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HtrA1, a potential predictor of response to cisplatin-based combination chemotherapy in gastric cancer

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Aims: HtrA1 is a member of the HtrA (high-temperature requirement factor A) family of serine proteases. HtrA1 plays a protective role in various malignancies due to its tumour suppressive properties. The aim of this study was to determine HtrA1 expression as a predictor of chemoresponse in patients with advanced gastric cancer.

Methods and results: HtrA1 expression was determined by immunohistochemistry on specimens of primary gastric cancer from 80 patients treated consecutively with cisplatin-based combination chemotherapy. Response to chemotherapy was assessed according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria. Our population consisted of males/females [51/29; median age 64 years (range 32–82)]. A complete or partial response was observed in 71.4% [95% confidence interval (CI) 54.7–88.2], 66.7% (95% CI 47.8–85.5) and 28.6% (95 CI 11.8–45.3) of tumours showing high, medium and low HtrA1 expression, respectively. A statistically significant association between HtrA1 expression and the clinical response was observed ($P = 0.002$). The median overall survival for patients with high/-medium expression was 17 months compared to 9.5 months for patients with low HtrA1 expression ($P = 0.037$).

Conclusions: Identification of HtrA1 in gastric cancer prior to chemotherapy indicates that levels of HtrA1 could be used to predict response to platinum-based combination therapies. Further assessment of HtrA1 expression is highly warranted in large, prospective studies.

Keywords: chemoresistance, chemotherapy, cisplatin, gastric cancer, HtrA1

Abbreviations: 5-FU, 5-fluorouracil; CDDP, cisplatin; HR, hazard ratio; HtrA, high-temperature requirement factor A; OS, overall survival; RECIST, Response Evaluation Criteria in Solid Tumours; TTP, time to progession

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Introduction

Despite its decreasing incidence, gastric carcinoma is still one of the major causes of cancer death worldwide.[1] Many patients present at diagnosis with unresectable disease[1,2] and patients undergoing pathological R0 resection will also probably relapse. For advanced gastric cancer, evidence supports the use of palliative chemotherapy with the aims of improving symptoms, quality of life and possibly prolonging survival. Several chemotherapeutic agents are considered active in advanced gastric cancer and many combination chemotherapy regimens have been developed in the hope of improving response rate and overall survival. Unfortunately, the benefits of combination chemotherapy have been modest.[3] No globally accepted standard regimen has yet been established. The combination of 5-fluorouracil (5-FU) and cisplatin (CDDP) is generally accepted as the mainstay chemotherapy for gastric cancer patients.[2] Response rates with regimens containing CDDP are in the range of 25–45% and median overall survival rarely exceeds 11 months.[2,4,5] In Europe, 5-FU has been replaced successfully by capecitabine in combination with CDDP,[5] whereas in Japan, S-1, another oral fluoropyrimidine, plus CDDP is the most reasonable first-line standard chemotherapy based on recent randomized studies.[6,7]

Because of the notable toxicity of chemotherapy and the limited survival time for some patients with advanced gastric cancer, it would be useful to select those patients whose tumours will be sensitive to chemotherapy in order to avoid treatment-related toxicity in non-responding patients.

While patients respond initially to CDDP, most patients develop resistance. The mechanisms leading to CDDP resistance include intracellular and extracellular changes that interfere with the ability of DNA damage signals to activate the apoptotic machinery, alter expression of several key apoptotic regulators, promote drug metabolism, decrease cellular drug accumulation and increase repair of DNA adducts.[8–10] At the present time, there are no clinically accepted molecular markers that can predict the sensitivity or resistance of gastric cancer to chemotherapeutic agents.

The high-temperature requirement factor A (HtrA) family of serine proteases was identified initially in *Escherichia coli* by two phenotypes of null mutants that were unable to grow at elevated temperatures,[11] or failed to digest misfolded protein in the periplasm (DegP).[12] Subsequently, homologues of HtrA/DegP have been described in a variety of species, including Gram-negative and -positive bacteria, plants and mammals. Until now, four human homologues of *E. coli* HtrA have been identified: HtrA1 (L56 or PRSS11),[13,14] HtrA2/Omi,[15,16] HtrA3 (PRSP)[17] and HtrA4. The HtrA family of serine protease appears to be involved in several important biological mechanisms in mammals, such as growth, apoptosis, arthritis, embryogenesis, neurodegenerative and neuromuscular disorder and cancer.[18] HtrA1 has a widespread pattern of expression, and its level in human tissues is modulated both in tissues with different physiological activities.[19–21] Data from our group indicate that HtrA1 acts as a tumour suppressor-like factor when overexpressed in cancer cell lines.[22,23] Consistently, meta-analyses of publicly available microarray data from Oncomine.org indicate that HtrA1 is down-regulated and shows allelic imbalance in cancer of diverse origins,[18] and it has been found to be down-regulated by immunohistochemistry in different cancer histotypes.[22,24,25] We have also shown that HtrA1 expression is regulated by chemotherapeutic drugs. Notably, in preliminary studies we have shown that expression of HtrA1 in primary tumours was associated with a better response to CDDP-based combination chemotherapy in ovarian cancer and gastric cancer, acting as an endogenous mediator of CDDP in cancer cells. Indeed, HtrA1 is activated during drug treatment *in vitro*, and active HtrA1 increases caspase 3/7 activity and participates in chemotherapy-induced cytotoxicity.[26]

The aim of the present expanded investigation was to determine whether in metastatic gastric cancer our preliminary findings could be confirmed.

Materials and methods

PATIENTS AND TREATMENT

This was a translational study which included 80 consecutive and unselected patients with recurrent or metastatic gastric cancer who underwent first-line CDDP-based combination chemotherapy at two oncology departments (Pesaro, Urbino, Italy). Patients were enrolled in prospective multi-institutional Phase II studies, where CDDP was used in weekly combination regimens at a dose of 35–40 mg/m^2,[27–29] or in bi-weekly combination regimens at a dose of 50 mg/m^2.[30,31] For all these patients, tumour tissues were available at the Institute of Pathology and fully assessable for immunohistochemical analyses. The medical and pathological reports of these patients were examined in detail for age and gender of the patients. The tumour site, histological subtype, grade, starting date of chemotherapy, first-line regimen used, response

rate to first-line CDDP-based chemotherapy, date of first disease progression and patient survival were analysed.

All radiological studies were reviewed to confirm treatment outcomes and to define clinical response according to Response Evaluation Criteria in Solid Tumours (RECIST) guidelines.[32] In the case of local relapse, objective response was assessed combining findings from both computerised tomography scan of the abdomen and endoscopy, including a new biopsy of the tumour, if still visible, or a biopsy of the area originally involved by the tumour. Patients were followed until the earliest of the following: their date of death, the date they were last known to be alive or the end of the follow-up period on 31 December 2008. Observations were censored at either the date of last known follow-up or the end date of the follow-up period if death had not occurred.

IMMUNOHISTOCHEMISTRY

Tissues from surgical resection specimens of gastric cancer were obtained for each of the 80 patients. A minimum of two and a maximum of four sections for each tumour were analysed. Sections from each specimen were cut at 5 μm, mounted on glass and dried overnight at 37°C. All sections were then deparaffinized in xylene, rehydrated through a graded alcohol series and washed in phosphate-buffered saline (PBS). PBS was used for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. For immunohistochemistry, tissue sections were heated twice in a microwave oven for 5 min each at 700 W in citrate buffer (pH 6) and then processed with the standard streptavidin–biotin–immunoperoxidase method (Dako Universal kit; Dako Corporation, Carpinteria, CA, USA). Anti-HtrA1 polyclonal antibody was used as described previously.[22] Diaminobenzidine was used as the final chromogen and haematoxylin as the nuclear counterstain. Negative control experiments for each tissue section were performed in the absence of the primary antibody. Positive controls included in each experiment consisted of tissue shown previously to express the antigen of interest. Three observers (A.B., P.M. and L.L.), blinded to treatment conditions, evaluated the staining pattern of the proteins separately and quantified HtrA1 expression in each specimen by scanning the entire section and estimating the number of positive cells in a high-power field 10×20 and described as: low (fewer than 1% of positive cells); medium (from 1% to 20% of positive cells); and high (more than 20% of positive cells). The level of concordance for the final scores, expressed as the percentage of agreement between the observers, was 95% (76 of 80 cases). In the remaining four specimens, the score was obtained by consensus review. This protocol of quantification for HtrA1 has been established and applied successfully by our research group in several scientific investigations.[19,22,23,25,26]

STATISTICAL ANALYSIS

The primary endpoint of the present analysis was the association between HtrA1 expression and tumour response. Additional analyses were addressed to time-to-progression (TTP) and overall survival (OS). The association between the expression of HtrA1 expression and chemotherapeutic response was assessed using the χ^2 test, or Fisher's exact test where appropriate. TTP was calculated from the starting date of first-line chemotherapy to the date of progression (per investigator assessment), or death from any cause. OS was calculated from the starting date of first-line chemotherapy until death of any cause, or censored at last follow-up visit. Survival data were analysed using the Kaplan–Meier product–limit method.[33] Comparison of survival curves was performed using the log-rank test. A multivariate analysis using the stepwise Cox proportional hazards regression modelling was performed considering those factors with prognostic significance on univariate analysis. P values <0.05 were considered statistically significant and all P values corresponded to two-sided significance tests. Approval of the study was obtained from the local research and ethics committee.

Results

The characteristics of patients and detailed chemotherapy protocols are shown in Tables 1 and 2. The group consisted of 51 males and 29 females (mean age 64 years; range 32–82 years). All patients underwent total or subtotal gastrectomy. Fifty-nine patients received a gastrectomy with curative intent and 21 a palliative gastrectomy (surgical treatment in the presence of at least one metastatic site of disease). Six patients received adjuvant chemotherapy. Fifty-one patients received CDDP in a weekly schedule of combination chemotherapy,[27–29] and 29 patients received CDDP in a bi-weekly schedule of combination chemotherapy.[30,31] As shown in Table 2, there was no difference in treatment outcomes between the two groups. Following first-line chemotherapy, 15 patients showed a complete response, 29 patients a partial response, 19 patients a stabilization of disease and 17 patients had progression of disease, for an overall

Table 1. Patient characteristics

Characteristic	Overall (*n* = 80)
Sex	
Male/female	51/29
Median age, years (range)	64 (32–82)
Previous surgery	
Partial gastrectomy	35
Total gastrectomy	45
Primary tumor site	
Proximal	14
Body	26
Distal stomach	37
Anastomosis	3
Histology	
Adenocarcinoma	64
Signet ring cell/indifferentiated	16
Lauren classification	
Intestinal type	43
Diffuse type	37
Grading	
Well–moderately differentiated	17
Low differentiated	53
Missing	5
Number of metastatic sites	
1	43
≥2	37
Stage of disease*	
I	3
IIA	11
IIB	12
IIIA	10
IIIB	18
IIIC	5
IV	21
Site of metastatic disease	
Liver	30
Peritoneum	22
Lymph node	37
Lung	2
local relapse	17
Other	18

*According to TNM classification, 7th edn, 2009.

chemotherapeutic response rate of 55.0% [95% confidence interval (CI) 44.1–65.9]. At present, 10 patients are still alive with a median follow-up of 8.6 years (range 5.5–10.6 years).

HTRA1 EXPRESSION ANALYSIS AND RESPONSE

Low HtrA1 expression was identified in 28 (35%) patients, medium HtrA1 expression in 24 (30%) patients and high HtrA1 expression in 28 (35%) patients. Representative intensities of immunoreactivity are shown in Figure 1A–C. There was no significant association between HtrA1 expression and clinicopathological characteristics, such as age, sex, tumour site, Lauren classification, histological grade, vascular or lymphatic invasion, lymph node metastasis (data not shown) and different CDDP-based regimens (Table 2). A complete or partial response was observed in 71.4% (95% CI 54.7–88.2) of tumours that had high HtrA1 expression, in 66.7% (95% CI 47.8–85.5) of tumours with medium HtrA1 expression and in 28.6% (95 CI 11.8–45.3) of tumours with low HtrA1 expression (Table 3). Response to first-line platinum-containing regimens was significantly different between the groups (Table 3). When considering patients with high and medium HtrA1 expression as a group, 69.2% (95% CI 56.6–81.7) of patients with high/medium HtrA1 expression responded to chemotherapy compared to 28.6% (95% CI 11.8–45.3) of patients with low HtrA1 expression ($P = 0.001$). The odds ratio for responders to first-line CDDP-based combination chemotherapy in the cohort with high and medium levels of HtrA1 was 5.62 (95% CI 2.05–15.43; $P = 0.0008$). Clinically determined response rate was not related to any other clinicopathological factors.

HTRA1 EXPRESSION AND SURVIVAL

The median TTP for all patients was 5.8 months (range 2–104 +) and the median survival time among all cases was 12.5 months, with 1- and 2-year overall survival of 53.8% and 26.2%, respectively.

Table 2. Overall response rate, HtrA1 expression, time-to-progression, and overall survival according to first-line cisplatin-based combination chemotherapy

	Weekly cisplatin combination* (n = 51)	Bi-weekly cisplatin combination† (n = 29)	P
Overall response rate, % (confidence interval, 95%)	56.8 (43.3–70.5)	51.7 (33.5–69.9)	0.833
HtrA1 expression (%)			
High	19 (37.2)	9 (31.0)	0.774
Medium	14 (27.5)	10 (34.5)	
Low	18 (35.3)	10 (34.5)	
Time-to-progression (months)	5.5	6.0	0.231
Overall survival (months)	12.2	12.6	0.212

*Weekly cisplatin 40 mg/m^2, epidoxorubicin 35 mg/m^2, 6S-leucovorin 250 mg/m^2, 5-fluorouracil 500 mg/m^2; weekly cisplatin 35 mg/m^2, 6S-leucovorin 250 mg/m^2, 5-fluorouracil 500 mg/m^2.
†Bi-weekly cisplatin 50 mg/m^2 day 1, 6S-leucovorin 100 mg/m^2 followed by 5-flurouracil 400 mg/m^2 bolus and 600 mg/m^2 in a 22-h infusion on days 1 and 2, plus mitomycin C 7 mg/m^2 on day 2 every 6 weeks; bi-weekly cisplatin 50 mg/m^2 day 1, 6S-leucovorin 100 mg/m^2 followed by 5-flurouracil 400 mg/m^2 bolus and 600 mg/m^2 in a 22-h infusion on days 1 and 2, plus pegylated liposomal doxorubicin 20 mg/m^2 on day 2.

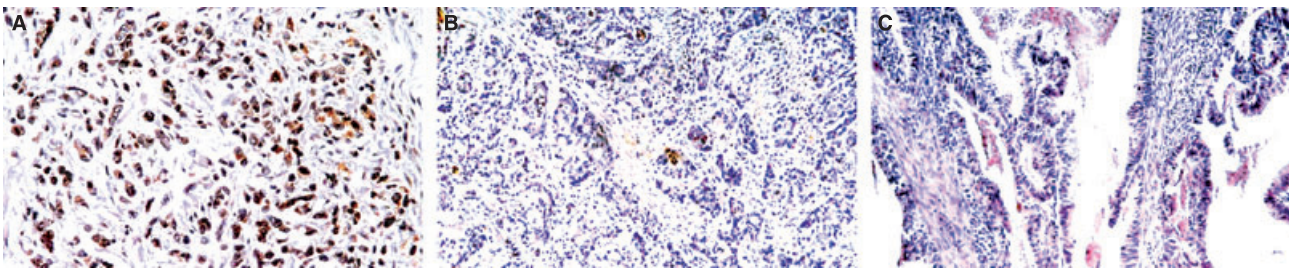

Figure 1. Immunohistochemical analysis of high-temperature requirement factor A1 (HtrA1) expression in primary gastric cancer. **A**, High HtrA1 expression (avidin–biotin complex); **B**, medium HtrA1 expression (avidin–biotin complex); **C**, low HtrA1 expression (avidin–biotin complex).

The secondary endpoint was the association between HtrA1 expression and survival outcomes. Due probably to the relative small sample size, no differences in TTP and OS were found between groups with low, medium and high HtrA1 expression levels (data not shown). However, considering patients with high/medium HtrA1 expression as a group, they showed longer TTP than patients with low HtrA1 expression (7.5 and 4.6 months, respectively). The hazard ratio (HR) for risk of progression for patients with high and medium HtrA1 expression compared to low HtrA1 expression was 0.52 (95% CI 0.29–0.93, P = 0.027) (Figure 2). Similarly, patients with high/medium HtrA1 expression had longer survival than patients with low HtrA1 expression (17.0 and 9.5 months; HR = 0.55; 95% CI 0.32–0.96, P = 0.037). The survival curves of low and high/medium HtrA1 expression are shown in Figure 3.

UNIVARIATE AND MULTIVARIATE ANALYSES

Univariate analysis (Table 4) identified both age and number of metastatic sites as two other variables in addition to HtrA1 expression as being associated with prolonged survival rates. Multivariate regression analysis included the three variables that were found to have prognostic significance on univariate analysis in 80 patients. The Cox proportional regression analysis revealed that only the number of metastatic sites had a significant impact on survival, whereas age and HtrA1 expression were of borderline significance (Table 5).

Discussion

Gastric cancer is still an incurable disease. Despite advances in chemotherapeutic intervention, only

Table 3. Association between the immunohistochemical HtrA1 expression and response rate (RECIST) following first-line cisplatin-based combination chemotherapy in patients with advanced gastric cancer

Response*	Low HtrA1 expression (n = 28)	Medium HtrA1 expression (n = 24)	High HtrA1 expression (n = 28)
Complete response	2	4	9
Partial response	6	12	11
Responders*,% (CI 95%)	28.6 (11.8–45.3)	66.7 (47.8–85.5)	71.4 (54.7–88.2)
Stable disease	11	4	4
Progressive disease	9	4	4
Non-responders	71.4%	33.3%	28.6%

CI, Confidence interval; RECIST, Response Evaluation Criteria in Solid Tumours (Therasse *et al.*)[32].
*Significant different distribution of responders and non-responders according to the HtrA1 status with P = 0.002 (result of the chi-square test with two degrees of freedom).

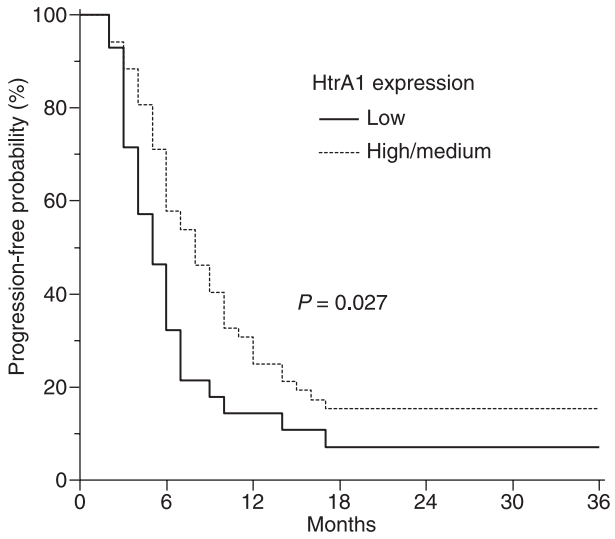

Figure 2. Kaplan–Meier plotting for the time to progression of the 80 patients with gastric adenocarcinoma, stratified according to high-temperature requirement factor A1 (HtrA1) expression.

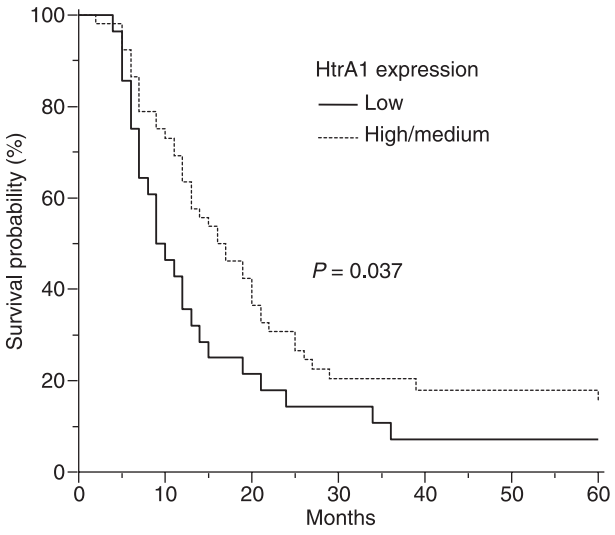

Figure 3. Kaplan–Meier plotting for the cumulative 5-year survival of 80 patients with gastric adenocarcinoma, stratified according to high-temperature requirement factor A1 (HtrA1) expression.

40–45% of the patients receiving palliative chemotherapy may achieve a response. CDDP has a broad range of activity in malignant disease and is used to treat many types of cancer, including gastric cancer. Unfortunately, the response rate to first-line CDDP-based chemotherapy rarely exceeds 45%.[2] The mechanism of resistance to CDDP-based regimens is multifactorial, including decreased drug uptake into the cell, increased drug inactivation and increased DNA repair.[34]

Predictive markers of chemoresponse are potentially useful to select those patients who may respond favourably to CDDP therapy and spare patients who can be predetermined not to respond to such treatment. For example, in colorectal cancer patients, k-ras status is used as a predictive marker of response to epidermal growth factor receptor (EGFR)-targeted agents. For many years researchers have focused attention upon many potentially related molecular markers in determining the response to traditional chemotherapeutic agents. However, due to conflicting results it has not been easy to derive a marker (or a set of markers) of response to a particular treatment. Better and more accurate definition of the biological characteristics of

Table 4. Clinicopathological characteristics and their association with overall survival in 80 patients with metastatic gastric cancer

Variable	*n*	Median survival (months)	*P*-value
Age			
≤64	40	19.0	0.026
<64	40	11.0	
Sex			
Male	51	12.5	0.479
Female	29	13.4	
Lauren classification			
Intestinal type	43	14.3	0.344
Diffuse type	37	11.8	
Invasion			
Yes	43	10.2	0.103
No	37	16.1	
Grading			
Well/moderately differentiated	17	14.4	0.675
Low differentiated	58	12.5	
Primary site			
GEJ/cardias	14	14.0	0.619
Body/distal stomach	63	12.5	
HtrA1 expression			
High/medium	52	16.8	0.037
Low	28	9.5	
Number of metastatic sites			
1	43	15.6	0.008
≥2	37	11.1	
Peritoneal carcinomatosis			
Yes	22	11.5	0.294
No	58	13.8	
Liver metastasis			
Yes	30	11.5	0.920
No	50	13.5	

GEJ, Gastroesophageal junction.

an individual tumour is needed, especially in the new era of molecular agents that target a specific biological pathway that is activated in a certain tumour. Recently, the addition of trastuzumab, a human epidermal growth factor receptor 2 (HER2)-directed drug, to standard chemotherapy has allowed patients to live longer than patients receiving chemotherapy alone.[35] The Phase III ToGA trial enrolled 594 patients whose tumours showed elevated levels of the protein HER2. Patients who received trastuzumab plus chemotherapy (CDDP and capecitabine or 5-FU) had a median survival of 13.8 months, compared with 11.1 months for those who received chemotherapy alone. This translated into a 26% reduction in the rate of death.[35]

Table 5. Cox regression survival analysis of factors predicting survival time of patients with gastric cancers (n = 80)

Variable	Hazard ratio	95% confidence interval	P-value
Age			
≤64 versus >64	1.58	0.97–2.58	0.065
HtrA1 expression			
High/medium versus low	1.54	0.93–2.54	0.089
Number of metastatic sites			
1 versus ≥2	2.53	1.13–5.65	0.024

Trastuzumab is the first targeted drug to improve overall survival for patients with gastric cancer in a Phase III trial. These results, together with those from ongoing Phase III trials including other biological agents, could be improved further in the presence of markers predicting efficacy of chemotherapy, as for CDDP-based therapy. HtrA1 may serve as a surrogate marker of response to CDDP treatment patients with metastatic gastric cancer. The assessment of this marker by immunohistochemistry on gastric tumour samples is able to select nearly two-thirds of patients who respond. By analysing other parameters that may be important for CDDP-based therapy regimens, for example the pathway of nucleotide excision repair[36] and the mismatch repair pathway,[37] it should be possible to individualise therapy by defining a panel of predictive markers based on the combination of different parameters.

The chemotherapeutic response is highly complex, depending on tumour-specific characteristics as well as on constitutional genetic factors of the individual patient. Thus, it is unlikely that only one specific parameter will be found that will predict therapeutic response precisely for all patients. Available data on biological tumour markers are promising, but all of them arise from retrospective studies, generally including a small number of patients. Well-designed, prospective trials are warranted in order to have a validated method of predicting chemosensitivity which can change the therapeutic approach from a general to an individual strategy.

Immunohistochemical evaluation of some markers may provide reproducible reliable information that could guide the therapeutic strategy. Identification of markers to predict chemotherapeutic response and subsequent survival could help to individualize cancer therapy and improve treatment outcomes.

The bacterial serine-protease HtrA, also known as DegP, is a heat shock-induced envelope-associated serine protease.[11,12] HtrA1, a member of the HtrA family of serine proteases, has been characterized recently for its effects on melanoma and ovarian cancer cells as a tumour suppressor-like protein.[22,23] Recent data have also shown that HtrA1 acts as an endogenous modulator of CDDP-induced cytotoxicity.[26]

The aim of our study was to determine if the response to CDDP-based combination treatments is associated with increased HtrA1 expression in gastric cancer. Our results indicate that high or medium HtrA1 expression is correlated significantly with response to first-line CDDP-containing regimens. Nearly 70% of cases with high and medium HtrA1 expression achieved a clinical response (complete or partial response) compared to fewer than 30% of patients whose tumours had low HtrA1 expression. The correlation between HtrA1 levels and response to first-line CDDP-based chemotherapy was of high statistical significance (P = 0.002). The result of our present study encompassing 80 gastric carcinomas compares favourably with our preliminary report on ovarian and gastric cancer patients.[26] In this study we have assessed HtrA1 expression in primary gastric tumours. Given the possible different expression of HtrA1 between primary tumours and metastatic sites, in future studies the assessment of HtrA1 levels from metastatic sites could be an interesting issue for characterizing more clearly the clinical role of this factor.

We have also found that HtrA1 expression predicts prolonged TTP and improved survival among CDDP-treated patients with gastric cancer. The median TTP was 7.5 and 4.6 months (P = 0.027) for the high/medium expression group and low HtrA1 expression group, respectively. Additionally, median overall survival was 17 months for patients whose tumours had high/medium levels of HtrA1 versus 9.5 months for patients with low expression, with a 45% risk reduction of death (P = 0.037). The results of this exploratory study are encouraging, but the limited sample size does not allow any firm conclusion on the prognostic role of HtrA1. Nevertheless, it seems to be a strong candidate for analysis in a large, prospective, independent series.

Our group has demonstrated that HtrA1 protein may modulate CDDP-induced cytotoxity, and that loss of HtrA1 may result in a chemoresistance phenotype.[26] The predictive role of HtrA1 for response to platinum chemotherapy has also been shown in human ovarian cancer cell lines.[38] Additional studies in ovarian cancer has implicated HtrA1 as a predictor of response to platinum-based therapy.[26] The mechanism of how

HtrA1 confers CDDP sensitivity to gastric cancer is not currently understood. However, based on previous reports,[18,26] we anticipate that HtrA1 may function in a serine protease-dependent manner to confer sensitivity to CDDP in gastric cancer. Future studies will focus upon this aspect using gastric cancer cell lines with and without HtrA1 expression. As a comparison, we also assessed p53 expression in the same cohort of patients. Unlike HtrA1, levels of p53 did not correlate with response or survival (data not shown).

In conclusion, our data show that HtrA1 expression is a useful marker for response prediction to CDDP-containing combinations in gastric cancer. Tumours with high and medium HtrA1 expression show a better response to CDDP-based combination chemotherapeutic than tumours with a low HtrA1 value. However, the predictive chemotherapy responsiveness of HtrA1 expression needs further evaluation in the context of large, prospective trials before accepting this marker for routine use.

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