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Precision Medicine and Pancreatic Cancer: A Gemcitabine Pathway Approach

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Abstract

Objectives—There is a need for validated predictive markers of gemcitabine response to guide precision medicine treatment in pancreatic cancer. We previously validated human Equilibrative Nucleoside Transporter 1 (hENT1) as a predictive marker of gemcitabine treatment response using Radiation Therapy Oncology Group (RTOG) 9704. Controversy exists about the predictive value of gemcitabine metabolism pathway biomarkers: deoxycytidine kinase (DCK), ribonucleotide reductase 1 (RRM1), ribonucleotide reductase 2 (RRM2) and p53R2.

Methods—RTOG 9704 prospectively randomized 538 patients after pancreatic resection to receive either 5-fluorouracil (5-FU) or gemcitabine. Tumor DCK, RRM1, RRM2 and p53R protein expression were analyzed using a Tissue Microarray (TMA) and Immunohistochemistry (IHC) and correlated with treatment outcome (overall survival and disease free survival) by unconditional logistic regression analysis.

Results—229 patients were eligible for analysis from both the 5-FU and Gemcitabine arm. Only RRM2 proteins expression, and not DCK, RRM1, or p53R2 protein expression was associated with survival in the gemcitabine treatment arm.

Conclusions—Despite limited data from other nonrandomized treatment data our data does not support the predictive value of DCK, RRM1, or p53R2. Efforts should focus on hENT1, and possibly RRM2 as a valid predictive marker of treatment response of gemcitabine in pancreas cancer.

Keywords

Pancreatic Cancer; Precision Medicine; hENT1; DCK; RRM1; RRM2

INTRODUCTION

The availability of both gemcitabine-based and non-gemcitabine-based treatment regimens for pancreatic cancer and the overall poor dismal prognosis associated with this disease necessitates the validation of predictive markers of treatment response in this disease, to

facilitate precision medicine options. (1–3) RTOG 9704 is a phase III randomized post-operative adjuvant study in patients with pancreatic cancer comparing pre- and post-chemoradiation 5-fluorouracil with pre- and post- chemoradiation gemcitabine, and represents an ideal study for evaluating predictive markers of gemcitabine treatment response.(4) Using it, we previously showed protein expression of hENT1, the predominant transmembrane transporter for gemcitabine, as a possible treatment predictive marker in the adjuvant setting for pancreatic cancer(5), which was further validated by several subsequent studies(Supplementary Table 1).(6–13)

There are several reasons for why there is conflicting data about the predictive value of other markers involved with gemcitabine metabolism (DCK,(7, 14, 15) RRM1(7, 9, 16–18), RRM2(17, 19) and p53R2) (20) (21) (22), some of which are being used in routine clinical practice to make decisions about precision medicine options for patients. Firstly, several gene expression studies have been performed without the appropriate microdissection of the tumor tissue to separate it from the stromal tissue. This suggests the protein-based markers by immunohistochemistry (IHC) represent the most reproducible methodology for these types of studies. Secondly, several studies used heterogeneous collection of data and disease stages, non-prospective and non-randomized treatment regimens often involving multiple drug regimens. Finally, different antibodies and IHC scoring systems have been used across several studies.

In view of the appropriate design and prospective tissue collection of the RTOG 9704 study, and its initial use in validating hENT1 as a promising predictive marker, we decided to use this study to analyze DCK, RRM1, RRM2 and p53R2 protein expression, correlate it with treatment outcome, and to compare our results with current existing published data to assess the predictive value of these markers for guiding precision medicine options with gemcitabine treatment.

METHODS

Patient Selection and Consent

The RTOG tissue bank received tumor blocks from a total of 229 of the 538 patients who had undergone surgical resection and were entered in the RTOG 9704 prospective adjuvant treatment trial. Permission to perform this study was obtained by the Institutional Review Board. (23) A tissue micro-array (TMA), which was also used for the original hENT1 study, was constructed using tissue core samples from the patients enrolled in the RTOG 9704 study, using three cores from different areas of a patient's tumor.

Immunohistochemistry and Scoring

Rabbit polyclonal antibodies were raised against a synthetic human DCK peptide, and tissue immunohistochemistry was performed per prior study.(24) Semi-quantitative scoring of tumoral cytoplasmic staining was used for the evaluation of DCK protein expression by a single pathologist (R.L.). Staining of DCK protein was assigned a score from 0 to 2 based on staining intensity (no staining = 0, weakly positive staining = 1, and strongly positive staining = 2). A final score (0–200) was determined by multiplying the intensity score and

the percentage of the positive cells in the specimen, as described previously.(15) The mean score of triplicate tissues from each patient was used for analysis, and the scores were dichotomized into low DCK and high DCK based on the median score of all the cytoplasmic means per patient data in the three TMAs.

Immunofluorescence combined with AQUA, using antiserum to RRM1 was generated from rabbits was used to assess in situ expression of nuclear RRM1 as described previously.(25) The final slides were scanned with SpotGrabber (HistoRx, New Haven, CT, USA), and images were analyzed with AQUA (version 1.6, PM-2000, HistoRx) by a pathologist with experience with RRM1 staining (ZZ). The AQUA scores ranged from 0 (no expression) to 3000 (maximal observed expression) for the triplicate TMA tissues for each patient. The median value of the RRM1 expression levels were used to divided the patients into high RRM1 and low RRM1 levels.

Tumoral protein expression of hRRM2 was performed using a mouse polyclonal antibody against hRRM2 (Convance, Princeton, NJ) as previously described.(26). Two independent scorers (YY, XL) experienced with RRM2 immunohistochemistry, assessed and scored the cytoplasmic RRM2 immunostaining intensities, ranging from 0 (no staining), to 3 (maximum staining), with a consensus score being agreed upon if there was initial discordance between the two scorers.(26) The mean cytoplasmic RRM2 score was assessed per patient, and the RRM2 cytoplasmic score was also categorized as “Low RRM2” expressing (mean cytoplasmic score <1.5 ($<\text{mean} + 1 \text{ S.D.}$)) and “High RRM2” expressing (mean cytoplasmic score ≥ 1.5 ($\geq \text{mean} + 1 \text{ S.D.}$)).

Due to non-specific targeting of commercial p53R2 antibody, a new highly specific p53R2 anti-rabbit antibody was used.(27) Details of the de-paraffinization protocol and IHC were described in a previous publication.(28). Each sample was scored by two independent investigators in a double-blind manner (XL, YY), with a consensus score being agreed upon if there was initial discordance between the two scorer. Cytoplasmic staining of p53R2 protein was assigned a score from 0 to 2 based on staining intensity (no staining = 0, weakly positive staining = 1, and strongly positive staining = 2) per prior study.(27) The percentage of adenocarcinoma cells stained at each intensity level was recorded for each, resulting in a weighted scores ranged between 0 and 200. For the purposes of statistical analysis, the scores were dichotomized into low p53R2 and high p53R2 based on the median score.

IHC Biomarkers Statistical Analysis

The DCK, RRM1, RRM2, p53R2 (Gemcitabine pathway biomarkers) immunohistochemistry scores were submitted to the RTOG Statistical Department for analysis without knowledge of patient demographics, treatment arm randomization or outcome. A statistical comparison to assess whether missing data were associated with dichotomized baseline characteristics (pathological t-stage (T1, T2 vs. T3, T4), AJCC stage (I, II vs. III, IV), primary tumor location (head vs. everything else)). was carried out using the chi-square test for each of the markers.

Association between the gemcitabine pathway biomarkers protein expression, dichotomized (“Low” vs. “High”) with tumor demographic details and treatment outcome (Overall

survival (OS), Disease Free Survival (DFS)) were sought by the chi-square test and the Cox proportional hazards model. Both treatment arms of the study were analyzed. OS and DFS were estimated using the Kaplan-Meier method and the biomarkers were compared using the log-rank test. The following variables were included in the multivariate analyses based on analysis of the original trial: age, gender, race, treatment arm (when appropriate), nodal involvement (no vs. yes), tumor diameter (< 3 vs. ≥ 3 cm), KPS (100, 90 vs. 60, 70, 80), and surgical margin status (negative vs. positive and negative vs. unknown). Results were expressed as Hazard Ratio (H.R.), (H.R. > 1 denoting increased risk of death) and considered significant at *p-value* ≤ 0.05.

Literature Search

A systematic literature search upto December 2014 was performed in MEDLINE and EMBASE to identify relevant studies. An initial search study using recognized search terms [hENT1 or DCK or RRM1 or RRM2 or p53R2] and [Pancreatic Cancer or Pancreatic Carcinoma] and [Gemcitabine] was conducted. Studies were considered eligible for comparison with the current study if there were performed using immunohistochemistry and in the adjuvant setting.

RESULTS

DCK Protein Expression

Of the 229 patient tumor samples evaluated, 186 had analyzable DCK immunostaining. There were no positive statistical associations between baseline characteristics and missing and determined DCK protein expression (Supplementary Table 2 and Supplementary Table 3). For cytoplasm mean across three TMAs, the median score was 125.00 (min-max: 0.00–200.00) to dichotomize the results in to “Low DCK” and “High DCK”. “High DCK” expression was seen in 50 of 88 patients in the gemcitabine treatment arm, and 44 of 98 patients randomized to the 5-FU treatment arm.

Univariate and multivariate analyses for dichotomized DCK: “Low DCK” vs. “High DCK” was performed for all patients (Table 1). By multivariate analysis, DCK expression was independently and significantly associated with OS despite adjusting for baseline characteristics in these multivariate models for the overall treatment group (HR = 0.71, *p*=0.044) (Table 1, Figure 1 A), but this was not seen with DFS. DCK proteins expression was not significantly associated with OS or DFS among those patients randomized to the gemcitabine treatment arm on multivariate analysis (Table 1, Figure 1B). However, multivariate analysis confirmed improved OS when comparing all patients with “High DCK” to patients with “Low DCK” (H.R. = 0.50, *p*=0.0026) (Table 1, Figure 1C), but no association was seen for DFS in the 5-FU treatment arm.

RRM1 Protein Expression

Of the 229 patient tumor samples evaluated, 196 had analyzable RRM1 immunostaining. There were no positive statistical associations between baseline characteristics and missing and determined RRM1 protein expression (Supplementary Table 4 and Supplementary Table 5). For nucleus staining maximum of the triplicate tissues from a single patient, the median

of the average of all TMA was 971.38 (min-max: 27.14–3370.34), which is used as the cut-off to divide patients into “Low RRM1” and “High RRM1”. “High RRM1” staining was seen in 44 of 92 patients in the gemcitabine treatment arm, and 54 of 104 patients randomized to the 5-FU treatment arm.

Univariate and Multivariate analyses for dichotomized RRM1: “Low RRM1” vs. “High RRM1” was performed for all patients (Table 1). RRM1 expression was independently and significantly associated with OS (H.R.= 0.71, p=0.031), but not DFS (H.R.=0.84, p=0.26) despite adjusting for baseline characteristics in these multivariate models for the overall treatment group (Table 1, Figure 2A). We observed statistically significant prolonged OS (H.R. = 0.51 p=0.0021) and DFS (H.R. = 0.65 p=0.036) for patients with “High RRM1” as compared to patients with “Low RRM1” for all patients treated with 5-FU (Table 1, Figure 2C). No association between RRM1 protein expression and OS and DFS was seen in the gemcitabine treated group (Table 1, Figure 2B).

RRM2 Protein Expression

Of the 229 patient tumor samples evaluated, 189 had analyzable RRM2 immunostaining. There were no positive statistical associations between baseline characteristics and missing and determined RRM2 protein expression (Supplementary Table 6 and Supplementary Table 7). “High RRM2” staining was seen in 9 of 88 patients in the gemcitabine treatment arm, and 12 of 101 patients randomized to the 5-FU treatment arm.

Univariate and multivariate analyses for dichotomized RRM2: “Low RRM2” vs. “High RRM2” was performed for all patients (Table 1). RRM2 expression was independently and significantly associated with DFS despite adjusting for baseline characteristics in these multivariate models for the overall treatment group (H.R. = 1.62 (95% CI: 1.01, 2.60) p=0.045)(Table 1, Figure 3A)). We observed prolonged disease-free survival which approached statistical significance for patients with “Low RRM2” as compared to patients with “High RRM2” for all patients treated with gemcitabine (H.R. = 2.24 (95% CI: 1.08,4.68) p=0.031)(Table 1, Figure 3B). No association between RRM2 protein expression and OS was seen in the gemcitabine treated group. In the 5-fluorouracil treatment arm, RRM2 expression was not significantly associated with OS or DFS after adjusting for baseline characteristics in these multivariate models. (Table 1, Figure 3C)

P53R2 Protein Expression

Of the 229 patient tumor samples evaluated, 163 had analyzable p53R2 immunostaining. There were no positive statistical associations between baseline characteristics and missing and determined p53R2 protein expression (Supplementary Table 8 and Supplementary Table 9). For cytoplasm mean, the median is 1.3 (min-max: 0.00–2.00) to dichotomize the results in to “Low p53R2” and “High p53R2”. “High p53R2” expression was seen in 44 of 74 patients in the gemcitabine treatment arm, and 55 of 89 patients randomized to the 5-FU treatment arm.

Univariate and multivariate analyses for dichotomized p53R2: “Low p53R2” vs. “High p53R2” was performed for all patients. P53R2 (Table 1) expression was not significantly associated with OS or DFS despite adjusting for baseline characteristics in these multivariate

models for the overall treatment group (OS: H.R. 1.07, $p=0.71$, DFS (H.R. 1.01, $p=0.97$)) (Table 1, Figure 4A). Again, p53R2 proteins expression was not significantly associated with OS or DFS among those patients randomized to either the gemcitabine treatment arm (OS: H.R.=1.36, $p=0.25$, DFS: H.R.=1.62, $p=0.06$) or the 5-FU treatment arm (OS: H.R.=0.98, $p=0.92$, DFS: H.R.= 0.76 $p=0.23$ on multivariate analysis (Table 1 Figure 4B,C).

DISCUSSION

Several lessons were learned from the initial RTOG 9704 study of hENT1 which supported its role as a promising predictive marker of treatment response for precision medicine in pancreatic cancer, and its subsequent validation in other studies.(5) Firstly, the importance of stage of disease and treatment setting is important when discussing the predictive value of a possible marker. The studies supporting hENT1 are all in the adjuvant setting, (6–13) with no strong evidence to support its role in the advanced or metastatic stage,(12, 29) and unclear significance in the neoadjuvant setting.(30, 31). In addition, the methodology for quantitating the marker is important. It is important to know whether protein or RNA is being measured; what type of antibody is being used; whether a tissue micro-array to increase tumor representation is employed, and the marker scoring system used including marker location (e.g. cytoplasm vs. nuclear location) and appropriate cut-offs. For example, if mRNA is being measured, then microdissection of tumor is important.(32, 33) Finally the availability of well characterized prospectively treated patients with both gemcitabine and non-gemcitabine treatment arms is important to formally assess the predictive value of a marker, rather than just the prognostic value.(5, 11, 12) Hence this study aimed to validate other non-hENT1 gemcitabine related markers using the RTOG 9704 tissue in the adjuvant setting and using previously standardized IHC methodology.

DCK is the enzyme responsible for the rate limiting step which converts the prodrug gemcitabine to its active mono phosphorylated form.(34) One study correlated high DCK mRNA levels with prolonged disease free survival in patients with pancreas cancer receiving palliative gemcitabine(33), and another showing no correlation at all.(32) Our data does not support the predictive role for tumoral cytoplasmic DCK protein to determine outcome response to gemcitabine in the adjuvant setting in pancreatic cancer. This is in contradistinction to other IHC studies which have suggested DCK as a gemcitabine predictive marker (Table 2). (7, 14, 15). While these studies used a similar antibody and DCK cytoplasmic scoring system, two are small retrospective studies which did not use TMA analysis or included heterogeneous treatment groups.(14, 15) The more recent large study in the adjuvant setting is complicated by its retrospective tissue and data collection methodology, and the absence of a randomized non-gemcitabine treatment arm.(7) Interestingly, our current study did appear to support an association between high levels of DCK and overall survival for the entire study group (both treatment arms), likely due to the effect seen in the 5-FU treatment arm. There is some preclinical cell data supporting the association between high DCK levels and 5-FU sensitivity but very little is known about the precise mechanism.(35) Similarly cell data has shown that RRM1 significantly contributes to the induction of DNA damage by 5-FU possibly related to RAD51 focus formation.(36) It is possible that it reflects an increased sensitivity of proliferating cells to 5-FU with dCK and possibly RRM1 being key enzymes of DNA synthesis

RRM1, a multimeric enzyme that converts ribonucleotides to deoxyribonucleosides has been reported as a predictive marker of gemcitabine treatment response in pancreatic cancer in several preclinical studies.(37, 38). Several very small clinical studies in pancreatic cancer have demonstrated an association between low RRM1 expression (as determined by quantitative reverse transcription PCR,(37, 39) or immunohistochemistry.(16)) In the study showing an association with survival in a gemcitabine treated cohort and low RRM1 protein expression, only nuclear expression was measured and the association was not seen for patients with high expression of RRM1. Similar to our larger study, there are also several other large RNA based and immunohistochemistry studies which do not demonstrate the predictive value of RRM1 (Table 2) (7, 17, 18, 32, 40). Two studies which have demonstrated the predictive value of combining low RRM1 and high hENT1 to predict improved survival with or without the expression of other markers such as RRM2 and dCK. (9, 33).

We demonstrated an association between high tumor RRM2 protein expression and decreased disease free survival in patients with pancreatic cancer receiving adjuvant therapy in a prospective randomized trial. This effect was not seen in patients treated with 5-FU alone, suggesting that tumor RRM2 protein levels may function as a predictive marker of response to treatment with gemcitabine. This is in keeping with previous non-pancreas cancer data which associated high levels of RRM2 expression (either by protein or gene expression) with gemcitabine chemoresistance and worse survival.(27, 41) The importance of the ribonucleotidase reductase RRM2 enzyme in cancer prognosis and as a potential predictive marker for response to gemcitabine treatment, has been reported in several preclinical cancer models including lung cancer (25, 42–44) and pancreatic cancer.(32, 34, 37, 45–47) However, the other clinical data supporting the role for RRM2 as a predictive marker of gemcitabine response is conflicting. Lower pre-treatment RRM2 mRNA expression of endoscopic ultrasound-fine needle aspiration biopsy specimens from patients with pancreatic cancer was associated with improved median survival and overall response rate after gemcitabine treatment..(45) However, in a larger study of both RRM1 and RRM2 gene expression in laser microdissected pancreatic surgical or biopsy specimens, there was no association between either gene expression and pancreatic cancer disease survival after treatment with gemcitabine.(32)A recent non-TMA retrospective study RRM2 protein expression study in the adjuvant setting with pancreatic cancer, supports the concept of high RRM2 expression corresponding with worse overall and disease free survival in the overall patient group as well as the 74 patients receiving adjuvant therapy (Table 2).

The p53-inducible p53R2 gene also plays an important role in DNA repair and synthesis after DNA damage.(48).(49, 50) Expression of p53R2 has been variably correlated with overall prognosis in patients with esophageal squamous cell carcinoma, early stage non-small cell lung cancer and colon cancer.(27, 41) To date there has been no study of p53R2 in pancreatic cancer, either as a prognostic or predictive biomarkers. This current study does not favor p53R2 to be either prognostic or predictive of treatment response in the adjuvant pancreatic cancer setting.

In conclusion, our current study does not support the predictive roles of DCK, RRM1, or p53R2 in the precision medicine guided treatment of pancreatic cancer with gemcitabine in

the adjuvant setting, but does support the possible predictive value of RRM2. While our study did not combine multiple markers as has been done with other studies, it is unlikely to have proven of benefit in this particular study. For now, efforts should focus on hENT1 as a valid predictive marker of treatment response of gemcitabine in pancreas cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

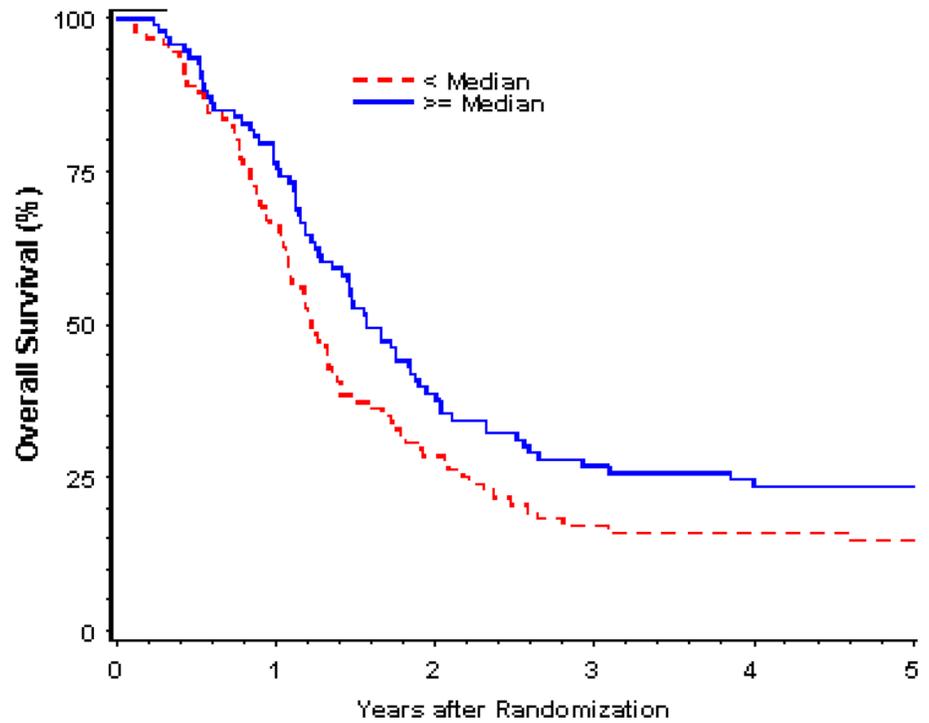
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Figure 1A: All Patients



H.R=0.69(95%CI 0.50,0.95) P=0.021

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hENT1 Markers

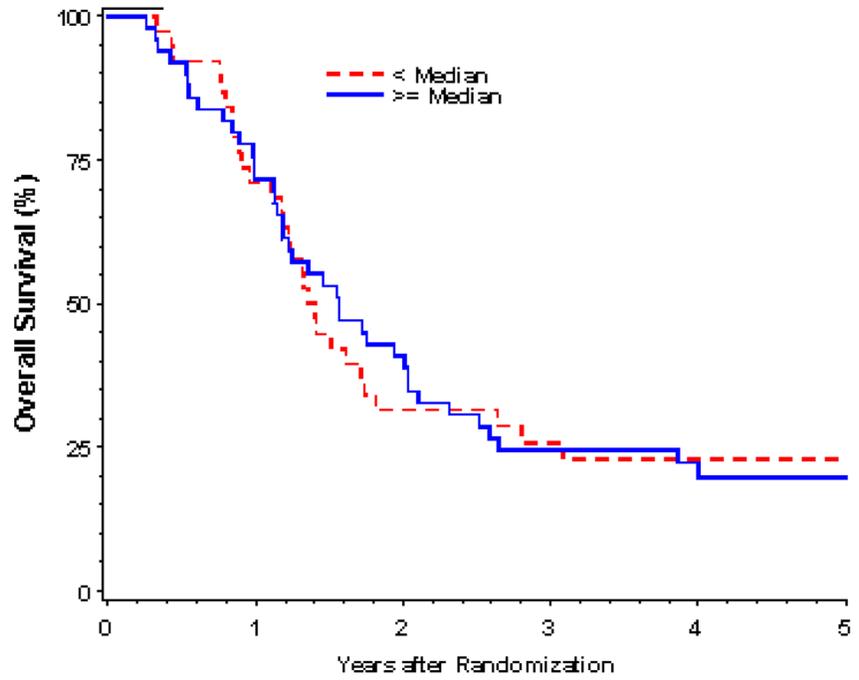
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Figure 1B: Gemcitabine Arm



H.R=0.97(95%CI 0.61,1.54). P=0.88

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Figure 1C: 5-FU Arm

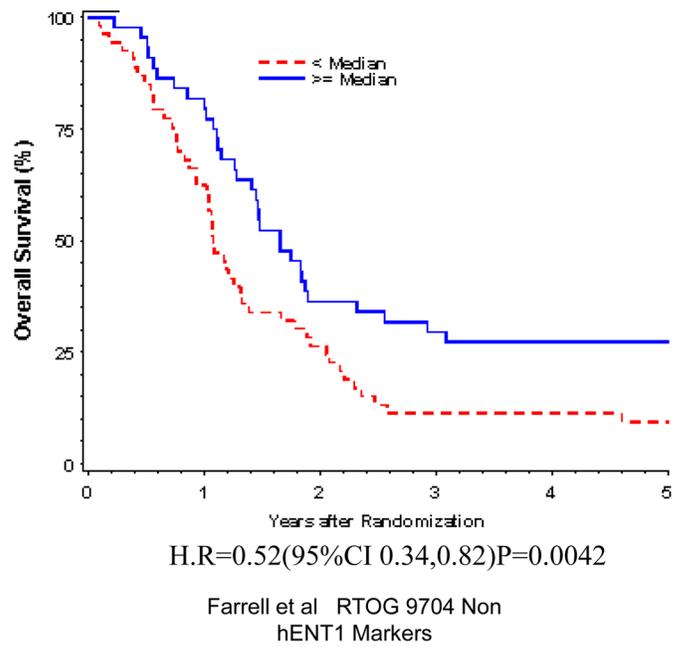
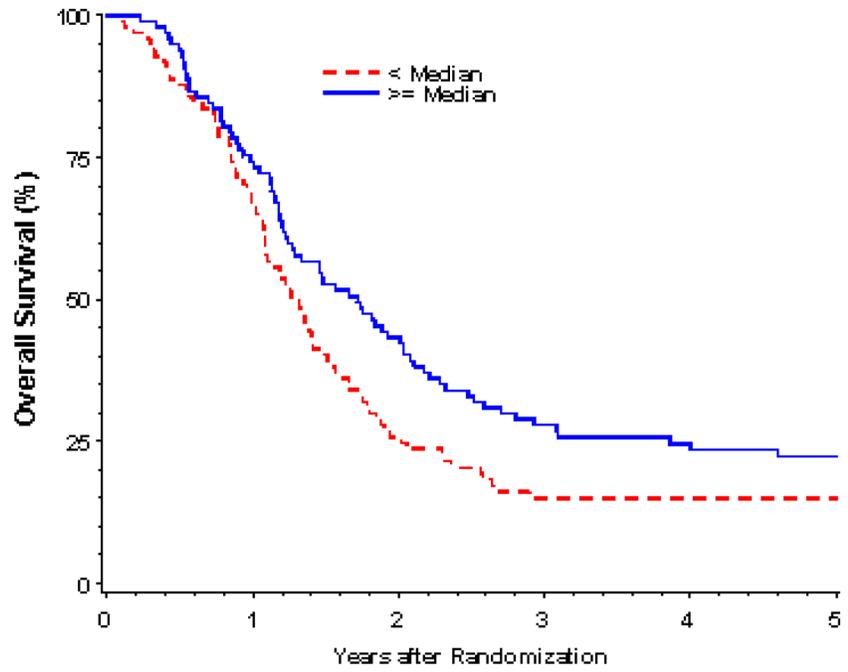


Figure 1. Overall survival for DCK protein expression, multivariate analysis. A. All patients. B Gemcitabine Treatment Arm. C 5-FU treatment arm

Figure 2A:All Patients



H.R=0.72(95%CI 0.53,0.99) P=0.039

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Figure 2B: Gemcitabine Arm

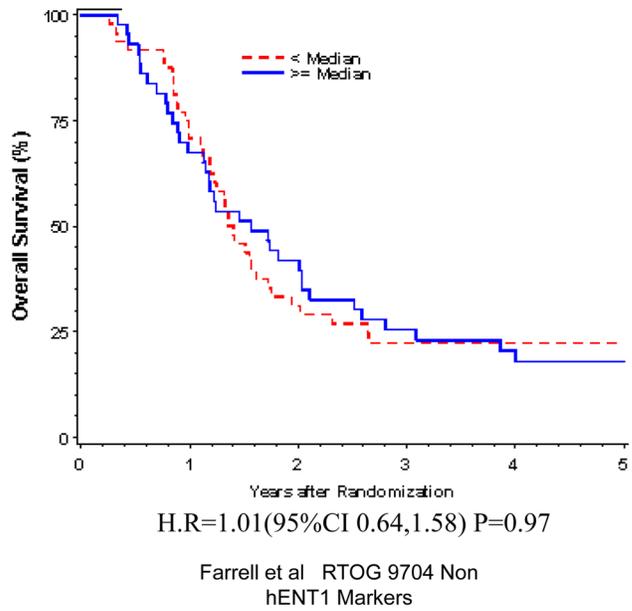


Figure 2C:5-FU Arm

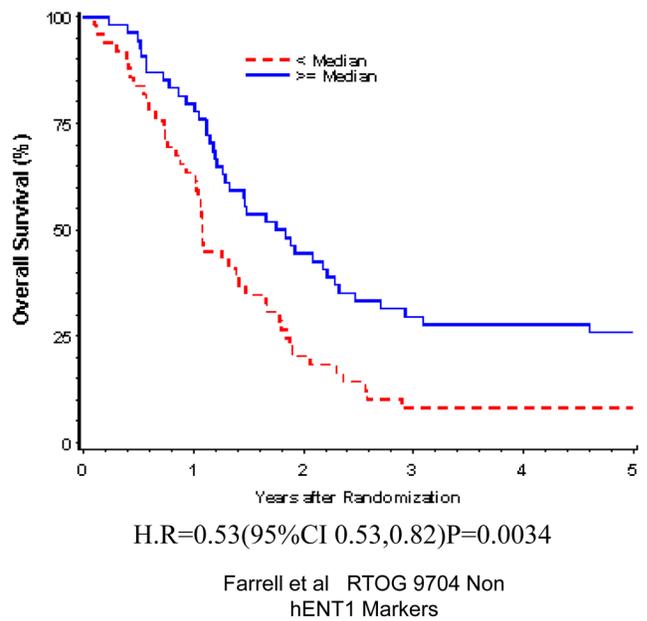


Figure 2.

Overall survival for RRM1 protein expression, multivariate analysis. A. All patients. B Gemcitabine Treatment Arm. C 5-FU treatment arm

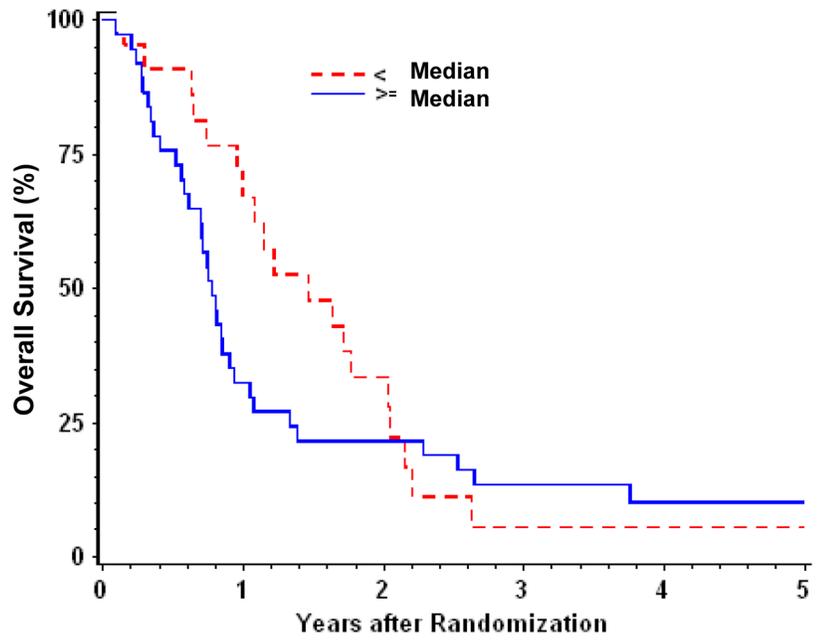
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Figure 3A:All Patients



H.R=1.41(95%CI 0.86,2.34)P=0.18

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hENT1 Markers

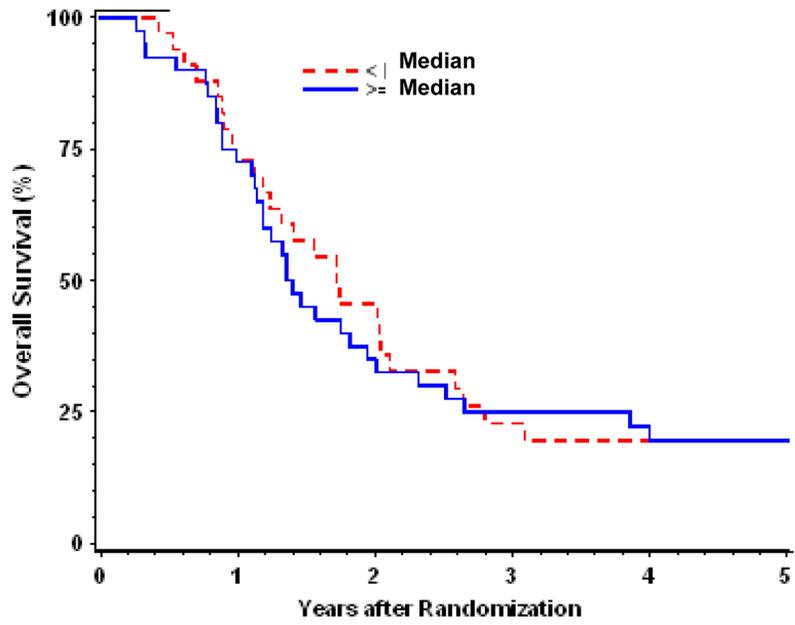
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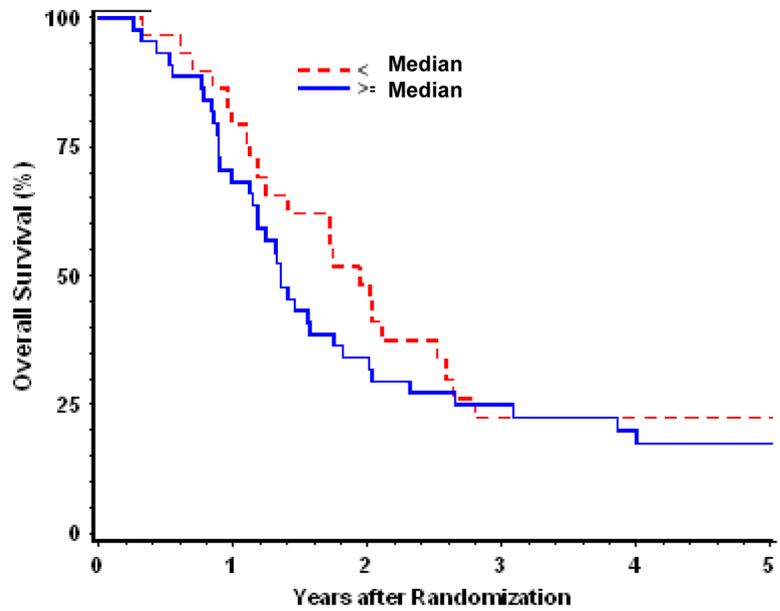
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Figure 3B:Gemcitabine



H.R=1.17(95%CI 0.53,2.56)P=0.70

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hENT1 Markers

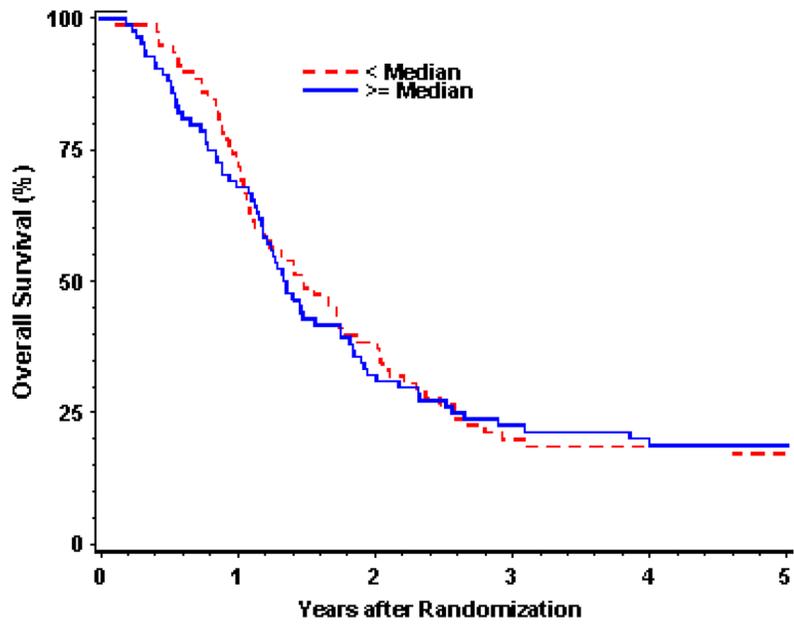
Figure 3C:5-FU

H.R=1.35(95%CI 0.69,2.64)P=0.38

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hENT1 Markers

Figure 3. Overall survival for RRM2 protein expression, multivariate analysis. A. All patients. B Gemcitabine Treatment Arm. C 5-FU treatment arm

Figure 4A:All Patients



H.R=1.01(95%CI 0.72,1.41) P=0.96

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hENT1 Markers

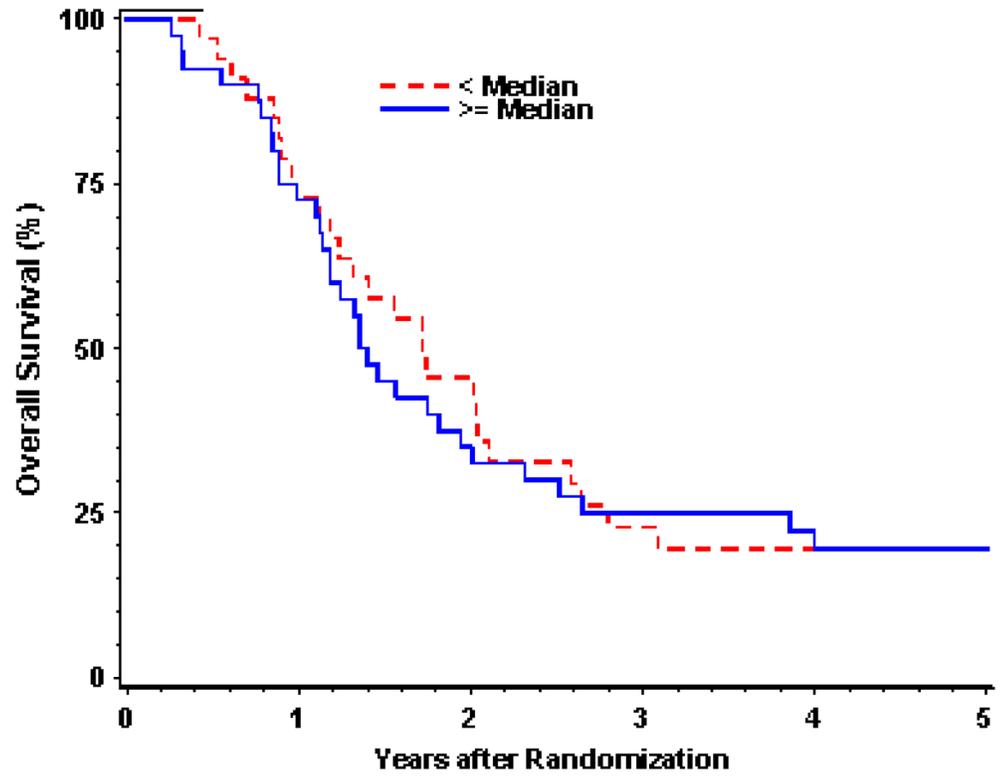
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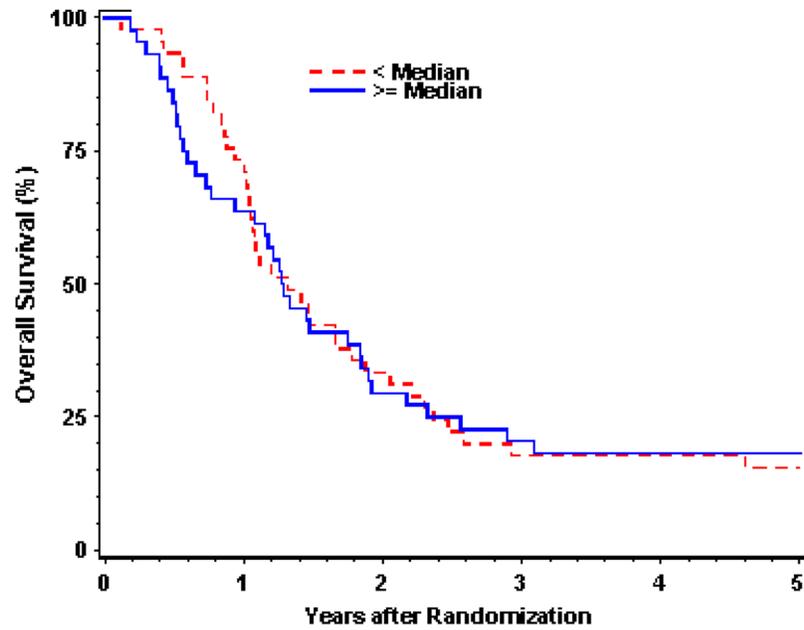
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Figure 4B: Gemcitabine



H.R=1.09(95%CI 0.66,1.80) P=0.73

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hENT1 Markers

Figure 4C:5-FU

H.R=0.97(95%CI 0.62,1.52) P=0.89

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hENT1 Markers

Figure 4.
Overall survival for p53R2 protein expression, multivariate analysis. A. All patients. B
Gemcitabine Treatment Arm. C 5-FU treatment arm

Table 1
Overall survival and Disease-Free Survival for hENT1, dCK, RRM1, RRM2 and p53R2 (univariate and multivariate analysis)

	hENT1(ref 5)		dCK		RRM1		RRM2		P53R2	
	Low/High vs No hENT1	p	High vs Low dCK	p	High vs Low RRM1	p	High vs Low RRM2	p-value	Low vs High p53R2	p
Univariate										
Overall Survival										
Both Groups	0.73	0.09	0.69	0.022	0.72	0.04	1.42	0.17	1.05	0.80
RT+5-FU	0.93	0.75	0.52	0.0048	0.53	0.0039	1.72	0.10	0.90	0.64
RT+GEM	0.51	0.02	0.97	0.88	1.01	0.97	1.10	0.81	1.28	0.35
Disease Free Survival										
Both Groups	0.74	0.09	0.84	0.25	0.82	0.19	1.62	0.045	1.05	0.79
RT+5FU	0.88	0.60	0.67	0.061	0.65	0.036	1.50	0.21	0.76	0.23
RT+GEM	0.57	0.05	1.11	0.66	1.06	0.80	1.74	0.12	1.50	0.11
Multivariate										
Overall Survival										
Both Groups	0.66	0.03	0.71	0.044	0.71	0.031	1.58	0.069	1.07	0.71
RT+5-FU	0.78	0.31	0.50	0.0026	0.51	0.0021	1.78	0.078	0.98	0.92
RT+GEM	0.40	0.004	0.92	0.72	1.20	0.44	1.42	0.39	1.36	0.25
Disease Free Survival										
Both Groups	0.61	0.009	0.88	0.40	0.84	0.26	1.62	0.0045	1.01	0.97
RT+5FU	0.72	0.18	0.67	0.06	0.65	0.036	1.78	0.21	0.76	0.23
RT+GEM	0.39	0.003	1.37	0.19	1.26	0.34	2.24	0.031	1.62	0.06

HR = Hazard Ratio* a hazard ratio of 1 indicates no difference between the two subgroups such that a HR < 1 indicates an increased survival for the first variables listed. p-value from Chi-square test using the Cox proportional hazards model.

Table 2
DCK, RRM1 and RRM2 Expression by Immunohistochemistry in Pancreatic Cancer

DCK Protein Expression									
Author	Year	Gemcitabine	Non-Gemcitabine	Antibody	IHC	Scores Used	TMA	Treatment Design	O.S. association with DCK
Farrell	Current	88	98	Rabbit Polyclonal	Semiquant	Cyto only	Yes	Prospective Randomized	No
Sebastiani	2006	44	0	Rabbit Polyclonal	Semiquant		No	Retrospective Non Randomized	Yes/p=0.04 (UniVar)
Marechal	2010	45	0	Rabbit Polyclonal	Semiquant	Cyto only	Yes	Prospective Non Randomized	Yes/p=0.002
Marechal	2012	243	49	Rabbit Polyclonal	Semiquant	Cyto only	Yes	Retrospective Non Randomized	Yes/p=0.012

RRM1 Protein Expression									
Author	Year	Gemcitabine	Non-Gemcitabine	Antibody	IHC	Scores Used	TMA	Treatment Design	O.S. association with RRM1
Farrell	Current	92	104	Rabbit Polyclonal	Quantitative	Nuclear	Yes	Prospective Randomized	No
Fisher	2012	74(33)*	0	Rabbit Polyclonal	Semiquant	Cyto and Nuclear	No	Retrospective Non Randomized	No
Marechal	2012	243	49	Rabbit Polyclonal	Semiquant	Nuclear	Yes	Retrospective Non Randomized	No
Valsecchi	2012	82	0	Rabbit Polyclonal	Semiquant	Cytoplasmic	No	Retrospective Non Randomized	No
Nakagawa	2012	109	0	Rabbit Polyclonal	Semiquant	Nuclear	Yes	Retrospective Non Randomized	Yes/p=0.019
Akita	2009	28	40	Rabbit Polyclonal	Quantitative	Nuclear	Yes	Retrospective Non Randomized	Yes/p=0.0010

RRM2 Protein Expression									
Author	Year	Gemcitabine	Non-Gemcitabine	Antibody	IHC	Scores Used	TMA	Treatment Design	O.S. association with RRM1
Farrell	Current	88	101	Rabbit Polyclonal	Semiquant	Cytoplasm	Yes	Prospective Randomized	No
Xie	2012	44	0	Goat polyclonal	Semiquant	Nuclear	Yes	Retrospective Non Randomized	No
Fisher	2012	74(33)*	0	Mouse monoclonal	Semiquant	Cyto and Nuclear	No	Retrospective Non-Randomized	Yes/p=0.02

* 33 of the reported 74 patients definitely received gemcitabine

IHC, immunohistochemistry

Semiquant, semiquantitative

Cyto, cytoplasm

TMA, tissue microarray

O.S., overall survival