer patients treated with gemcitabine. Some limitations of the review have to be taken into consideration, the majority of the included studies have a retrospective design, and there is no standardised scoring protocol for hENT1-expression.

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**Key words**: Pancreatic cancer; Gemcitabine; hENT1; Predictive; Survival

**Core tip**: hENT1 is a predictive marker for pancreatic cancer patients treated with gemcitabine.

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**INTRODUCTION**

Gemcitabine is the standard chemotherapy treatment for pancreatic cancer[[1-3](#_ENREF_1)], but its efficacy is limited, only 15% of patients with advanced pancreatic cancer[[4](#_ENREF_4)] and up to 30% in general[[5](#_ENREF_5)] can be expected to respond to treatment. Gemcitabine is hydrophilic and therefore passive diffusion through hydrophobic cellular membranes is slow[[1](#_ENREF_1)]. Permeation through the membranes requires specialised membrane transporters[[1](#_ENREF_1),[3](#_ENREF_3)], where human equilibrative nucleoside transporter 1 (hENT1) is the most important one for gemcitabine[[6](#_ENREF_6),[7](#_ENREF_7)]. Because gemcitabine is a prodrug, it has to be phosphorylated after intracellular uptake [[1](#_ENREF_1)] in order to have a cytotoxic effect[[6](#_ENREF_6)]. This rate-limiting step is carried out by the enzyme deoxycytidine kinase (dCK)[[8](#_ENREF_8)].

Recent research has revealed that different gene-expressions, including hENT1[[9](#_ENREF_9), [10](#_ENREF_10)] and enzymes involved with gemcitabine metabolism, such as dCK, may be predictors of the efficacy of gemcitabine treatment for pancreatic cancer[[11](#_ENREF_11)]. Several studies have indicated that high expression of hENT1 is associated with longer overall survival (OS) and longer disease free survival (DFS)[[9](#_ENREF_9),[10](#_ENREF_10),[12](#_ENREF_12)].

The aim of this review was to evaluate and summarize the potential predictive value of hENT1-expression in pancreatic tumour cells in patients treated with gemcitabine.

**STUDY SELECTION**

To identify all relevant English-language articles published from 1966 to March 2013, a computerised search of the PubMed, Embase and Cochrane databases was performed. The following search terms were used: (hENT1 OR nucleoside transporter), (gemcitabine OR gemzar), (pancreatic OR pancreas), (cancer OR adenocarcinoma OR neoplasm). The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[[13](#_ENREF_13)] was used as a guideline for the processing and reporting of results. The initial search yielded 230 publications (54 in PubMed, 1 in Cochrane, 175 in Embase). To find studies that might have been missing in the database search, a manual search was made by reading through reference lists of relevant articles and systematic reviews. The results of the search and the selection of studies are shown in (Figure 1). The quality of the included articles was assessed using the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK)[[14](#_ENREF_14)].

**ELIGIBILITY CRITERIA**

For inclusion in this systematic review the following criteria had to be met: Retrospective or prospective studies of patients with pancreatic cancer, all stages, treated with gemcitabine with or without additional radiation. The expression of hENT1 had to be reported and related to patient outcome. The articles had to be available in full-text and published in English. Exclusion criteria were conference abstracts, overlapping patient cohorts and studies in which the relevant outcomes of interests were not addressed.

**DATA EXTRACTION**

A data extraction form was completed before the extraction process began. The form was reviewed by a second author (RA) to ensure that all relevant information was being extracted. The data extraction was done by a single reviewer (SN) and the following data were extracted from each study: publication details (author(s), date of publication, location, study centre), study design, population details (age, sex, pT-stage, pN-stage), patient number, type of intervention (dose, schedule, duration), method to determine hENT1-expression, hENT1-scoring, primary and secondary outcome measurements and results correlated to hENT1-expression.

**OUTCOMES OF INTEREST AND DEFINITION**

The primary outcome measures were overall survival (OS) and disease-free survival (DFS) correlated to hENT1 expression. Secondary outcome measures were toxicity according to the Common Toxicity Criteria (<http://www.eortc.be/services/doc/ctc/>) and response rate according to RECIST[[15](#_ENREF_15)] criteria.

**LITERATURE SEARCH**

The search identified 230 references in the databases PubMed, Chochrane and Embase. Of these, 120 were excluded after identification of duplicates and exclusion based on irrelevant title. Abstracts from 110 articles were screened and 75 of them were excluded. The main reasons for exclusion were: nonclinical trials (such as review articles), hENT1-expression was not being assessed, and irrelevant outcome measurements. The remaining 35 studies were retrieved for further assessment. Of these, 25 references were excluded for the following reasons: because they were conference abstracts and not full-text articles; hENT1-subgroups were measured[[16](#_ENREF_16), [17](#_ENREF_17)]; hENT1 was evaluated as a prognostic factor rather than a predictive factor of gemcitabine treatment[[18](#_ENREF_18)]; overlapping patient populations[[19](#_ENREF_19)]; and too small sample size/case reports[[20](#_ENREF_20)]. An additional five article abstracts were screened for eligibility after identification in a manual search of reference lists of relevant articles. All of these were excluded based on irrelevance. In total 10 studies fulfilled our inclusion criteria and were included in this systemic review.

**CHARACTERISTICS OF SELECTED STUDIES**

The included articles were published between 2004 and 2012. They originated from Belgium (two studies)[[1](#_ENREF_1),[3](#_ENREF_3)], Canada (one study)[[9](#_ENREF_9)], USA (one study)[[12](#_ENREF_12)] and Japan (five studies)[[2](#_ENREF_2),[8](#_ENREF_8),[21-23](#_ENREF_21)]. The five studies from Japan originated from five different universities, Kyushu, Osaka, Yokohama, Mie and Hiroshima. One author had published two articles[[1](#_ENREF_1),[3](#_ENREF_3)], for one of these the patient population was recruited from two centres, while for the other the patient population was recruited from five centres. The potential bias of overlapping patient populations was therefore small, but must nevertheless be taken into consideration in the analysis of the results.

Nine of ten studies were retrospective[[1-3](#_ENREF_1), [8-10](#_ENREF_8),[21-23](#_ENREF_21)] and one was a post-hoc analysis of a randomised controlled study[[12](#_ENREF_12)]. The ten involved a total of 855 patients and the sample size varied from 21 to 234 *(*Tables 1-2*).*

Four studies[[1](#_ENREF_1), [8](#_ENREF_8), [10](#_ENREF_10), [12](#_ENREF_12)] used parallel groups, while the remainder were single-arm studies. The treatment protocols, which differed between the studies, included adjuvant gemcitabine monotherapy, palliative gemcitabine treatment, neoadjuvant gemcitabine chemotherapy, adjuvant gemcitabine chemotherapy + radiation, neoadjuvant gemcitabine + radiation (and adjuvant 5-FU), resection only and neoadjuvant gemcitabine based chemoradiation + adjuvant gemcitabine *(*Table 2*).* All protocols were based on gemcitabine treatment and resection of the tumour.

**hENT1-EXPRESSION**

To quantify the hENT1-expression, eight studies used immunohistochemistry (IHC) and two used reverse transcription PCR. Grading of the expression differed between the studies, as is described in more detail in Table 3. The majority of the studies dichotomised the expression in high/positive versus low/negative hENT1 expression. There are no standardised scoring procedures available.

**OVERALL SURVIVAL**

Nine of the ten included studies had overall survival as an outcome measurement of interest. Kawada *et al*[[2](#_ENREF_2)] was the sole study that only reported DSS (disease specific survival) as the primary outcome. The definition of DSS is the length of time from either the date of diagnosis or start of treatment for a specific disease (*e.g*., pancreatic cancer), and that the patients with the disease still are alive. The difference with OS being that OS measures death from any cause, not the just death from a particular disease. Survival times for the individual studies were calculated based on: diagnosis, in one study[[10](#_ENREF_10)]; the start of gemcitabine treatment in two studies[[9](#_ENREF_9),[22](#_ENREF_22)]; resection, in five studies[[1](#_ENREF_1),[3](#_ENREF_3),[8](#_ENREF_8),[21](#_ENREF_21),[23](#_ENREF_23)]; and randomisation in one study[[12](#_ENREF_12)].

In univariate analyses all nine studies that had overall survival as an outcome measurement of interest showed a survival benefit with gemcitabine treatment and high/positive expression of hENT1 compared to patients with low/negative hENT1 expression. Multivariate analyses were conducted in eight of the nine studies. Seven of these identified high/positive hENT1 as an indicator of longer overall survival in patients with pancreatic cancer who received gemcitabine treatment. One study[[8](#_ENREF_8)] indicated a trend towards better OS in the multivariate analysis, but this was not statistically significant, *P =* 0.2.

**DISEASE-FREE SURVIVAL**

In seven studies disease-free survival (DFS) was reported as an outcome measurement. DFS was calculated from the same starting points as reported above for OS. Six of these studies showed a statistically significant longer DFS in univariate analyses. One study[[8](#_ENREF_8)] was not statistically significant in regards to hENT1. Multivariate analyses were conducted in five studies but of these only three[[3](#_ENREF_3), [12](#_ENREF_12), [23](#_ENREF_23)] reported statistically significant results with longer DFS in regards to hENT1 in patients with pancreatic cancer treated with gemcitabine. Morinaga *et al*[[21](#_ENREF_21)] and Murata *et al*[[22](#_ENREF_22)] also performed multivariate analyses, but the results did not prove to be statistically significant with reported *P-*values ranging from 0.129 to 0.232.

**TOXICITY**

Only two studies addressed the issue of toxicity[[3](#_ENREF_3),[12](#_ENREF_12)], but the numbers published were inadequate for further analysis. Farrell *et al*[[12](#_ENREF_12)] tried to find a relationship between hENT1-levels and the incidence of grade 3 or higher toxicities; however no relationship was found using a logistic regression model. The analysis data were not shown in the article. Maréchal *et al*[[3](#_ENREF_3)] reported that grade III/IV haematological toxicities were noticed in 10/45 patients and grade III/IV nonhematological in 3/45 patients. They did not relate this finding to hENT1 expression and no further data or data analysis was shown in the article with regard to toxicity.

**RESPONSE RATE**

Of the ten included studies only two[[10](#_ENREF_10), [22](#_ENREF_22)] reported the outcome measurement response rate (RR). Giovannetti *et al* evaluated RR in 34/36 patients in the group of patients receiving palliative treatment with gemcitabine. Two of the patients were not evaluable because of early death and refusal. The results showed that five patients had shown a partial response (PR), thirteen had stable disease (SD) and sixteen had progressive disease (PD). No further evaluation or data analysis was made with respect to RR. Murata *et al* evaluated RR in correlation to hENT1 expression; radiographic RR was judged according to RECIST (Response Evaluation Criteria in Solid Tumors). Radiographic RR was not significantly correlated to hENT1 expression, *P = 0.665.*

**DISCUSSION**

Based on the data collected from the selected studies, there is evidence that hENT1 expression is a predictive marker for gemcitabine treatment in pancreatic patients treated with gemcitabine. Patients with high expression of hENT1 had significantly longer OS in all included studies that evaluated this outcome measurement. These results are in accordance with other studies of gemcitabine outcome correlated to hENT1 expression in other types of tumours including biliary tract cancer[[24](#_ENREF_24)], cholangiocarcinoma[[25](#_ENREF_25)], bladder cancer[[26](#_ENREF_26)] and non-small cell lung cancer[[27](#_ENREF_27)].

Since there is no standardised protocol for the grading of hENT1 expression, the methods used differed between the included studies (Table 3). The majority used immunohistochemistry (IHC) to evaluate hENT1 expression in the tumour cells. This is the major method for assessing biomarkers in histopathology. Most studies in this review using this method of evaluation had two independent assessors (blinded to each other and to patient outcomes) to make the grading of a higher quality and better precision. The different ways of grading protein expression is an issue that needs to be considered when looking at the results of the independent studies as well as the results of this review. There is a need for standardised protocols to achieve better homogeneity across studies when it comes to grading the protein-expression of hENT1 in pancreatic tumour cells.

Gemcitabine is the standard treatment for patients with pancreatic cancer. This is based on several studies where gemcitabine has shown a survival benefit compared to other treatment regimes[[1](#_ENREF_1), [28-30](#_ENREF_28)]. In this review the treatment differed between the included studies, but they all used gemcitabine as the base for chemotherapy. Most studies were done in resectable patients but the predictive value of hENT1 was also confirmed in unresectable patients[[9](#_ENREF_9),[10](#_ENREF_10)]. Kawada *et al*[[2](#_ENREF_2)] used neoadjuvant chemoradiation and their results showed, in opposite to all the others, a trend towards better disease-specific survival in patients with low expression of hENT1, although the results were not statistically significant. They did use a slightly different outcome measurement that may have influenced the results. Even though the result was not statistically significant it raises some questions that are important in the discussion about the different treatment regimens across the studies. In the case of neoadjuvant treatment or neoadjuvant chemoradiation, the tumour cells with high expression of hENT1 may be destroyed before the tumour samples are collected and will therefore give misleading information. This creates interesting issues as to when and how the tumour cells should be analysed. Fine needle aspiration is discussed as an option for retrieving tumour cells for evaluation of hENT1. This method can be used before resection and may therefore be an effective tool to identify which patients may benefit from neoadjuvant treatment with gemcitabine. Future studies are needed in this area.

One study[[12](#_ENREF_12)] was a post-hoc analysis of a randomised controlled trial, which is of course rated higher methodologically than are retrospective cohort studies. The common opinion is that a systematic review exhibits the greatest strength if the majority of the included studies are randomised controlled trials or at least prospective trials. However, no such trials have been made within this area, but the need for a review was still considered to be strong. The retrospective design of the included studies implies that we need to consider reporting and selection bias when analysing the results.

REMARK[[14](#_ENREF_14)], which is a relative new assessment tool, was used for quality evaluation in this review. The maximum score in REMARK is 20, and the average score in the included articles was 12.6 within the range of 9-17. Since this is a relatively new tool, there is not much information as to what quality is considered high, and what low low. In this review the included articles were of relatively similar quality (Table 2*)* according to REMARK.

The results of this review are important to the consideration of future treatment options for patients with pancreatic cancer. Since according to this review, hENT1 has proven to be a predictive marker for gemcitabine outcome there are a few considerations to be made. First, if we can alter the expression of hENT1 in the tumour cells to create a higher expression, more patients would benefit from gemcitabine treatment and survive longer. Pretreatment with thymidylate synthase inhibitors has proven to increase the expression of hENT1 in tumour cells in vitro[[6](#_ENREF_6)]. This might be a way to alter the expression in vivo as well, but further studies are needed. The second option is to find another way for gemcitabine to enter the tumour cells there exert its toxic power. Research in this area is currently under way, and progress would enable more personalised treatment options for pancreatic cancer patients.

Another aspect for the future is the cost of overtreatment with gemcitabine in patients who do not benefit from it. According to one study conducted on a Swedish pancreatic cancer cohort, EUR 8.6 million would be saved each year in Sweden if hENT1 testing were used to select patients for gemcitabine therapy[[31](#_ENREF_31)].

**CONCLUSION**

This review provides evidence that hENT1 is a predictive marker for pancreatic cancer patients treated with gemcitabine. However, standardised procedures for evaluating and grading hENT1 expression need to be established. Additionally, more research and preferably prospective trials or RCTs in this area are needed to strengthen the results of this review.

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**P-Reviewers:** Macedo FI, Rossi RE, Yun S, Zhang Q,  **S-Editor:** Wen LL  **L-Editor:**  **E-Editor:**

**Figure 1 Flowchart showing article selection process.**

**Table 1 Characteristics of identified studies**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Year of publication** | **Country** | **Inclusion period** | **No. of patients** | **Study design** | **Follow-up median (mo 95% CI)** |
| Spratlin *et al*[[9](#_ENREF_9)] | 2004 | Canada | 1998-2002 | 21 | RS | NR |
| Giovannetti *et al*[[10](#_ENREF_10)] | 2006 | Italy | 2001-2004 | 1021 | RS | 11.2 (0.4-32.1) range |
| Farrell *et al*[[12](#_ENREF_12)] | 2009 | United States | 1998-2002 | 91 | Post hoc2 | NR |
| Maréchal *et al*[[3](#_ENREF_3)] | 2009 | Belgium | 2000-2003 | 45 | RS | 21.9 (3.3-107.4) |
| Fujita *et al*[[8](#_ENREF_8)] | 2010 | Japan | 1992-2007 | 70 | RS | 15.7 (0.5-114) |
| Maréchal *et al*[[1](#_ENREF_1)] | 2012 | Belgium | 1996-2009 | 234 | RS | 55.7 (46.4-61.2) |
| Kawada *et al*[[2](#_ENREF_2)] | 2012 | Japan | 2002-2007 | 63 | RS | 31 |
| Morinaga *et al*[[21](#_ENREF_21)] | 2012 | Japan | 2006-2008 | 27 | RS | NR |
| Murata *et al*[[22](#_ENREF_22)] | 2012 | Japan | 2005-2010 | 93 | RS | 15 (3.5-57.2) |
| Nakagawa *et al*[[23](#_ENREF_23)] | 2012 | Japan | 2002-2011 | 109 | RS | 39.7 (2-122) |
|  |  |  |  | Tot: 855 |  |  |

1*n =* 81 with complete hENT1; 2Post hoc analysis of RCT. NR: Not reported; RS: Retrospective.

**Table 2 Characteristics of identified studies**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Age median****yr(range)** | **Sex m/tot** ***n* (%)** | **hENT1 method** | **Chemotherapy** | **Radiation dose****Gy /(Gy/frac)**  | **Outcome measurement** | **Quality REMARK** |
| Spratlin *et al*[[9](#_ENREF_9)] | 58 (51-64)1 | 11 (52) | IHC | Pall Gem | No | OS | 10 |
| Giovannetti *et al*[[10](#_ENREF_10)] | 65 (22-83) range | 53 (50) | RT-PCR | Pall Gem orNeo gem | No | OS, DFS, TTP, RR | 12 |
| Farrell *et al*[[12](#_ENREF_12)] | 53/63/652 | 45 (49) | IHC | Adj Gem | 50.4 | OS, DFS, Tox | 17 |
| Maréchal *et al*[[3](#_ENREF_3)] | 56 (34-83) range | 23 (51) | IHC | Adj Gem | No | OS, DFS, Tox | 13 |
| Fujita *et al*[[8](#_ENREF_8)] | 65 (36-86)  | 42 (60) | RT-PCR | Adj Gem orResection only | No | OS, DFS | 12 |
| Maréchal *et al*[[1](#_ENREF_1)] | NR | 129 (53) | IHC | Adj Gem | No | OS | 15 |
| Kawada *et al*[[2](#_ENREF_2)] | - (41-81) | 33 (52) | IHC | Neo Gem Adj 5-FU | 50/2 | DSS  | 9 |
| Morinaga *et al*[[21](#_ENREF_21)] | 64 (45-74) | 17 (63) | IHC | Adj Gem | No | OS, DFS | 12 |
| Murata *et al*[[22](#_ENREF_22)] | 68 (44.87) | 38 (69) | IHC | Neo GemAdj Gem | 45/2 | OS, DFS, RR | 13 |
| Nakagawa *et al*[[23](#_ENREF_23)] | 67 (41-83) | 52 (48) | IHC | Adj Gem + S1 | No | OS, DFS | 13 |

195%CI; 2Medians in the different hENT1 expression groups. IHC: Immunohistochemical; RT-PCR: Reverse transcription polymerase chain reaction; Adj: Adjuvant; Neo: Neoadjuvant; Radio: Radiotherapy; Gem: Gemcitabine; Pall: Palliative; OS: Overall survival; DFS: Disease specific survival; Tox: Toxicity; TTP: Time to progression; DSS: Disease specific survival; RR: Response rate.

**Table 3 hENT1 expression levels, cut-offs and grouping**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study** | **Method** | **Grading** | **Reference cells** | **Groups** **(*n*)** |
| Spratlin *et al*[[9](#_ENREF_9)] | IHC | 0-2 based on relative intensities of staining.0 = absence of staining1 = intermediate staining2= most intense staining | Langerhans cells, lymphocytes. | Dichotomised:Low = 0 (9)High = 1 and 2 (12) |
| Giovannetti *et al*[[10](#_ENREF_10)] | RT-PCR | Gene-expression ratio with GAPDH, expressed as tertiles.GAPDH/target gene ratio |  | Gene expression tertiles:low <1.06 (27)intermediate 1.06 – 1.38 (28)high ≥ 1.38 (28)Dichotomised: by mediansLow <1.23 (44)High ≥1.23 (37) |
| Farrell *et al*[[12](#_ENREF_12)] | IHC | Based on relative intensities. High = strong reactivity in > 50% of neoplastic cells.No = No staining in >50%Low = all cases between High and No. | Lymphocytes | Dichotomised: No (18)1*vs*Low/High (73)1 |
| Maréchal *et al*[[3](#_ENREF_3)] | IHC | 0-3 based on staining intensities.0 = No staining1= weakly positive2= moderately positive3 = strongly positiveFinal score calculated: multiplying intensity score and the percentage of the specimen. Weighted score 0-300. | Langerhans cells.Lymphocytes | Dichotomised:Low ≤ 80 (19)(final score)High = ≥80 (26) |
| Fujita *et al*[[8](#_ENREF_8)] | RT-PCR | Level of mRNA calculated from standard curve constructed with total RNA from Capan-1, a human pancreatic cancer cell line. |  | mRNA split into high/low groups using recursive descent partitioning. Cut-off 0.5.Low (26)1High (14) |
| Maréchal *et al*[[1](#_ENREF_1)] | IHC | 0-2 based on staining intensities.Quantified as Farrell.  | Lymphocytes | Dichotomised:Low/moderate (136)1High (86)1 |
| Kawada *et al*[[2](#_ENREF_2)] | IHC | 0-2 based on staining intensities.1 = same intensity as control. | Langerhans cells | Negative = 0-1 (41)Positive = 2 (22) |
| Morinaga *et al*[[21](#_ENREF_21)] | IHC | Staining intensity and percentage of positive tumour cells scored and given a hENT1-score by calculating the two.Staining 0-3 where 0 = no1= weakly pos2 = moderately pos3 = strongly posPercentage:0 = no positive1 ≤ 50% positive cells2 = 50-80% positive cells3 = ≥80% |  | Low = hENT1score 0-3 (11)High = hENT1score 4-6 (16) |
| Murata *et al*[[22](#_ENREF_22)] | IHC | Staining intensity + extent of positive staining. Intensity:0 = No staining1 = weakly positive2 = moderately positive3 = strongly positiveExtent staining:High = score 3 >50% cellsLow = score 0 or 1 >50%Intermediate = all others | Langerhans cells | Dichotomised:Negative = low and intermediate (16)Positive = high (39) |
| Nakagawa *et al*[[23](#_ENREF_23)] | IHC | Staining intensities:0 = not stained1 = faintly stained2 = weakly stained 3 = as strongly as islet cells | Langerhans cells | Low = grade 0 or 1 in >50% (31)High = grade 2 or 3 in >50% of cells (78) |

1In gem-arm. IHC: Immunohistochemical; RT-PCR: Reverse transcription polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

**Table 4** **Results**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Median survival all patients (m, 95% CI)** | **OS****Univariate analysis****median (*m,* 95% CI)****or****HR (95% CI)*****P-*value** | **Multivariate analysis****HR (95% CI)** | **DFS****Univariate analysis****median (*m*, 95%CI)****or****HR (95% CI)*****P-*value** | **Multivariate analysis****HR (95% CI)** | **Main conclusions** |
| Spratlin *et al*[[9](#_ENREF_9)] | 11.01 (6.8-17.5)5.01 (2.8-12.2) | (mo):High = 13 (4.2-20.4)Low = 4 (1.5-6.9)*P =* 0.01 |  | NR |  | Pat with detectable hENT1 had sig longer OS compared with pat with low hENT1.  |
| Giovannetti *et al*[[10](#_ENREF_10)] | 13.3 (10.9-15.7) | (mo):Low = 8.48 (7.01-9.95)Inter = 15.74 (13.84-17.63)High = 25.69 (17.64-33.74)*P ≤* 0.0012 groups:Low = 12.42 (8.18-16.66)High = 22.34 (16.34-28.34)*P ≤* 0.001 | Low = 5.34 (2.28-12.50)Inter = 1.07 (0.46-2.49)High = 1*P <* 0.00012 groups:HR = 4.21*P ≤* 0.001 | Palliative (mo):Low = 5.85 (2.75-8.95)Inter = 10.09 (9.63-10.54)High = 12.68 (2.89-22.47)*P =* 0.02Adjuvant (mo):Low = 9.26 (3.86-14.67)Inter = 12.91 (9.31-16.51)High = 20.43 (13.27-27.60)*P ≤* 0.01 |  | hENT1 expression was significantly correlated with outcome – pat with high hENT1 had longer OS. |
| Farrell *et al*[[12](#_ENREF_12)] | NR | (HR):Low/High = 0.51 (0.29-0.91)No = 1*P =* 0.02 | Low/High = 0.40 (0.22-0.75)No = 1*P =* 0.03 | (HR):Low/High = 0.57 (0.32-1.001)No = 1*P =* 0.05 | Low/High = 0.39 (0.21-0.73)No = 1*P =* 0.003 | hENT1 expression was ass with longer OS, DFS in pat receiving gem. hENT1 is a relevant predictive marker for gem outcome. |
| Maréchal *et al*[[3](#_ENREF_3)] | 21.9 (3.3-107.4) |  (HR):High = 1Low = 3.88 (1.78-8.92)*P =* 0.0007 | High = 1Low = 3.42 (1.44-8.81)*P =* 0.0005 | (HR):High = 1Low = 3.55 (1.65-7.63)*P =* 0.02 | High = 1Low = 3.17 (1.43-6.73)*P =* 0.0004 | Pat with high hENT1 had sig longer OS and DFS compared to low hENT1. |
| Fujita *et al*[[8](#_ENREF_8)] | NR | (mo):High = 45Low = 16.5*P =* 0.011 | (RR):Low = 2.980 (0.964-10.86)*P =* 0.2 (not sig) | (mo):High = 25Low = 8*P =* 0.11 (not sig) | NR | Low hENT1 ass with shorter OS in gem-group.  |
| Maréchal *et al*[[1](#_ENREF_1)] | 32.0 (26.4-34.3) (GEM-group) | (HR):High = 0.43 (0.29-0.63)Low/Mod = 1*P <* 0.0001 | *n =* 2222High = 0.34 (0.22-0.53)Low/Mod = 1*P <* 0.0001 | NR | NR | High hENT1 predicts longer OS in pat treated with adj gem. Absence of gem - hENT1 lacks prognostic value. |
| Kawada *et al*[[2](#_ENREF_2)] | NR | Positive *vs* Negative*P =* 0.352 | Positive/negative*P =* 0.503 | NR | NR | DSS tended to be better in the hENT1-neg group but not statistically sig.  |
| Morinaga *et al*[[21](#_ENREF_21)] | NR | (mo):Low = 11.8 (6.9-16.6)High = 22.2 (11.5-32.9)*P =* 0.024 (HR):Low = 1High = 0.366 (0.148-0.906)*P =* 0.030 | Low = 1High = 0.327 (0.128-0.835) *P =* 0.019 | (mo):Low = 7.3 (3.6-11.1)High = 9.3 (4.2-14.5)*P =* 0.022 (HR):Low = 1High = 0.362 (0.146-0.898)*P =* 0.028 | Low = 1High = 0.558 (0.214-1.452)*P =* 0.232 | High hENT1 sig ass with longer OS in pat receiving adj gem after resection.  |
| Murata *et al*[[22](#_ENREF_22)] | 24.3  | (HR):Positive = 1Negative = 3.04 (1.45-6.37)*P =* 0.0037 | Positive = 1Negative = 3.15 (1.35-7.37) *P =* 0.008 | (HR):Positive = 1Negative = 2.34 (1.22-4-47)*P =* 0.011 | Positive = 1Negative = 1.76 (0.85-3.66)*P =* 0.129 | Sig longer OS, RFS in pat with pos hENT1. |
| Nakagawa *et al*[[23](#_ENREF_23)] | OS: 34.9DFS: 17.8 | (5y-SR %):High = 38 Low = 13*P =* 0.001 | High = 1Low = 3.16 (1.65-6.06)*P =* 0.001 |  (5y-SR %):High = 30Low = 17*P =* 0.004 | High = 1Low = 2.70 (1.52-4.83)*P =* 0.001 | hENT1 expression is predictive of the efficacy of adj gem-based chemotherapy after resection.  |

1From diagnosis/from treatment; 2*n* = 222 in multivariate analysis. Ass: Associated; pat: Patient; gem: Gemcitabine; sig: Significant; adj: Adjuvant; pos: Positive; op: Operation; DFS: Disease-free survival; OS: Overall surviva.