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CyclinD1 and *interleukin-1 receptor antagonist* polymorphisms are associated with prognosis in neoadjuvant-treated gastric carcinoma

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ABSTRACT

Purpose: We evaluated DNA polymorphisms in genes related to DNA repair, cell-cycle control and tumour microenvironment to determine possible associations with response and survival in neoadjuvant-treated gastric cancer patients.

Patients and methods: One hundred and seventy eight patients who received platinum/5FU-based chemotherapy were genotyped for 10 polymorphisms in nine genes (*ERCC1*: Asn118Asn, *C* > *T*; *ERCC1*: 8092C > A; *TP53*: Arg72Pro, *G* < *C*; *cyclinD1*: Pro241Pro, *G* > *A*; *STK15*: Phe31Ile, *A* > *T*; *VEGF*: 936*C* > *T*; *TNF-α*: -308*G* > *A*; *interleukin-1b (IL-1B)*: -511*C* > *T*; *IL-1 receptor antagonist (IL-1RN)*: variable tandem repeat; *IL-8*: -251*T* > *A*). Genotypes were correlated with histopathological and clinical response and overall (OS) and progression-free survival (PFS).

Results: Only the *cyclinD1* genotypes were associated with clinical response ($P_{\chi^2} = 0.044$). Significantly worse survival rates were noted in patients homozygous for the G-allele as compared to patients with the AG or AA genotypes of the *cyclinD1* polymorphism (OS: $P_{log\text{-}rank} = 0.024$; PFS: $P_{log\text{-}rank} = 0.007$) and in patients homozygous for the short allele compared to all other genotypes at the *IL-1RN* polymorphic locus (OS: $P_{log\text{-}rank} = 0.026$; PFS: $P_{log\text{-}rank} = 0.013$). The combination of both unfavourable genotypes demonstrated strong prognostic relevance (OS: $P_{log\text{-}rank} = 0.006$; PFS: $P_{log\text{-}rank} = 0.001$). Multivariate analysis for OS in the group of completely resected patients ($n = 139$) revealed statistical significance for ypM ($P < 0.001$), histopathological response ($P < 0.001$) and the combined *cyclinD1/IL-1RN* genotypes ($P = 0.043$).

Conclusion: The *cyclinD1* and *IL-1RN* polymorphisms were associated with survival. The combination of specific *cyclinD1* and *IL-1RN* genotypes showed a particular prognostic relevance and should be considered an independent prognostic marker for neoadjuvant-treated gastric cancer patients.

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1. Introduction

Multimodal treatment protocols are increasingly employed to improve the survival of advanced gastric cancer patients. In Europe, the current standard treatment is perioperative chemotherapy.[1] However, as only 30–40% of patients respond to therapy, there is an urgent need for diagnostic methods that allow for a more personalised selection of active treatment.

Constitutional genetic variants in tumour and/or therapy-related genes may contribute to the success of chemotherapy, which in gastric cancer is often based on cisplatin and 5-fluorouracil (5FU). Previously, we analysed polymorphisms in genes related to the action of 5FU and found an association between a polymorphism in the promoter region of the *thymidylate synthase* gene, which codes for a key enzyme in 5FU metabolism, and survival in neoadjuvant-treated gastric cancer patients.[2] In addition, a polymorphism in the *glutathione-S-transferase M1* gene, related to the detoxification of cisplatin, was associated with a better prognosis in completely resected patients.[3]

The nucleotide excision repair (NER) pathway is thought to be responsible for the repair of DNA damage induced by cisplatin. Proper repair is in turn dependent on adequate cell-cycle control.[4] DNA polymorphisms with putative functional significance have been reported in the NER gene *ERCC1*, as well as in genes related to cell-cycle control, such as *cyclinD1*, *p53* and *STK15*. Some of these polymorphisms have been associated with a particular chemotherapy response.[4–9]

Increasing evidence suggests that in addition to specific features of tumour cells, the tumour microenvironment is involved in drug resistance.[10] In this context, inflammation is considered important and proinflammatory gene products have been demonstrated to play a role in apoptosis, cell proliferation, angiogenesis, invasion and resistance to chemotherapy.[11] Proinflammatory cytokines, including interleukin-1 (IL-1) proteins, have been shown to modulate cisplatin resistance *in vitro*.[12] IL-1α and IL-1β are two major agonistic proteins whose effects are counterbalanced by the IL-1 receptor antagonist (IL-1RN). Several polymorphisms in the *IL-1* gene complex have been demonstrated to have prognostic relevance for gastric cancer patients receiving palliative chemotherapy.[13] Polymorphisms with putative functional relevance have also been identified in the *tumour necrosis factor alpha* (*TNF-α*) gene, a central mediator of inflammation, as well as in the *IL-8* and *vascular endothelial growth factor* (*VEGF*) genes, both involved in angiogenesis,[14,15] and thus represent candidates that might influence chemotherapy response. However, data demonstrating associations between these polymorphisms and response or survival in neoadjuvant-treated gastric cancer patients are not yet available in the literature.

The goal of our study was to perform a comprehensive analysis of a panel of genetic variants related to DNA repair, cell-cycle control and tumour microenvironment, and to determine whether they might be useful for predicting responses to therapy and/or prognosis in this group of patients.

2. Patients and methods

2.1. Patient characteristics

This analysis was done as a part of consecutive phase II studies conducted in a single centre evaluating preoperative chemotherapy in patients with advanced gastric carcinomas from October 1991 to January 2005.[16] Primary end-point of these trials were the activity and feasibility of the preoperative chemotherapy. The molecular study was done as a retrospective exploratory analysis.

Eligibility requirements included the presence of locally advanced adenocarcinoma (tumour stage T3–4) of the stomach and cardia with or without metastases in local lymph node (Nx), but without peritoneal or distant metastases (M0). Staging procedures included endoscopy, endoluminal ultrasound, laparoscopy and computed tomography (CT) of the chest, abdomen and pelvis. Eligible patients had to be fit for platin-containing chemotherapy and older than 18 years.[16,17] DNA samples from 178 patients receiving more than 50% of the projected dose of chemotherapy were available for analysis. The protocol was reviewed and approved by the local Institutional Review Board and an informed consent was obtained according to institutional regulations.

2.2. Preoperative chemotherapy

All patients received platinum/5FU-based chemotherapy. The preoperative chemotherapy protocol for 131 patients contained two cycles, each consisting of cisplatin (50 mg/m^2 body surface area (BSA)) at weeks 1, 3 and 5, and both leucovorin (500 mg/m^2 BSA) and 5FU (2000 mg/m^2 BSA) at weeks 1, 2, 3, 4, 5 and 6. Additionally, at weeks 1, 3 and 5, 14 patients received paclitaxel (85 mg/m^2 BSA) and 16 patients received docetaxel (40–50 mg/m^2 BSA). Ten patients received epirubicin (30 mg/m^2 BSA) at weeks 2, 4 and 6. In seven patients, cisplatin was replaced by oxaliplatin (85 mg/m^2 BSA).

2.3. Response evaluation and surgical therapy

Response evaluations were performed histopathologically and clinically as previously described.[17,18] In brief, for histopathological response evaluations, all patients with less than 10% viable tumour cells (regression scores 1a and b) were classified as responders. All other patients were classified as non-responders (regression scores 2 and 3).[18] Patients were also classified as non-responders when tumour progression occurred during chemotherapy. Clinical response evaluations were done by measuring the size of the primary tumour by computer tomography scan, endoluminal ultrasound and endoscopy as described.[17] Responders were defined as having at least a 50% reduction in the size of the primary tumour. Surgery was performed as previously described.[17]

2.4. Patient follow-up

After surgical resection, patients were assessed by computer tomography of the chest and abdomen and endoscopy at 3-month intervals during the first year, 6-month intervals

during the second and third years and 12-month intervals during the fourth and fifth years. Follow-up was calculated from the first day of chemotherapy until last contact of the patients. Median follow-up was defined as the time until the survival rate falls below 0.5 and was calculated by the reverse Kaplan–Meier method.

Overall survival (OS) was defined as the time between the first day of chemotherapy and death by any cause. Progression-free survival (PFS) was defined as the time between the first day of chemotherapy and to progressive disease or death from any cause, whichever came first. Median OS and PFS were defined as the time at which the respective survival rate calculated by the Kaplan–Meier method falls below 0.5. No patient was lost to follow-up.

2.5. DNA isolation

DNA was isolated from blood lymphocytes or formalin-fixed, paraffin-embedded non-tumourous tissues as previously described.[2]

2.6. Genotyping

Polymorphisms were selected based on previous associations made with treatment response or survival and on functional characterisations suggesting possible effects related to platinum/5FU-based chemotherapy. The genes and selected polymorphisms as well as the method used for genotyping are shown in Table 1.

PCR reactions were run as 25 μl reaction mixtures consisting of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTP and 0.4 μM of each primer. After initial denaturation at 94 °C for 4 min, 35–40 cycles were performed of 30 s at the indicated annealing temperature and 30 s at 72 °C, followed by a final extension of 7 min at 72 °C. Primer sequences, annealing temperatures and restriction enzymes are included in Table 1.

The *IL-1RN* gene contains a penta-allelic 86 base pair tandem repeat in intron 2, which was analysed by agarose gel electrophoresis. Alleles were conventionally divided into short (s) and long (l) categories. The short category included the allele with two repeats, and the long category was comprised of alleles with three or more repeats, as previously described.[13,19,20]

Optimal conditions for genotyping by RFLP or DHPLC analysis were established using at least 10 samples with known genotypes, previously determined by direct sequencing. DNA sequencing was performed by cycle sequencing with fluorescent-labelled dye terminators and separation with an automated sequencing system (Genetic analyzer 2100, Applied Biosystems). DHPLC was performed with the Wave analyzing system (Transgenomic) as previously described.[21] To allow for a univocal discrimination of the homozygous variants CC and AA of the ERCC1 8092C > A polymorphism, an additional DHPLC run was performed after mixing the sample PCR products with the PCR product of a sample with a known CC genotype. This method allowed for the formation of heterozygotes, which gave a clearly aberrant elution pattern.

2.7. Statistical analysis

Associations between the different genotypes and clinical and histopathological features were tested by the χ^2 test. Survival rates were estimated according to Kaplan–Meier curves. Comparisons between different groups of patients

Table 1 – Primer sequences, annealing temperatures and methods used for polymorphism analysis.

Gene	Polymorphism	db SNP Reference No.	Primer sequences	Temp. (°C)	Method: (restriction enzyme)	Ref.
ERCC1	Asn118Asn 354C>T	rs11615	F: 5′-gcagaggtcacctgaggaac-3′ Re: 5′-gaggtgcaagaagaggtgga-3′	55	RFLP: (BsrDI)	[5]
ERCC1	8092C>A	rs3212986	F: 5′-gggcaccttcagctttcttt-3′ Re: 5′-cagagacagtgccccaagag-3′	62	DHPLC	[9]
TP53	Arg72Pro 215G>C	rs1042522	F: 5′-gactgctcttttcacccatc-3′ Re: 5′-cagcccctcagggcaactga-3′	63	RFLP: (AccII)	[26]
CyclinD1	Pro241Pro 870G>A	rs9344	F: 5′-gtgaagttcatttccaatccgc-3′ Re: 5′-gggacatcaccctcacttac-3′	58	RFLP: (ScrF1)	[27]
STK15	Phe31Ile 91T>A,	rs2273535	F: 5′-ctttcatgaatgccagaaagtt-3′ Re: 5′-ctgggaagaatttgaaggaca-3′	55	RFLP: (ApoI)	[28]
VEGF	936C>T	rs3025039	F: 5′-aaggaagaggagactctgcgc-3′ Re: 5′-tatgtgggtgggtgtgtctacagg-3′	60	RFLP: (NlaIII)	[15]
TNF-α	-308G>A	rs1800629	F: 5′-aggcaataggttttgagggccat-3′ Re: 5′-tcctccctgctccgattccg-3′	60	RFLP: (NcoI)	[29]
IL-1B	-511C>T	rs16944	F: 5′-gcctgaaccctgcataccgt-3′ Re: 5′-gccaatagccctccctgtct-3′	65	RFLP: (AvaI)	[19,30]
IL-1RN	Penta-allelic VNTR	rs2234663	F: 5′-cccctcagcaacactcc-3′ Re: 5′-ggtcagaagggcagaga-3′	62	Agarose gel	[19]
IL-8	-251T>A	rs4073	F: 5′-cactctagtactatatctgtcacatgg-3′ Re: 5′-ccttatcaaatacggagtatgacg-3′	60	RFLP: (Mfel)	[14]

Abbreviations: db SNP, database of single nucleotide polymorphism; Temp., annealing temperature in the PCR reaction; VNTR, variable number of tandem repeats; F, forward; R, reverse; RFLP, restriction fragment length polymorphism; DHPLC, denaturing high pressure liquid chromatography; mod., modifies according to.

were performed with the log-rank test. Relative risks were estimated by calculating hazard ratios (HRs) from Cox proportional hazard models. Prognostic significance of potential parameters was determined by univariate and multivariate analyses. In the multivariate analysis, stepwise selection of the categorical variables was performed on likelihood ratio tests. The ratio of the number of variables to events was limited to 1:10.

For internal validation of the multivariate models, 1000 bootstrap samples were created and stepwise Cox regression analysis was applied to each sample. The relative frequencies of inclusions of the respective factors were calculated.

Discrimination c-indices were computed to quantify the predictive accuracy of the multivariate model by a bootstrap resampling approach. The data were drawn with replacement and used for variable selection and model fitting. The left out samples were used for the computation of the c-index and its' confidence interval.

All statistical testing was two-sided and conducted at the 0.05 significance level. As our study is an exploratory study, no adjustments for multiple tests were performed. SPSS 16.0 software (SPSS Inc., Chicago, IL 11.5) and *R* were used.

3. Results

3.1. Patients, treatment and follow-up

One hundred and seventy eight patients who received preoperative chemotherapy were included in the study. Patient characteristics are summarised in Table 2. One hundred and seventy one patients (96%) could be resected and 139 patients (78%) were completely resected (R0). Regarding histopathological response, there were 51 responders (29%) and 127 non-responders (71%), whereas for clinical response, there were 53 responders (30%) and 125 non-responders (70%). Histopathological and clinical responses were significantly associated with OS and PFS (both $P_{\text{log-rank}} < 0.001$). The median follow-up was 68.1 months (range: 26.5–137.2 months). The median OS was 55.9 months (95% confidence interval (CI), 26.7–85.0 months; range: 3.8–137.3) and the median PFS was 31.4 months (95%CI, 20.2–42.7 months; range: 0.7–137.3). Analysis of the three patient groups treated with the variant chemotherapeutic protocols as listed in Table 2 revealed no association with clinical response ($P_{\chi^2} = 0.659$), histopathological response ($P_{\chi^2} = 0.310$), OS ($P_{\text{log-rank}} = 0.588$) and PFS ($P_{\text{log-rank}}$ $P = 0.99$).

3.2. Allele frequencies and response

The allele frequencies of the polymorphisms are included in Tables 3 and 4.

All allele frequencies were in the Hardy–Weinberg equilibrium.

Only the *cyclinD1* 870G > A variant demonstrated a correlation with clinical response. Heterozygous patients were more frequently found among the responding group (32/53; 60%) as compared to the patients with AA (10/53; 19%) or GG (11/53; 21%) genotypes ($P_{\chi^2} = 0.044$). None of the other polymorphisms demonstrated a statistically significant association with histopathological or clinical response.

Table 2 – Patient characteristics.

	No. of patients	%
Preoperative characteristics		
Total	178	100
Age ± standard deviation	55.9 ± 10.3	
Range	23.1–77.2	
Sex		
Female	46	26
Male	132	74
Localisation		
Proximal third	117	65
Middle third	35	20
Distal third	14	8
Linitis	12	7
Laurén classification		
Intestinal	74	41
Non-intestinal	104	59
Grading		
G1/2	28	16
G3/4	150	84
Response		
Histopath. responder	51	29
Histopath. non-responder	127	71
Clinical responder	53	30
Clinical non-responder	125	70
Chemotherapy regimens		
E-PLF	10	6
P-PLF, D-PLF	30	17
PLF, OLF	138	77
Postoperative characteristics		
Resection	171/178	96
R-category		
R0	139/171	81
R1/2	32/171	19
ypT-category		
ypT0	13/171	8
ypT1	12/171	7
ypT2	103/171	60
ypT3	38/171	22
ypT4	5/171	3
ypN-category		
ypN0	70/171	41
ypN1	59/171	34
ypN2	29/171	17
ypN3	13/171	8
ypM-category		
ypM0	135/171	79
ypM1	36/171	21

Abbreviations: E-PLF, epirubicin, cisplatin, leucovorin, 5FU; P, paclitaxel; D, docetaxel; O, oxaliplatin.

3.3. Genotypes and survival

3.3.1. DNA repair and cell-cycle control associated genes

Statistically significant worse OS and PFS rates were found for patients homozygous for the G-allele of the *cyclinD1* 870G > A polymorphism as compared to the patients carrying the A-allele (AA and AG). The median survival of the patients with

the respective genotypes was 31.97 months (95%CI 23.8–40.1 months) versus 87.8 months (95%CI, 44.7–131.0 months) for OS ($P_{log\text{-}rank}$ = 0.024) and 16.2 months (95%CI, 4.6–27.8 months) versus 39.4 months (95%CI, 15.9–62.9 months) for PFS ($P_{log\text{-}rank}$ = 0.007) (Fig. 1A and B). An analysis of an association of these genotypes with OS was also performed for the completely resected patients (n = 139), but statistical significance was not achieved in this subgroup ($P_{log\text{-}rank}$ = 0.145).

No other significant associations were observed. The results of univariate Cox-regression analyses and relative risks with the 95%CI for the whole study group are summarised in Table 3.

3.3.2. *Genes related to the tumour microenvironment*

In this group of genes, worse OS and PFS were observed in patients homozygous for the short allele of the *IL-1RN* polymorphism as compared to the patients with other genotypes. The median survival of the patients with the respective genotypes was 32.1 months (95%CI, 31.2–32.9 months) versus 74.1 months (95%CI, 46.8–101.4 months) for OS ($P_{log\text{-}rank}$ = 0.026) and 14.2 months (95%CI, 0.2–28.2 months) versus 37.7 months (95%CI, 21.8–53.6 months) for PFS ($P_{log\text{-}rank}$ = 0.013) (Fig. 1C and D). In the subgroup of R0 resected patients, these genotypes demonstrated no statistical significance (OS: $P_{log\text{-}rank}$ = 0.07).

No significant associations were found for the polymorphisms in the *IL-1β*, *TNF-α*, *VEGF* or *IL-8* genes. The results of univariate Cox-regression analyses and relative risks with the 95%CI of the polymorphisms are summarised in Table 4.

3.3.3. *Combined analysis of* **cyclinD1 and IL-1RN genotypes**

Combining the most adverse prognostic genotypes of the *cyclinD1* and *IL-1RN* polymorphisms revealed a strong, statistically significant association with OS and PFS. Patients homozygous for the G-allele of the *cyclinD1* 870A > G polymorphism or homozygous for the short allele of the *IL-1RN* gene demonstrated far worse survival as compared to the patients carrying any other genotype. The median OS was 32.3 months (95%CI, 26.7–37.9 months) versus 87.9 months (95%CI, 41.9–133.9 months) ($P_{log\text{-}rank}$ = 0.006) and the median PFS was 16.7 months (95%CI, 8.5–24.9 months) versus 47.5 months (95%CI, 20.2–42.7 months) ($P_{log\text{-}rank}$ = 0.001) (Fig. 1E and F). The relative risks with the 95%CI of univariate Cox regression analysis are included in Table 4. Analysis of this genotype combination for an association with OS in completely resected patients confirmed statistical significance ($P_{log\text{-}rank}$ = 0.022).

3.3.4. *Multivariate analysis*

Multivariate Cox-regression analyses included the established clinical variables with prognostic significance for OS and PFS namely, ypT, ypN, ypM and R-category and histopathological and clinical response (all P-values < 0.001). In addition, the genotypes of the single polymorphisms of *cyclinD1* and *IL-1RN* and the combination of the *cyclinD1/IL-1RN*

Table 3 – Allele frequencies of polymorphisms in genes related to DNA repair and cell-cycle control and their associations with survival.

Genes and genotypes	No. of patients (n = 178) n (%)	Overall survival			Progression-free survival		
		HR	95% CI	P-value	HR	95% CI	P-value
ERCC1 Asn118Asn[a]							
CC	24 (13)	1		0.200	1		0.412
CT	88 (50)	0.72	0.40–1.31		0.78	0.44–1.37	
TT	65 (37)	1.07	0.59–1.95		1.01	0.57–1.81	
ERCC1 8092C > A[a]							
AA	10 (6)	1		0.294	1		0.125
AC	75 (42)	0.56	0.25–1.26		0.47	0.22–1.01	
CC	92 (52)	0.71	0.32–1.56		0.61	0.29–1.27	
AC + CC	167 (94)	0.64	0.29–1.38	0.254	0.54	0.26–1.12	0.099
TP53 Arg72Pro[b]							
GG	89 (50)	1		0.986	1		0.776
GC	67 (38)	0.98	0.63–1.50		0.92	0.61–1.38	
CC	15 (8)	0.95	0.45–2.00		1.16	0.61–2.22	
STK15 Phe31Ile[a]							
TT	116 (65)	1		0.313	1		0.284
TA	51 (29)	0.77	0.48–1.23		0.90	0.59–1.37	
AA	10 (6)	0.50	0.16–1.59		0.40	0.13–1.28	
TA + AA	61 (35)	0.73	0.47–1.14	0.163	0.81	0.53–1.22	0.302
CyclinD1 Pro241Pro							
GG	49 (28)	1		0.079	1		0.024
GA	82 (46)	0.63	0.40–1.01		0.62	0.40–0.95	
AA	47 (26)	0.58	0.34–1.01		0.52	0.31–0.87	
GA + AA	129 (72)	0.61	0.40–0.94	0.025	0.58	0.39–0.86	0.008

Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Successful amplification of DNA from 177/178 patients.
[b] Successful amplification of DNA from 171/178 patients.

Table 4 – Allele frequencies of polymorphisms in genes related to the tumour microenvironment and their associations with survival.

Genes and genotype	No. of patients (n = 178) n (%)	Overall survival			Progression-free survival		
		HR	95% CI	P-value	HR	95% CI	P-value
VEGF 936C > T							
CC	136 (76)	1		0.477	1		0.510
CT	37 (21)	0.93	0.56–1.54		0.93	0.58–1.48	
TT	5 (3)	1.80	0.66–4.95		1.74	0.64–4.75	
CT + TT	42 (24)	1.01	0.63–1.62	0.956	1	0.64–1.55	0.989
TNF-α –308G > A							
GG	132 (74)	1		0.975	1		0.811
GA	44 (25)	1.05	0.67–1.67		1.04	0.67–1.60	
AA	2 (1)	–	–		1.58	0.39–6.43	
GA + AA	46 26)	0.96	0.61–1.51	0.850	1.06	0.70–1.62	0.778
IL-8 –251T > A							
TT	55 (31)	1		0.146	1		0.326
TA	91 (51)	1.51	0.93–2.44		1.34	0.86–2.08	
AA	32 (18)	1.00	0.53–1.91		1.00	0.56–1.80	
IL-1RN[a]							
s/s	16 (9)	1		0.065	1		0.053
l/s	61 (34)	0.59	0.31–1.12		0.50	0.27–0.94	
l/l	100 (57)	0.48	0.26–0.89		0.49	0.27–0.89	
l/l + l/s	161 (91)	0.52	0.29–0.94	0.029	0.50	0.28–0.88	0.015
IL-1B –511C > T							
TT	21 (12)	1		0.142	1		0.096
CT	85 (48)	0.55	0.31–1.00		0.59	0.33–1.06	
CC	72 (40)	0.69	0.38–1.25		0.86	0.48–1.53	
CC + CT	157 (88)	0.61	0.35–1.06	0.082	0.71	0.41–1.22	0.209
CyclinD1 and IL-1RN							
Other genotypes	116 (65)	1		0.007	1		0.001
GG (*cyclinD1*) or s/s (*IL-1RN*)	62 (35)	1.75	1.17–2.64		1.9	1.29–2.78	

Abbreviations: HR, hazard ratio; CI: confidence interval; s, short alleles; l, long alleles.
[a] Successful amplification of DNA from 177/178 patients.

genotypes, with the most significant associations with OS and PFS in univariate analysis were included. The major result was that the combined *cyclinD1/IL-1RN* genotypes were identified as an independent prognostic markers for OS (P = 0.023) and PFS (P = 0.004). Results and relative risks with the 95%CI are summarised in Table 5. The inclusion frequencies of the first five variables calculated by the bootstrap analysis for OS were ypM 96.9%, histopathological response 92.7%, clinical response 50.5%, combined *cyclinD1/IL-1RN* genotypes 42.8%, R0 category 42.5%. Regarding PFS they were ypM 98.1%, histopathological response 92.7%, R0 category 88.3%, combined *cyclinD1/IL-1RN* genotypes 72.0%, ypN 33.9%. The discrimination c-indices were 0.737 (95%CI, 0.664–0.806) for OS and 0.748 (95%CI, 0.677–0.815) for PFS.

Multivariate analysis for OS performed in the group of the completely resected patients identified the combined *cyclinD1/IL-1RN* genotypes as an independent prognostic factor (P = 0.043, HR 1.73, 95%CI 1.02–2.94) after ypM (P < 0.001, HR 3.88, 95%CI 1.93–7.79), histopathological response (P < 0.001, HR 4.57, 95%CI 2.15–9.73). Inclusion frequencies in the bootstrap analysis were ypM 80.1%, histopathological response 77.1%, ypT 39.1%, *IL-1RN* polymorphism 39.1%, combined *cyclinD1/IL-1RN* genotypes 37.7%. The c-index was 0.671 (95%CI, 0.575–0.761).

4. Discussion

In this study, we analysed a panel of polymorphisms in DNA repair and cell cycle-associated genes as well as genes related to the tumour microenvironment for associations with response and survival of advanced gastric cancer patients treated with platinum/5FU-based neoadjuvant chemotherapy. The most interesting result of our analysis was that the *cyclinD1* and *IL-1RN* polymorphisms each demonstrated a prognostic relevance, allowing us for the first time to identify a specific pattern of genotypes encompassing both polymorphisms as an independent prognostic marker in neoadjuvant-treated gastric cancer patients.

Determination of the combination of these genotypes might be very useful for the classification of patients into different risk groups with specific therapeutic consequences.

We found a significant relationship between worse rates of survival and patients homozygous for the short allele of the *IL-1RN* polymorphism, as compared to patients carrying at

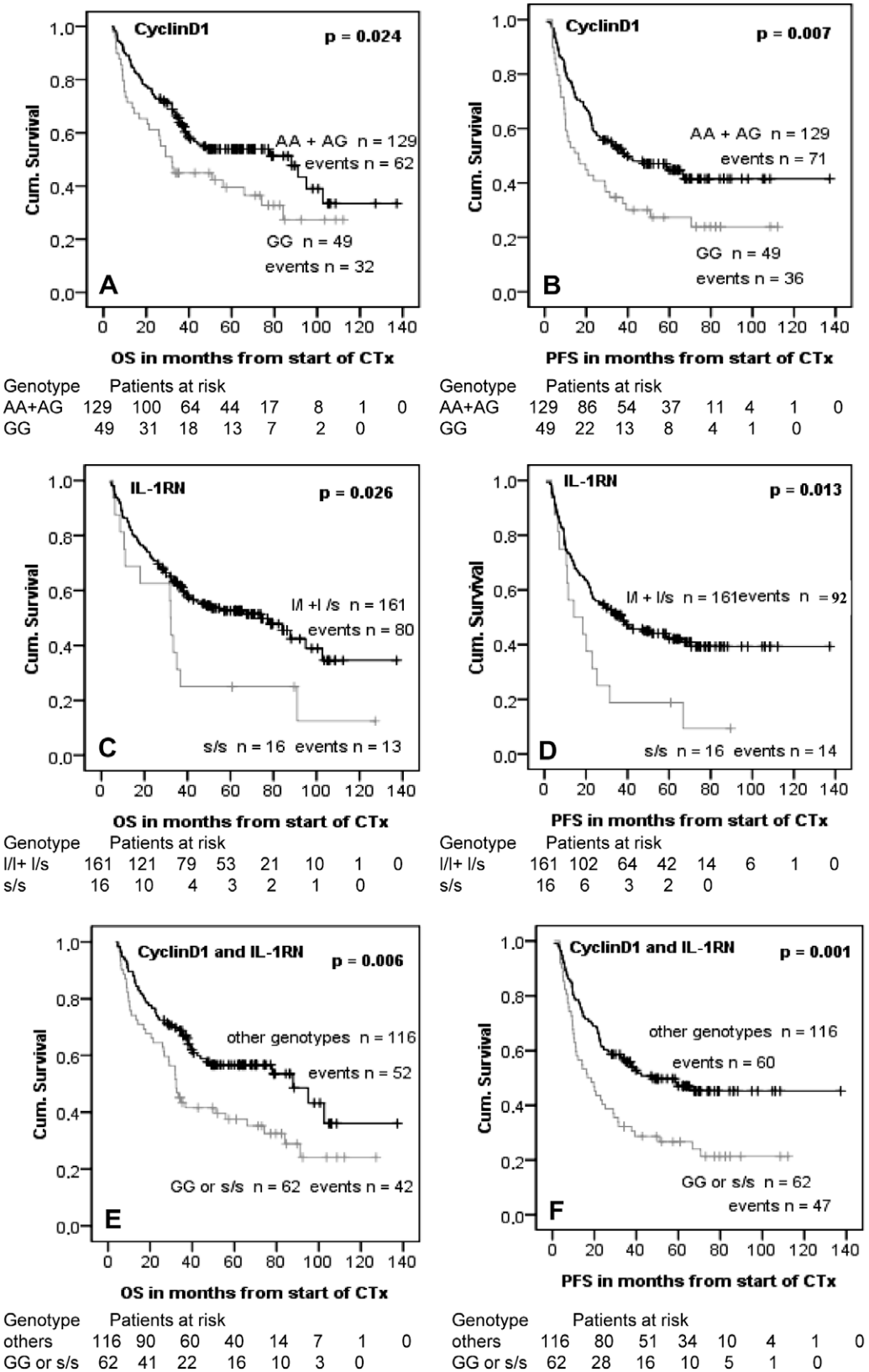

Fig. 1 – Survival curves with respect to polymorphic genotypes. The numbers of events during the period and the number of patients at risk after each 20-month interval are indicated. *cyclinD1* genotypes: (A) overall and (B) progression-free survival; *IL-1RN* genotypes: (C) overall and (D) progression-free survival; *cyclinD1/IL-1RN* genotypes: (E) overall and (F) progression-free survival.

Table 5 – Multivariate Cox-regression analysis and overall- and progression-free survival.[a].

Factor	Overall survival $n = 178$			Progression-free survival $n = 178$		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ypM-category						
ypM0	1		<0.001	1		<0.001
ypM1	4.7	2.97–7.49		4.20	2.53–6.96	
Histo. responder						
Responder	1		<0.001	1		<0.001
Non-responder	5.2	2.54–11.03		3.75	2.03–6.95	
R-category						
R0	–			1		0.001
R1/2				2.39	1.46–3.91	
CyclinD1 and IL-1RN						
Other genotypes	1		0.023	1		0.004
GG (*cyclind1*) or s/s (*IL-1RN*)	1.65	1.07–2.54		1.81	1.21–2.71	

Abbreviations: HR, hazard ratio; CI, confidence interval; M, metastasis; Histo., histopathological; R, resection.

[a] Included parameters are ypT-,ypN-, ypM-, and R-category, histopathological and clinical response, *IL-1 RN* (s/s versus l/l and l/s), *cyclinD1* (GG versus AA and GA) and *cyclinD1* (GG) or *IL-1 RN* (s/s) versus the other genotypes.

least one long allele. Few data on the prognostic relevance of *IL-1* polymorphisms are available in the literature, though associations with the survival of gastric cancer patients treated with palliative chemotherapy have indeed been reported for the *IL-1RN* and *IL-1β* gene polymorphisms. However, direct comparison with our study is complicated, as grouping of the patients according to various genotypes was different and an additional polymorphism in the *IL-1β* gene was analysed in this study.[13] The short allele of the *IL-1RN* polymorphism has been linked to enhanced IL-1β production and thus is assumed to favour inflammation, although the data regarding this effect are somewhat controversial.[22,23] The association detected in our study of this *IL-1RN* genotype with a worse prognosis is reminiscent of the results of several reports demonstrating that proinflammatory genotypes of *IL-1* variants are associated with increased gastric cancer risk.[19,20] Our data thus support and contribute to the broader concept that inflammation is critical for tumour progression and may also have a strong impact on patient prognosis.[11]

Regarding the *cyclinD1* polymorphism, patients homozygous for the G-allele had a significantly worse prognosis as compared to the patients carrying an A-allele. The cyclinD1 protein has an important regulatory function in cell cycle progression. The 870G > A polymorphism in the gene is located at the conserved splice donor site of exon 4/intron 4 and leads to an alternatively spliced transcript that lacks exon 5. This protein product demonstrated a prolonged half-life in the nucleus and as a consequence is assumed to promote accelerated cell cycle progression and increased proliferation,[24] which would in turn suggest an association of the A-allele with worse prognosis. In a study of primarily resected adenocarcinomas of the oesophagus, the A-allele was shown to be associated with worse prognosis,[25] in contrast to our results. The reasons for this discrepancy may be manifold, but one of the most obvious differences between the two studies lies in the patient population, as the previous study analysed primarily resected patients with adenocarcinoma of the oesophagus,[25] whereas we studied gastric cancer patients treated with a platinum/5FU-based neoadjuvant chemotherapy. The association of the A-allele with better survival seen in our study might derive from the possibility that the effects of a DNA-damaging agent such as cisplatin might be more profound and might more easily lead to cell death in the context of increased cell cycle progression than in a more normal cell cycle pattern. Our results are similar to those from a study of rectal cancer patients treated with neoadjuvant radiotherapy, in which an association of the A-allele with response and better prognosis was detected.[6]

In our study, the polymorphisms in the *ERCC1* gene did not demonstrate a significant association with response or survival. These results are similar to a study of gastric cancer patients treated with a 5FU/cisplatin palliative chemotherapy, where no significant associations between various polymorphisms in *NER* genes and the clinical outcomes of the patients were found.[8]

Considering our results with respect to histopathological or clinical response, only a weak association between the *cyclinD1* polymorphism and clinical response was found. In light of the prognostic relevance of the *cyclinD1* and *IL-1RN* polymorphisms, the lack of an association with response was surprising, and suggests that the observed prognostic impact is independent of the chemotherapy and instead refers primarily to the genetic constitution of the patient. However, the chemotherapeutic agents may also exert some specific effects on the molecular or cellular level that do not necessarily translate into measurable tumour shrinkage.

Our study has some limitations. First, from a statistical perspective, multiple tests have been performed. However, our study has to be considered as an exploratory study and therefore no corrections for multiple testing have been performed. Internal validation of the multivariate Cox regression model by bootstrap analysis demonstrated a moderate inclusion frequency of the combined *cyclinD1/IL-1RN* genotypes regarding OS in the whole study and in the group of the completely resected patients (42.8% and 37.7%, respectively). The genotype combination, however, was chosen at a relatively

high frequency of 72% for PFS, which points to a more important role of the genotype combination for tumour progression. The discrimination c-indices of all models were in the range between 0.671 and 0.748. As the C-index of the model can range between 0.5, which indicates random choice, and 1.0, which represents perfect discrimination, our results indicate fairly good discrimination performance. However, analysis of the relevant results in a prospective study is important and necessary to validate the data. Second, the DNA samples used in this study were from 178 patients demonstrating a relatively long median survival of 55.9 months. This may reflect a preferential inclusion of surviving patients at the beginning of our study. One strength of our study is the comprehensive analysis of a panel of polymorphisms encompassing different functional groups of genes in a relatively high number of quite homogeneously treated gastric cancer patients. The thorough documentation of the clinical and histopathological data of the patients allowed the analysis to be made with respect to OS and PFS, and permitted the detailed determination of the prognostic relevance of the analysed polymorphisms.

In conclusion, our study shows for the first time a prognostic relevance of the *cyclinD1* and *IL-1RN* polymorphisms for neoadjuvant-treated gastric cancer patients. In the future, analysis of these polymorphisms could substantially contribute to patient-specific, tailored therapies.

Conflict of interest statement

None declared.

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