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The Predictive Value of *ERCC1*, *RRM1*, and Thymidylate Synthase in Advanced Malignant Pleural Mesothelioma Patients Treated with Platinum-Based Chemotherapy

Nagwa Ismail Wahba[1]*, Ola Khorshid[1], Fatma Abo Elkasem[1], Abeer Bahnassy[2], Rabab Gaafer[1,3]

Abstract

Background: A rare yet severe neoplasia called malignant pleural mesothelioma (MPM) typically manifests itself in advanced stages. Despite some improvements in the treatment of patients with MPM, this malignancy continues to have a detrimental prognosis. The primary goal of the present study was to assess the associatin between *ERCC1, RRM1*, and thymidylate synthase (*TS*) expression and disease outcome in patients with malignant pleural mesothelioma (MPM) treated with either pemetrexed plus cisplatin or gemcitabine plus cisplatin. **Methods:** This prospective cohort study was done on ninety-one advanced MPM patients. The patients received either pemetrexed plus platinum (55 of 91) or gemcitabine plus platinum chemotherapy (36 of 91). Tissue biopsy was taken at time of diagnosis. Immunohistochemistry was used to assess the levels of *ERCC1, RRM1,* and *TS* transcription in tissue biopsies (paraffin embedded). **Results:** Based on the findings, 70% of patients with low expression of *TS* had low expression of *ERCC1*, and 68% of patients with high expression of *TS* had high expression of *ERCC1*, suggesting a significat association between *ERCC1* expression and *TS* expression (p=0.005). However, there was no relation between *ERCC1* and *RRM1* expression patterns (p= 0.296). In patients underwen platinum-based theraphy (n 91), a significant correlation was detected between low *ERCC1* median High-scoring and longer progression time (TTP;9.6 v 5.3 months;P< 0.001) or overall survival (OS) (OS;18.1 v 9.1 months; P<0.001). A significant correlation was found between low TS protein expression and longer time progression (TTP; 11.8 v 5.4 months; P< 0.001) or OS (OS; 19.8 v 8.5 months; P <0.001) in patients undergoing pemetrexed plus platinum chemotherapy (n 55). Low RRM1 expression was accompanied by high progression free survival (TTP; 10.6 v 3.8 months) and OS (OS; 20.6 v 8.6 months) in patients receiving gemcitabine plus platinum chemotherapy. Based on the multivariate test results, the independent variables significantly affecting OS were tumor stage (HR: 2.3; 95% CI: 1.1-4.9; p= 0.021) and *ERCC1* (HR: 2.7; 95% CI: 1.7-4.3; p < 0.001). **Conclusion:** Decreased TS protein expression can be indicative of greater responsivness to pemtrexed and of longer TTP and OS in individuals with advanced MPM (locally progressed or metastatic) who are receiving pemetrexed-based chemotheraphy. Low *ERCC1* expressions in individuals with advanced MPM can predict increased PFS and OS, as well as a better responsivness to platinum-based chemotherapy. In patients with progressed MPM receiving gemcitabine plus cisplatin chemotherapy, lower *RRM1* expression was associated with a better prognosis, longer PFS, and longer OS.

Keywords: *RRM1- ERCC1-* immunohistochemistry- biomarkers- mesothelioma

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Introduction

Malignant pleural mesothelioma (MPM) is an aggressive tumor characterised with difficulty of diagnosis in early stage, poor prognosis, and no effective treatment. MPM has a growing tendency worldwide (Cihan et al., 2014), and its peak incidence is expected to occur between 2015 and 2030 (Neumann et al., 2013). Apart from cisplatin and the antifolates, most single-agent chemotherapy regimens? demonstrated low mean survival durations and low response rates. On the other hand, combined chemotherapy regimens frequently showed higher efficacy. Pemetrexed, as a a novel multitargeted antifolate, inhibits multiple targets of folic acid metabolic pathway, especially thymidylate synthase (*TS*) (Shih et al., 1997). Increased baseline *TS* expression in non-small-cell lung cancer (NSCLC) cell lines can predict poor response to pemetrexed, in a way that TS rate is connected to pemetrexed effectiveness in a range of solid tumours (Gomez et al., 2006).

*[1]Department of Medical Oncology, National Cancer Institute, Cairo University, Egypt. [2]Department of Pathology, NCI, Cairo University, Egypt. [3]Member of Chest Tumor Committe, Esmo Member, Egypt.*For Correspondence: nagwawahba313@gmail.com*

Excision repair cross-complementation group 1 (*ERCC1*), as a key element in the nucleotide excision repair (NER) pathway, can eliminate cisplatin-induced DNA adducts (Friedberg 2001). *ERCC1* mRNA concentrations and treatment response to cisplatin-based therapy have been demonstrated to be inversely correlated in multiple retrospective studies, in which tumour specimens from ovarian cancer clinical trials (Weberpals et al., 2009), colorectal cancer (Kim et al, 2009), and NSCLC (Olaussen et al., 2006) and (Vilmar et al., 2010) were investigated.

An enzyme called ribonucleotide reductases transforms ribonucleotides triphosphates into deoxyribonucleotides diphosphates, which is necessary for the production of DNA. The holoenzyme of ribonucleotide reductases is composed of a large subunit, namely RRM1, and a small subunit, namely RRM2. The cytoplasmic proteins are present during the entire cell division process (Pontarin et al., 2008).

This study aimed to evaluate the prognostic and predictive significance of *ERCC1*, *RRM1*, and *TS* in patients with advanced mesothelioma (patients with locally advanced and metastatic cancers).

Materials and Methods

Study Population

Ninety-one chemotherapy-naive patients with advanced MPM (either locally advanced or metastatic MPM) were prospectively selected amomg those who were treated at the National Cancer Institute, Cairo, Egypt. All patients signed a written consent form before participating in the study. The ethical committee at national cancer institute approved the study.

The patients were diagnosed with MPM based on clinical data and pathological assessment using immunohistochemistry. They had inoperable tumors. Patients' clinical and pathological information, such as gender, age, histopathological findings, medical history, and physical examination results, and Karnofsky functioning condition were gathered. Chemotherapy-naive patients with ECOG performance status 0-2 were considred as inclusion citeri. involved in the study.

Eligible patients were classified into 2 groups
First group

Patients received Pemetrexed 500mg/m^2 plus cisplatin 75mg/m^2 on day1 (n= 55).The treatment cycle was repeated every 21 days. *ERCC1* and *TS* were assessed at the time of diagnosis and treatment management of participants. Treatment responsiveness was assesed after 4 treatment cycles by using chest CT with contrast.

Second group

Patients received Gemcitabine 1000mg/m^2 on day1 and day 8 plus cisplatin 75 mg/m^2 on day 1 (number of patients 36). The treatment cycle was repeated every 21 days. *ERCC1* and *RRM1* were studied at the time of diagnosis. If there was a satisfactory treatment response following four cycles, chemotherapy was resumed every 3 weeks for a total of 6 rounds. Dexamethasone prophylaxis, along with vitamins supplements, were given to all patients who received pemetrexed. Folic acid and vitamin B_{12} supplementations began 7–14 days before the first chemotherapy treatment session. On the day before every chemotherapy dosage, the day of the dosage, and the day following the dosages, dexamethasone (4 mg p.o. twice daily) was given. Folic acid was prescibed for an additional 21 days following the last chemotherapy treatment session.

Majority of patients with regressive diseases underwent surgical resection. Four to eight weeks after the final chemotherapy treatment session, surgery was carried out. The patients with resectable conditions underwent pleurectomy/decortication. PFS was the main outcome. PFS was described as the duration between the intiation of treatment and the first clinically or radiologically obvious sign of progression of the disease, such as a pleural effusion or metastasis. Patients who had no evidence of disease progresion were assessed at the time of the last follow-up . OS served as the secondarily ending point. The time from diagnosis to mortality or the last follow-up was considered as the mean survival rate.

Pathological assessment of the markers

Specific monoclonal mouse antihuman antibodies for *ERCC1* (clone 8F1, dilution 1:50, Santa Cruz Biotechnology) and TS were used in immunohistochemistry examinations (clone 106, dilution 1:100, DAKO). The samples were paraffin-embedded and formalin-fixed and then cut into 2-mm-thick cell slices.

Antigen retrievals were carried out for both *ERCC1* and *TS* for thirty minutes at room temperature in W-CAP pH 8.0 buffers (Bio-Optica) in order to improve the immunoreactivity. After blocking endogenous peroxidase activities for thirty minutes using three percent hydrogen peroxide, the antibodies were incubated for an hour at room temperature. The immunoreactions were detected using a biotin-free detecting method (EnVision, Dako Cytomation) based on the activities of the peroxidase enzymes and a dextran chains coupled to the secondarily antibodies while diaminobenzidine (Dako Cytomation) was served as the chromogens.

The samples were fixed, dehydrated, and counterstained with hematoxylins. Two physicians who were blinded to the patients' identities and clinical outcomes read and evaluated the portions. A method used the staining strength and quantity of cells to analyze the findings. Tumor cells were deemed positively for *ERCC1* staining when they had nuclear reactivity, although TS positivity was determined by both nuclear and cytoplasmic reactions.

A semiquantitative histological scoring was used to determine the proportion of tumour cells that were positive with intens staining (H-score). In specifically, the proportion of positive neoplastic cells was multiplied by the stained intensity of the tumor, which ranged from low (score 1) to moderately and higher (scories 2 and 3). (Giving values from 0 to 300). Regarding RRM1, every biopsy sample was sliced into thin (2 m) pieces and placed on glass plates. Deparaffinization of the sections was followed by heat-induced antigen retrieval in the DAKO-PT-link modules using a pH 9 target retrievals solutions for twenty minutes at 97°C.

After blocking endogenous peroxidase, slices were treated with rabbits polyclonal anti-human RRM1 (Proteins Tech Europe) mixed 1:50 at room temperature for twenty minutes. Using EnVision Flex+ kit from DAKO and diaminobenzidine as the chromophore, staining was done. Mostly, cytoplasm marking was seen, but lesser nuclear stains were also present. The bigger vessels' fibrous connective tissue acted as negative controls, and no stains could be seen there. In order to categorise the specimens into positive (H-scoring upper≥quartiles) and negative (H-scoring upper<quartiles) groups, the upper quartile level of the H-score was predetermined.

Statistical Analysis

IBM SPSS® Statistical edition 22 (IBM® Corp., Armonk, NY, USA) was used for data analysis. Data were presented using means, standard deviations, median, and range. Frequencies and percentages were also used to represent qualitative data. To investigate the relationship between various markers and qualitative factors, chi-squared test or Fisher's exacting test was applied. Kaplan-Meier methodology was used to do survival analysis, and the log-rank testing was used to compare two survival curves. To investigate variables influencing mortality on a univariate analysis, a Cox-proportional hazards regression modelling was used. For estimating risk, the hazard ratios (HR) and 95% confidence level (CI) were considered. A p-value less than 0.05 was regarded as significant.

Results

The current study was done on 91 patients suffering from mesothelioma. In terms of gender, there were 51 males (56%) and 40 females (44%). The patients' mean age was 54.4±10.9 years. The patients'sdemographic and clinical characteristics are shown in Table 1.

ERCC1 expression was tested in all the patients (n 91). RRM1 expression was tested in patients treated with Gemcitabine + cisplatin (n 36) and TS expression

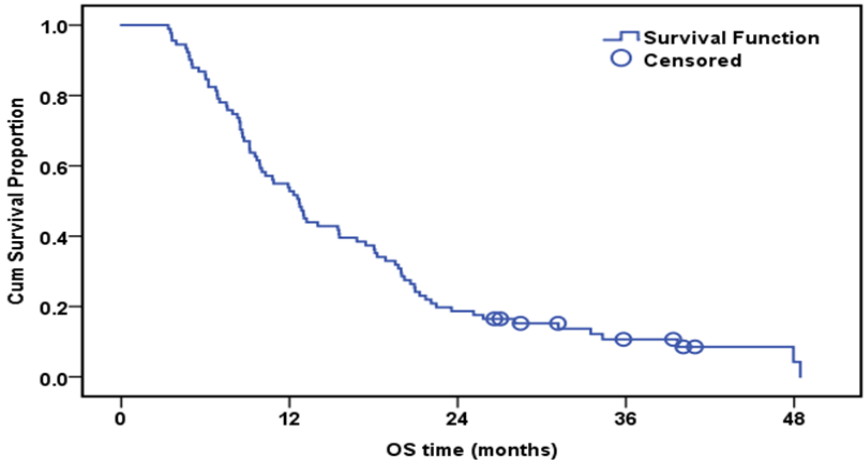

Figure 1. Overall Survival of the Whole Studied Group (n=91)

Table 1. Relation of *ERCC1* Expression with Demographic Characteristics of the Studied Group

		ERCC1 Expression		
		Low	High	p-value
Age (years)	≤ 55	27 (54.0%)	23 (46.0%)	0.974
	> 55	22 (53.7%)	19 (46.3%)	
Sex	Male	25 (49.0%)	26 (51.0%)	0.297
	Female	24 (60.0%)	16 (40.0%)	
Residence	Shobra	13 (61.9%)	8 (38.1%)	0.556
	Helwan	10 (45.5%)	12 (54.5%)	
	Others	26 (54.2%)	22 (45.8%)	
Smoking	Non- or Ex-smoker	30 (58.8%)	21 (41.2%)	0.282
	Current Smoker	19 (47.5%)	21 (52.5%)	
Pathology	Epithelioid	44 (57.1%)	33 (42.9%)	0.139
	Sarcomatoid/biphasic	5 (35.7%)	9 (64.3%)	
Treatment Protocol	GEM	20 (55.6%)	16 (44.4%)	0.791
	Alimta	29 (52.7%)	26 (47.3%)	
Response	Regressive	24 (68.6%)	11 (31.4%)	0.019
	Progressive	13 (36.1%)	23 (63.9%)	
	Stable	12 (60.0%)	8 (40.0%)	

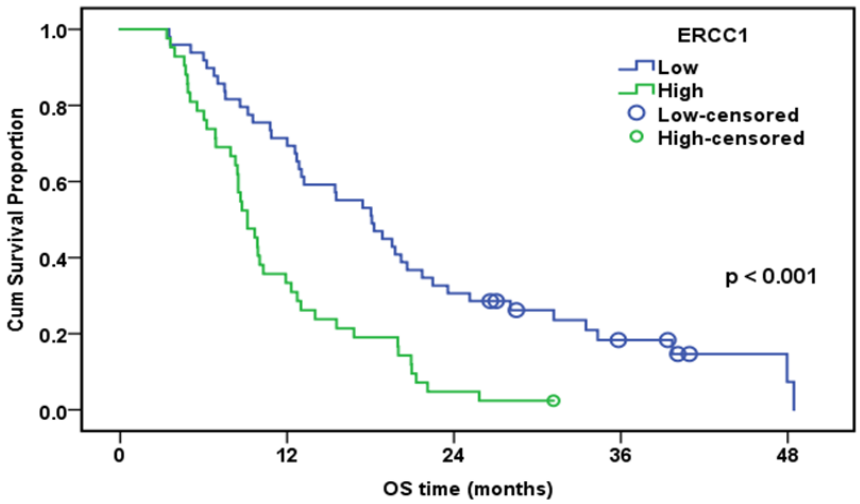

Figure 2. Overall Survival of the Whole Studied Group in Relation to *ERCC1* Expression (n=91)

Table 2. Expression Level of ERCC1, RRM1, and TS in the Studied Group

	Number	Percentage
ERCC1		
Low	49	53.8
High	42	46.2
RRM1 (n=36)		
Low	17	47.2
High	19	52.8
TS (n=55)		
Low	30	54.5
High	25	45.5

was asssed in those treated with Pemetrexed + cisplatin (n 55) (Table 2).

There was a positive association between *ERCC1* expression and TS expression (p=0.005). It was found that 70% of patients with low expression of TS had low expression of *ERCC1*, and 68% of patients with high expression of TS had high expression of *ERCC1*. Howver, there was no relation between *ERCC1* expression and *RRM1* expression (Table 3).

Table 3. Relation of the Expression of *ERCC1* with that of *RRM1* and *TS*

		ERCC1 Expression		
		Low	High	p-value
RRM1 Expression	Low	11 (64.7%)	6 (35.3%)	0.296
	High	9 (47.4%)	10 (52.6%)	
TS Expression	Low	21 (70.0%)	9 (30.0%)	0.005
	High	8 (32.0%)	17 (68.0%)	

Survival analysis

The median follow-up duration was 12.7 months (range: 3.4-48.5). By the end of the follow-up, only eight patients were alive. The cumulative one-year overall survival (OS) of the patinets in both groups was 53.8% (Figure 1, Table 6). The median survival was 12.7 months. High expression of *ERCC1, RRM1*, and *TS* was associated with worsen OS (Table 5, Figure 2).

There was a positive association betweeen *ERCC1* expression and *TS* expression (p=0.005); 70% of patients

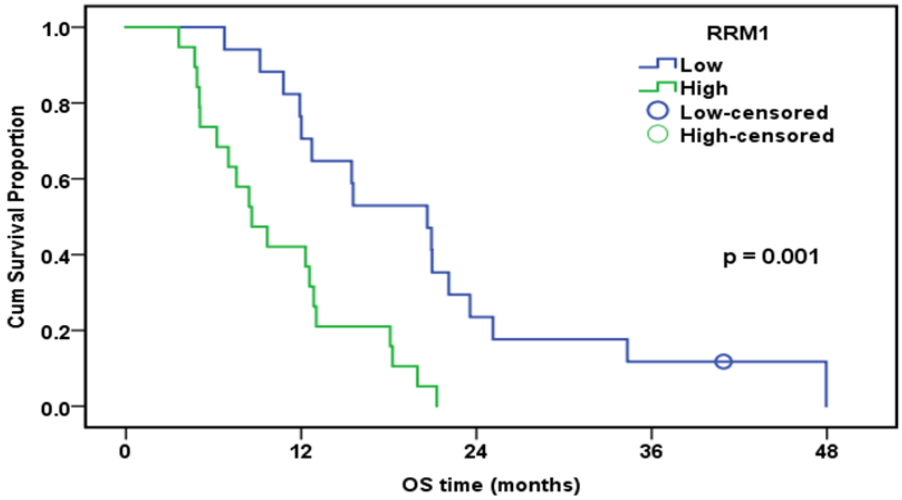

Figure 3. Overall Survival of Patients Treated with GEM in Relation to *RRM1* Expression (n=36)

with low TS had low *ERCC1*, and 68% of high TS was associated with high *ERCC1* (Table 4). On the other hand, there was no relation between *ERCC1* and *RRM1* expression pattern (p= 0.296). In patients underwen platinum-based theraphy (n 91), a significant correlation was detected between low *ERCC1* median High-scoring and longer progression time to (TTP; 9.6 v 5.3 months; P< 0.001) or OS (OS;18.1 v 9.1 months; P<0.001) (Figure 2, 3). A significant correlation was found between low TS protein expression and longer progression time (TTP; 11.8 v 5.4 months; P< 0.001) or OS (OS; 19.8 v 8.5 months; P <0.001) (Figure 4) in patients undergoing pemetrexed plus platinum chemotherapy (n 55) .

Low *RRM1* expression was accompanied by high progression free survival (TTP; 10.6 v 3.8 months) and OS (OS; 20.6 v 8.6 months)in patients receiving gemcitabine plus platinum chemotherapy (Table 7). Based on the multivariate Cox regression analysis, the independent factors significantly affecting OS were tumor stage (HR: 2.3; 95% CI: 1.1-4.9; p= 0.021) and *ERCC1* (HR: 2.7; 95% CI:1.7-4.3; p < 0.001).

Table 4. Relation of *ERCC1, TS* and *RRM1* Expression with Response to Treatment of the Studied Group

	ERCC1 Expression		
	Low	High	p-value
Response			
Regressive	24 (68.6%)	11 (31.4%)	
Progressive	13 (36.1%)	23 (63.9%)	0.019
Stable	12 (60.0%)	8 (40.0%)	
	RRM1 Expression		
	Low	High	p-value
Response			
Regressive	7 (87.5%)	1 (12.5%)	< 0.001
Progressive	2 (11.1%)	16 (88.9%)	
Stable	8 (80.0%)	2 (20.0%)	
	TS Expression		
	Low	High	p-value
Response			
Regressive	16 (59.3%)	11 (40.7%)	0.047
Progressive	6 (33.3%)	12 (66.7%)	
Stable	8 (80.0%)	2 (20.0%)	

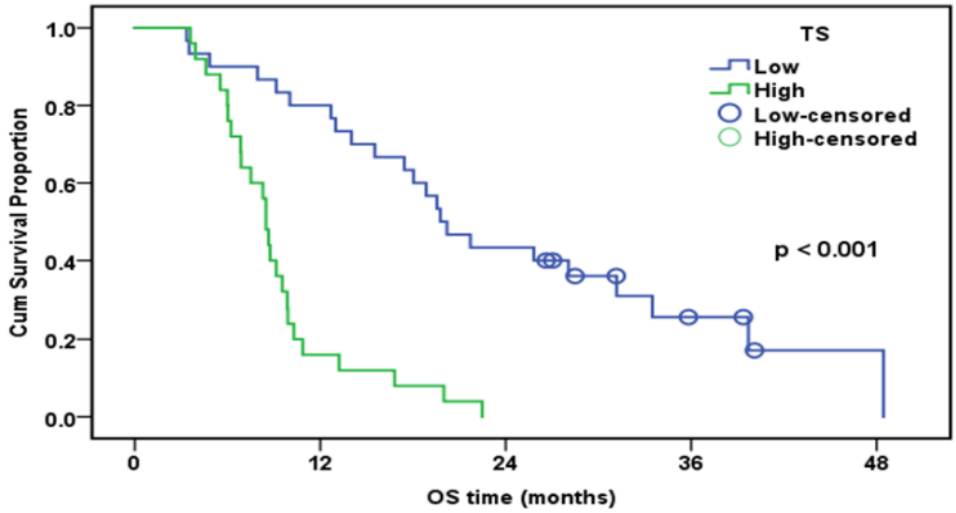

Figure 4. Overall Survival of Patients Treated with Alimta in Relation to *TS* Expression (n=55)

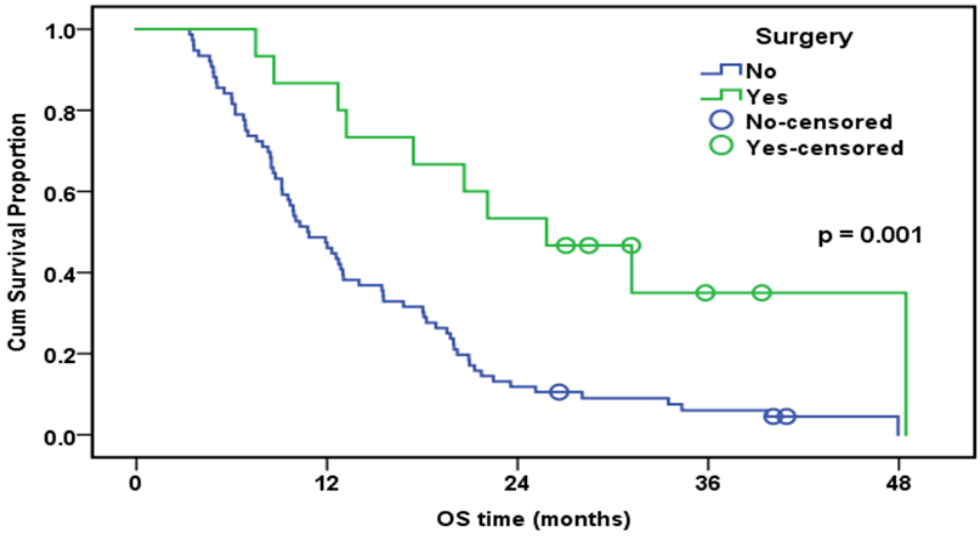

Figure 5. Overall Survival of the Whole Studied Group in Relation to Surgical Treatment (n=91)

Table 5. Overall Survival and Its Relation to the Three Markers *ERCC1, RRM1* and *TS*

	N	No of events	Cumulative survival at one year (%)	Median survival (months)	p-value
Whole group	91	83	53.90%	12.7	
ERCC1					< 0.001
Low	49	42	71.40%	18.1	
High	42	41	33.30%	9.1	
RRM1 (n=36)					0.001
Low	17	16	76.50%	20.6	
High	19	19	42.10%	8.6	
TS (n=55)					< 0.001
Low	30	23	80.00%	19.8	
High	25	25	16.00%	8.5	

Discussion

In the present study, immunohistochemistry was used to detect *ERCC1* expression in 91 MPM patients. It was found that decreased expression of *ERCC1* proteins was associated with improved chemotherapeutic responsiveness, prolonged PFS, and extended OS. High expressions of *ERCC1* was shown to be associated with

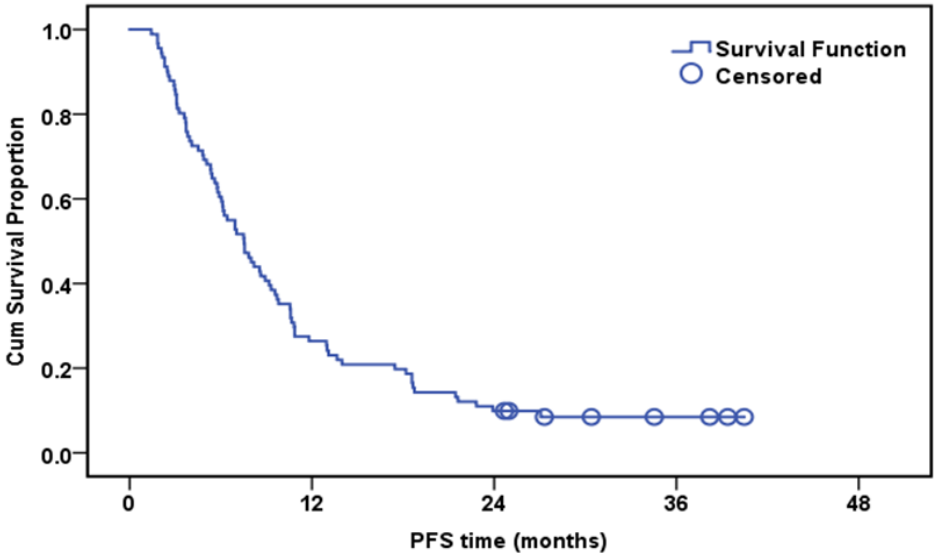

Figure 6. Progression-Free Survival of the whole Studied Group (n=91)

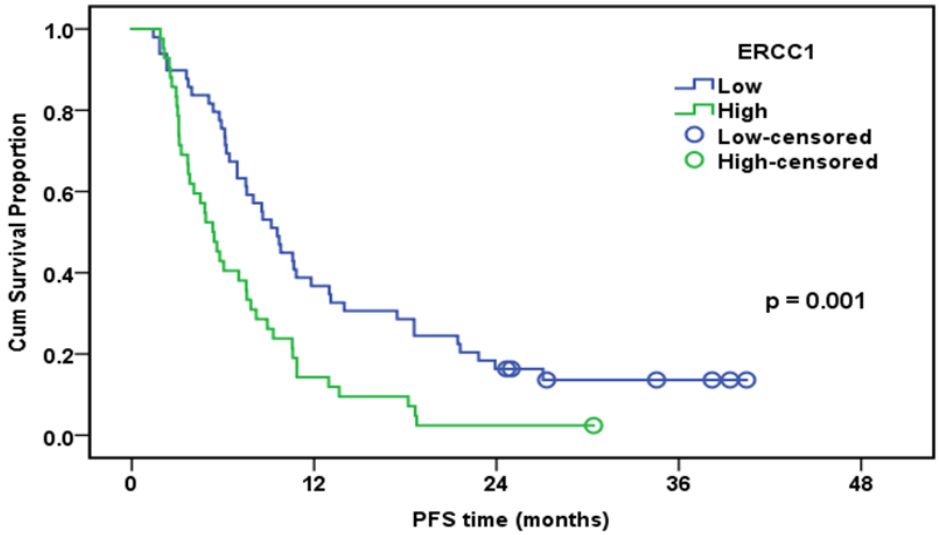

Figure 7. Progression-Free Survival of the whole Studied Group in Relation to *ERCC1* Expression (n=91)

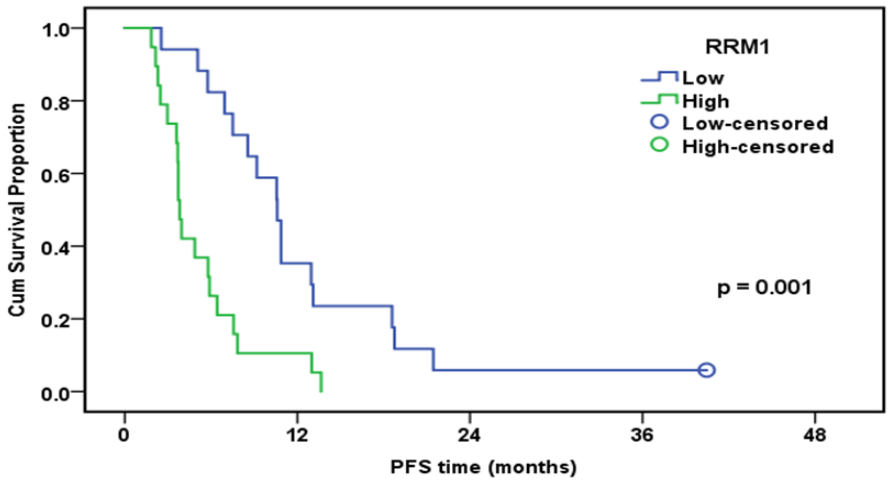

Figure 8. Progression-Free Survival of Patients Treated with GEM in Relation to *RRM1* Expression (n=36)

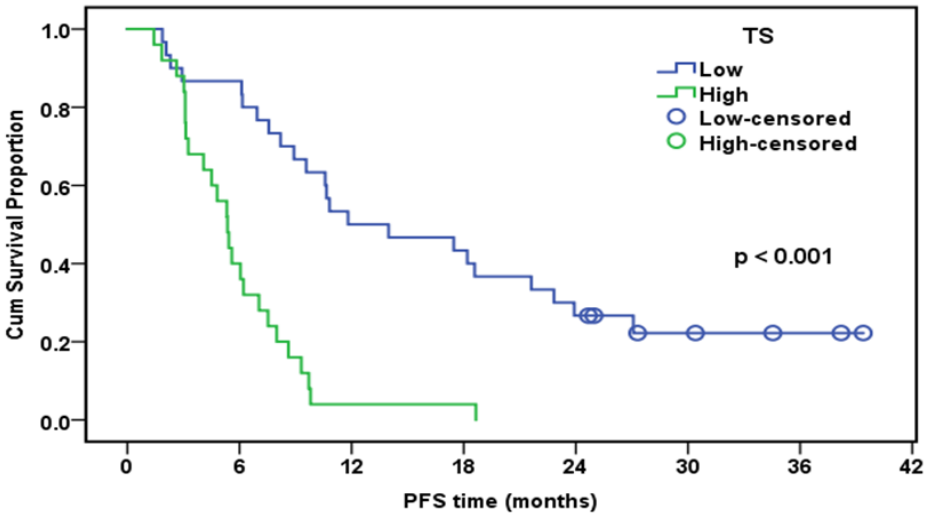

Figure 9. Progression-Free Survival of Patients Treated with Alimta in Relation to *TS* Expression (n=55)

Table 8. Factors Independently Affecting Progression-Free Survival Using Multivariate Analysis

	p-value	HR	95.0% CI for HR	
			Lower	Upper
ERCC1	0.001	2.25	1.42	3.56
Tumor stage	<0.001	6.77	3.02	15.21
Surgery	<0.001	4.53	2.13	9.66
Residence	0.002			
Residence (Helwan vs Shobra)	<0.001	3.71	1.8	7.65
Residence ((Others vs Shobra)	0.075	1.7	0.95	3.04

disease progression (p = 0.019). *ERCC1*- negative tumors had a median PFS of 9.6 months, whereas *ERCC1*-positive tumors had a median PFS of 5.3 months (p=0.001). Multivariate Cox regressions results revealed thatpatients with *ERCC1*-positive tumors had a considerably lesser disease progression than those with *ERCC1*-negative tumors (HR, 2.25; 95 percent CI, 1.42 - 3.56; p =0.001) (Table 8).

According to findings reported by Zimling et al., MPM cases, who received cisplatin-vinorelbin treatment and had low *ERCC1* expressions, had a greater OS. PS and OS were signi

ficantly correlated in this study according to multivariable regression analysis (HR, 2.15; 95%ci, 1.04 - 4.42; p= 0.03). In *ERCC1*-negative tumors group, the median PFS was 10.9 months (95%CI, 5.6 -16.7 months), while it was 6.7 months in *ERCC1*-positive tumor group (95% CI, 4.4 -7.2 months) (p=0.053). In comparison to to *ERCC1*-negative tumor group, multivariable Cox modelling revealed that *ERCC1*-positive patients had a substantially lesser disease progression or lower mortality rate (HR, 2.24; 95% CI, 1.16 - 4.34; p=0.0163) (Zimling

et al, 2012) (Table 8).

In the current research, based on multivariable Cox logistic regression results, tumor staging (HR: 2.3; 95 % CI:1.1-4.9; p=0.021) and *ERCC1* (HR: 2.7; 95 percent CI:1.7-4.3; p<0.001) were found as independent predictors substantially affecting OS. The median follow-up time in this research was 12.7 months (range: 3.4-48.5). Exactly eight of the patients were alive at the end of the follow-up. Collectively, the group's one-year OS rate was 53.8%. Poorer OS was correlated with high expression of *ERCC1*. According to our findings, OS was significantly higher in women, non- or ex-smokers, and patients who underwent surgery (Figure 5). In a nutshell, it seems that patients who have long-term disease survival and are responsive to chemotherapy are more probably to have negative *ERCC1* expression (Figure 7).

Similarlary, Cihan et al., (2014) reported that 36% of patients with negative *ERCC1* expression and 54% of patients with positive *ERCC1* expression had disease progression . With respect to the course of treatment, the mean OS was determined to be 11.7 in patients who had positive *ERCC1* expression, while it was 19.2 in patients with negative *ERCC1* expression, suggesting an association between *ERCC1* expression and disease prognosis.

Immunohistochemistry was used in Zucali et al., (2011)'s study to identify *ERCC1* expression in 67 MPM patients who received pemetrexed and carboplatin treatments. They discovered no correlation between the presence of the ERCC1 proteins and PFS and OS (Figure 6).

Righi et al., (2010) also used immunohistochemistry to identify *ERCC1* expression in 45 MPM cases who received various platinum-based treatments. According to their findings, increased *ERCC1* expression could be a prognostic factor regardless of the chemotherapy regimen. Neverthelese, they reported no correlation between *ERCC1* expression and treatment responsivness. Furthermore, they yielded that patients in the lowest tertile had considerably shorter survival rate. They did not detect a relationship between survival rate and *ERCC1* median-H scoring (HR: 3.06; % CI: 1.08-8.69; p=0.035).

Low TS protein expresion in MPM cases were found to be an indicator of extended TTP and OS by Righi et al. It was discovered that individuals with reduced *TS* expression had greater OS and DFS (Figure 9). On the other hand, they did not discover a significant association between *TS* expression and outcomes in MPM cases who did not receive pemetrexed treatment (Righi et al, 2010).

In the current research, disease progression was noted in 33.3% of patients with low TS protein expressions and in 66.7% of paients with elevated TS protein expression (p= 0.047), revealing a significant association between low *TS* expression and a better treatment response with pemetrexed cisplatin (in 55 individuals).The mean OS in the current research was 12.7 months. The median PFS (11.8 months vs 5.4 months) and OS (19.8 months vs 8.9 months) were both significantly longer in patients with low *TS* expression level (p< 0.001). Lower TS protein expression was significantly correlated with DCR given that disease progression was observed in 33.3% of cases with low *TS* expression (p <0.001). The median OS was 10.9 months in men 18.3 months in women (p =0.004), suggesting gender a significant predictor of OS and PFS.

MPM was managed in 85 patients by using pemetrexed. out of these 85 patients, 75 received pemetrexed together with platinum and 10 received just pemetrexed. Gender did not have any significant effect on TTP or OS in this study. Reduced survivability was associated with non-epithelioid histology, but not with reduced TTP (HR 3.03; 95% CI - 1.64 - 5.62; p< 0.001). IHC was used to determine the degree of *TS* expression. The findings revealed no difference between higher and lower TS concentrations in terms of disease management ratios (p = 0.73). Even though patients with poor TS ratings had statistically higher median TTP (8 months vs. 6 months; p=0.93) and OS (14 mo vs. 11.25 mo; p=0.59) compared to those with greater TS ratings , this difference was not statistically significant (Lustgarten et al., 2013).

Overexpression of *RRM1* has been linked to resistance to gemcitabine, a powerful ribonucleotide reductase inhibitor used in chemotherapy. Higher nuclear RRM1 protein level had a predictive relevance for extended FFR in MPM individuals after platinum/gemcitabine and platinum/pemetrexed standard chemotherapy accompanied by EPP (Jordheim et al., 2011).

Nuclear RRM1 plays a more significant predictive role in the subset of individuals undergoing platinum/gemcitabine induction chemotherapy. Prolonged FFR and OS were both substantially correlated with higher nuclear *RRM1* expressions (Frischknecht et al, 2015).

The current study evaluated the prognostic and predictive values of RRM1in thirty six advanced MPM patients treated with six cycles of gemcitabine and cisplatin. The findings showed a significant correlation between decreased *RRM1* expression and improved therapeutic responses (Figure 8). Additionally, decreased *RRM1* expression was associatedwith prolonged PFS (p= 0.001). Lower *RRM1* expression was associated with a better prognosis, longer PFS, and longer OS.

In conclusion, Lower TS protein expression can be indicative of greater responsivness to pemtrexed and of longer TTP and OS in individuals with advanced MPM (locally progressed or metastatic) who are receiving pemetrexed-based chemotheraphy. Low *ERCC1* expressions in individuals with advanced MPM can predict increased PFS and OS, as well as a better responsivness to platinum-based chemotherapy. In patients with progressed MPM receiving gemcitabine plus cisplatin chemotherapy, lower *RRM1* expression was associated with a better prognosis, longer PFS, and longer OS.

Recommendation

Analysis of ERCC1 protein expression should be done in MPM patients before starting platinum-based chemotherapy. It is recommend to avoid platinum-based regimens if there is high expression of *ERRC1* to avoid resistance to treatment or early disease progression.In these cases, immunotherapy is suggested. Analysis of TS protein expression should be done in MPM patients before starting pemetrexed- based chemotherapy. It is suggested to avoid pemetrexed-based regimens if high expression of

TS was detected to avoid resistance to treatment or early disease progression.

Analysis of *RRM1* expression should be done before gemcitabine-base chemotherapy administration. In case of high expression of RRM1, gemcitabine should be avoided.

Author Contribution Statement

Nagwa Ismail collected the clinical data and tissue specimens and wrote the manuscript. Ola Khorshid supervised overall research, provided, and interpreted data. Fatma abu Alkassem helped in supervision, data analysis, and writing of the manuscript. Abeer Bahnassy provided the pathological assessment and results. Rabab Gaafar designed the research plan and supervised overall research.

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Ethical statement

This study was approved by the ethical committee of national cancer institute Cairo University, Egypt.

Availability of data

The data supporting this study results are available from the corresponding author upon reasonable request.

Conflicts of interest

All authors declare that there is no conflict of interest.

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