Syndecan-1 expression is an independent favourable prognostic marker in oesophageal adenocarcinoma and represents a potential therapeutic target

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Abstract. Because of the poor prognosis of oesophageal adenocarcinoma (EAC), there is an urgent need for additional therapeutic approaches. Syndecan-1 (CD138) is a cell-surface heparan sulphate that is overexpressed in multiple myeloma cells and several carcinomas. Specific drugs targeting CD138 [e.g. antibody-linked drug conjugates (ADCs)] are currently being assessed; however, the significance and implication of CD138 expression in EAC is mostly unknown. In the present study, CD138 expression was assessed using immunohistochemistry, and its association with histopathological parameters and *ERBB2* (Her2/neu) amplification status in patients treated with primary resection and neoadjuvant (radio-)chemotherapy was investigated. Of the 723 cases of EAC included, 232 tumours (32.1%) expressed CD138, with 96 tumours displaying strong expression (13.3%). Patients with CD138-positive tumours had less invasive carcinoma, fewer lymph mode metastases and a significantly longer overall survival (OS) than patients with CD138-negative tumours (P=0.002). In multivariate analysis, strong CD138 expression was an independent favourable prognostic factor (P=0.02). Patients who received neoadjuvant CROSS (carboplatin, paclitaxel and intensity modulated radiotherapy) therapy and had CD138-positive tumours lived significantly longer (P=0.04). In tumours without Her2/neu amplification, CD138 expression was associated with a longer OS (P=0.02). In conclusion, CD138 in cancer is already used as a target for ADCs, such as indatuximab ravtansine, the effectiveness of which depends on the extent of CD138 on tumour cells. This indicates that CD138 is also a predictive, therapeutically relevant biomarker. Regardless of the favourable prognostic effect of CD138 in EAC, there is an urgent clinical need for personalized therapeutics in relapse. Future clinical trials now need to show how effective the corresponding ADCs are in CD138-positive EACs.

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Introduction

Oesophageal cancer is the seventh most common cancer globally, responsible for 1 in 18 cancer-related deaths and over 500,000 deaths every year (1). The two most common histological subtypes are squamous cell carcinoma and adenocarcinoma (2). Squamous cell carcinoma is the predominant type worldwide but the incidence of oesophageal adenocarcinoma (EAC) exceeds that of squamous cell carcinoma in higher-income countries (3). The 5-year survival rate of EAC is approximately 20% (4,5).

Curative treatment intents can only be performed for operable patients. For inoperable patients chemo-(radio)-therapy alone is the preferred treatment option (6). All operable EAC patients should be treated with neoadjuvant chemotherapy or chemoradiotherapy, before surgery if possible since it improves survival significantly (7,8). Therapy with a combination of cisplatin and fluorouracil the anti-HER2 monoclonal antibody trastuzumab has also been shown to be efficient in HER2-positive advanced disease (9). In addition, patients with PD-L1-positive oesophageal cancer who were treated with pembrolizumab or nivolumab had promising results. The prognosis of our patients could be improved by the introduction of neoadjuvant therapies. Today, about 20-25% of operable patients are still alive five years after primary diagnosis (10,11). However, the majority of patients die as a result of their tumour disease due to (late) relapses. The chemotherapies then available are only therapeutically effective to a very limited extent. Primarily inoperable patients or (palliative) patients with hematogenous metastases show a

disastrous prognosis, only very few patients survive five years. According to national guidelines, patients who have received neoadjuvant CROSS and have no vital tumour postoperatively do not receive further adjuvant therapy. Despite improvements described above there is still a great need for therapies in the recurrent/metastatic situation. Syndecan-1 (CD138) is cell-surface heparan sulphate (12), one of four members of the syndecan family, and includes 288 amino acids which make it the second largest among (13). Syndecans play a role in adjusting cell-cell and cell-matrix interactions (14). They also play roles such as modulation of cell proliferation and invasive growth (15,16). CD138 is physiologically expressed on plasma cells and various epithelial cells (17). CD138 expression is best known to be highly specific for multiple myeloma (18). With that said, CD138 overexpression is also reported in various carcinomas such as breast, pancreatic, gallbladder, endometrial, ovarian, prostate and urinary bladder cancers (14).

Specific drugs targeting CD138 have recently been assessed in various tumours. For instance, indatuximab ravtansine, a monoclonal antibody-linked cytotoxic agent that targets CD138, is reported to be very efficient on multiple myeloma (19,20). This antibody-drug conjugate (ADC) uses the CD138 binding site on the cancer cell to hijack the cell and place the actual therapeutic agent in the tumour cell in a targeted and highly concentrated manner. The same principle of ADC is successfully used with trastuzumab deruxtecan (target protein Her2/neu) and also with sacituzumab govitecan (target protein TROP2) in breast carcinoma (21,22).

Our group recently demonstrated that sacituzumab govitecan is also effective in EAC and that the efficacy is dependent on the level of expression of TROP2 on the tumour cell (23). The same relationship was shown for CD138 in breast carcinoma. Indatuximab ravtansine led to an increased complete response in xenografts of triple-negative breast carcinomas that strongly expressed CD138 in immunohistochemistry (24,25). This makes CD138 an ideal predictive therapy-relevant biomarker. Another recently identified agent, VIS823, showed promising results in a preclinical study on multiple myeloma cell lines (26).

Almost nothing is known about the significance and expression level of CD138 in EAC. We aim to answer the following questions with this work: i) how many EACs express CD138? ii) what are the histomorphological, molecular and clinical characteristics of CD138-positive EAC? iii) is CD138 suitable as a relevant prognostic marker in EAC? iv) does neoadjuvant therapy (after CROSS or FLOT) have an impact on the expression level of CD138 in EAC?

Materials and methods

Patient cohort. Patients were included in this analysis if they had undergone primary surgery with curative intent for primary EAC at the Department of General Surgery Department, University of Cologne between 1996 and 2020 and if sufficient formalin-fixed paraffin-embedded material of the primary tumour was available. Demographic, histopathological and survival data were retrieved from clinical records and histopathological reports with respect to tumour characteristics including the stage of disease at the time of diagnosis according to the AJCC TNM staging system (8th edition, 2020) (27). In case of missing data on follow-up, patients were phoned to follow up in terms of the current tumour status. The study was performed according to the regulations of the Ethics Committee of the University of Cologne (approval nos. 20-1583 and 10-242).

TMA preparation and immunohistochemical assessment of CD138 expression. One tissue cylinder (diameter 1.2 mm) per case was punched out from one tumour-bearing formalin-fixed, paraffin-embedded (FFPE) block using a semiautomated precision instrument. The cylinders were then transferred to an empty paraffin block. Tissue slides were stained with antibodies against CD138 (clone EP85, rabbit, 1:500 pretreatment with EDTA buffer, Epitomics, Burlingame, CA, USA). All immunohistochemical staining was carried out with a Leica BOND-MAX stainer (Leica Biosystems, Wetzlar, Germany) in accordance with the manufacturer's protocol. Counterstaining was done using haematoxylin and bluing reagent.

Two pathologists with special expertise in the field of EAC (DA, AQ) assessed the membranous expression of CD138 on tumour cells. A tumour cell was counted positive if ≥50% of the membrane showed CD138 expression. The percentage of tumour cells with CD138 expression in relation to all tumour cells was calculated. The categorization of our staining analyses was based on the previously published data on the subject (28). The staining intensity was determined semi-quantitatively and divided into four intensity levels (0, 1+, 2+, 3+). Four groups were then created to perform statistical analysis: tumours without expression of CD138 on their tumour cell membrane were classified as negative, tumours with a low expression intensity in ≤70% of tumour cells and moderate intensity in ≤30% of tumour cells were scored as weakly positive (1+), tumours with low staining intensity in >70% of tumour cells, moderate intensity in <30 to 70% or strong intensity in ≤30% of tumour cells were classified as moderately positive (2+) and tumours with moderate intensity in >70% of tumour cells or strong intensity in >30% were classified as strongly positive (3+). As also mentioned in the same paper, this classification corresponds to a standard categorization that has also been used by other research groups in various immunohistochemical studies (29).

Fluorescence in situ hybridization. Fluorescence *in situ* hybridization (FISH) to evaluate the *ERBB2* gene amplification status was performed with a Zytolight SPEC ERBB2/CEN 17 Dual Probe Kit (Zytomed Systems GmbH, Germany) according to the manufacturer's protocol. Sample processing was performed as previously described (30).

Statistical analysis. The expression of CD138 in tumour cells was dichotomized in two ways: first, negative (0) tumours were compared to positive tumours (1-3+). In the second step, tumours with strong expression of CD138 were analysed in comparison to the other tumours (0, 1+, 2+).

The expression levels were correlated to patients' sex, age and histopathological parameters including tumour stage (pT) and lymph node status (pN0, pN+). Additionally, CD138 expression data were correlated with *ERBB2*-amplification status (Her2/neu) and *MET* status.

Table I. Histopathological parameters of the patient cohort (n=723).

Characteristic	Primary surgery	Neoadjuvant treatment		Total
		CROSS	FLOT/NOS	
Sex, n (%)				
Male	234 (86.7)	199 (88.1)	199 (87.7)	632 (87.4)
Female	36 (13.3)	27 (11.9)	28 (12.3)	91 (12.6)
Median age at surgery, years (range)	68 (30-91)	59 (27-83)	62 (34-85)	64 (27-91)
pT/ypT, n (%)				
Submucosa-limited (pT1)	84 (31.1)	26 (11.5)	21 (9.3)	131 (18.1)
Extensive (pT2+)	186 (68.9)	200 (88.5)	206 (90.7)	592 (81.9)
pN/ypN, n (%)				
pN0	109 (40.4)	92 (41.1)	76 (33.5)	277 (38.4)
pN1+	161 (59.6)	132 (58.9)	151 (66.5)	444 (61.6)
UICC stage, n (%)				
I	64 (23.8)	18 (7.9)	14 (6.2)	96 (13.3)
II	34 (12.6)	27 (11.9)	28 (12.3)	89 (12.3)
III	102 (37.9)	112 (49.6)	111 (48.9)	325 (45.0)
IV	69 (25.7)	69 (30.5)	74 (32.6)	212 (29.4)
ERBB2 status, n (%)				
Wild type	222 (88.1)	181 (91.0)	193 (93.2)	596 (90.6)
Amplified	30 (11.9)	18 (9.0)	14 (6.8)	62 (9.4)
MET status, n (%)				
Wild type	257 (95.2)	203 (89.9)	207 (91.2)	667 (92.3)
Amplified	13 (4.8)	23 (10.1)	20 (8.8)	56 (7.7)

Patient cohort: n=723. CROSS, Chemoradiotherapy for Oesophageal Cancer followed by Surgery Study; FLOT, Fluorouracil, Leucorovin, Oxaliplatin, Docetaxel, followed by surgery; NOS, neoadjuvant chemotherapy, not specified. TNM classification was performed following UICC 2020, 8th edition criteria. For all 723 patients, data were available as follows: Tumour stage (pT/ypT) in all 723 patients (100%), lymph node status (N) in 721 cases (99.7%). UICC staging was available for 722 patients (99.9%). The ERBB2 status (Her2/neu) was available in 658 cases (91.0%), the MET status in 723 cases (100%).

For the statistical comparisons, chi-square test, Fisher's exact test and one-way ANOVA were used with a Bonferroni correction for multiple comparisons. $P<0.05$ was considered statistically significant, $P<0.1$ as a statistical trend.

For survival analysis, Kaplan-Meier curves were generated and overall survival between the CD138 expression subgroups was compared using a log-rank test, or, in the case of crossing curves, a Breslow test. Additionally, multivariate Cox proportional hazard analysis was performed including prognostic factors like age, sex, tumour stage, lymph node status and the application of neoadjuvant therapy.

For all analysis and data visualization, Python v. 3.9 was used on PyCharm Community Edition 2022.2 including commonly free available packages (numpy, pandas, scipy.stats, matplotlib, pingouin, lifelines).

Results

Characteristics of the patient cohort. The total cohort included 723 patients; 632 patients were male (87.4%) and 91 were female (12.6%); 453 patients (62.7%) received neoadjuvant treatment [CROSS regime, FLOT or not further specified (NOS)]. Table I contains detailed data for the total cohort as well as patient subgroups, respectively.

Extent of CD138 expression in adenocarcinoma of the oesophagus (EAC). When dichotomized into negative and positive tumours, the majority of EACs did not express CD138 [491 (67.9%) vs. 232 (32.1%)]. Ninety-six tumours displayed strong expression of CD138 (13.3%) (Fig. 1). These proportions were observed more or less in all treatment groups, with tumours slightly more often expressing strong levels of CD138 after neoadjuvant CROSS treatment (Tables II and III).

CD138 expression is correlated with a less extensive tumour and lower lymph node stage. When CD138-positive and CD138-negative tumours were compared, CD138-positive tumours were more frequently limited to the mucosa and submucosa (pT1), while CD138-negative tumours more often showed extensive infiltration (pT2+) ($P=0.008$, chi-square test). Additionally, tumours expressing CD138 less often metastasized to the lymph nodes (pN0 vs. pN+) than CD138-negative tumours ($P=0.014$, chi-square test).

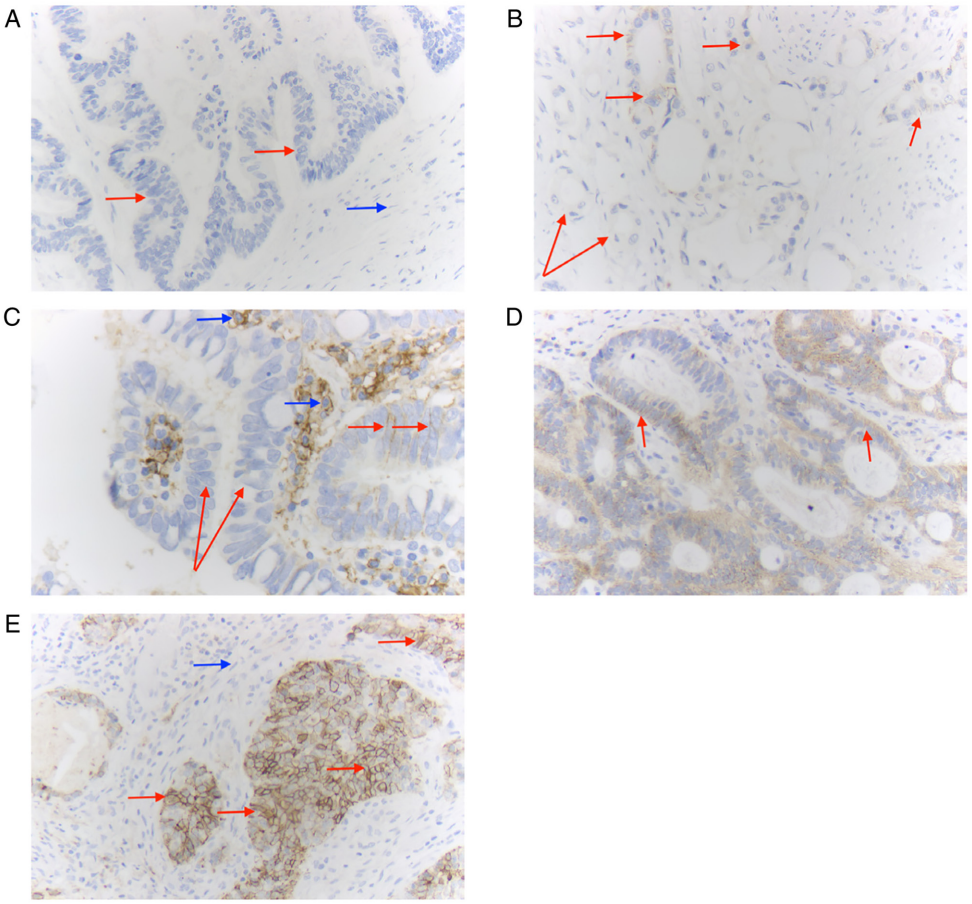

Figure 1. CD138 expression levels in EAC (all photos 200x; red arrows mark tumour cell clusters, blue arrows surrounding stromal cells). (A) Tumour is CD138-negative (both the carcinoma cells marked in red and a fibroblast marked in blue in the adjacent stroma show no CD138 expression). (B) Weak expression of CD138 in the EAC-the single arrows mark the weak membranous labelling (partly only laterally formed) of CD138 on carcinoma cells, the double arrow in the lower left of the image also shows CD138 negative tumour cells. (C) Partial and weak CD138 expression on carcinoma cells (red arrows); in contrast, plasma cells located in the peritumoral stroma are strongly CD138-positive (as a good additional internal control). (D) Moderate expression level of CD138 in EAC and (E) strong CD138-positivity on carcinoma cells. A simple expression level, already visible in the overview, can be seen in more than 90% of the tumour cells; this is membranous and in the majority of tumour cells circumferentially formed. In the stroma, exemplarily shown fibroblasts (blue arrow) show no labelling with CD138; few plasma cells next to the fibroblasts are weaker than the carcinoma cells also labelled (not marked with arrows). EAC, oesophageal adenocarcinoma.

This was also observed separately for strong expression levels of CD138 compared to no, weak or moderate CD138 expression: patients with high levels of CD138 in tumour tissue had a lower tumour stage and more often no lymph node metastasis (P=0.007, P<0.001, chi-square test).

No significant association between CD138 expression and age or sex of the patients was observed (ANOVA, chi-square test; data not shown). Furthermore, there was no interdependence observed between CD138 levels and the presence or absence of neoadjuvant treatment (chi-square test, Fisher's exact test, ANOVA; data not shown).

CD138 expression is correlated to ERBB2 amplification. While CD138-negative tumours only had an *ERBB2* (Her2/neu) amplification in 27.9% of cases, the majority of CD138-positive tumours were Her2/neu-amplified (58.1%, P<0.001, chi-square test).

Tumour tissue with strong CD138 expression more often displayed an Her2/neu amplification than tumours with no or weaker expression (27.4% vs. 10.7%, P=0.004, chi-square test).

Tumours treated with primary surgery and CD138-positive tumours were more frequently Her2/neu-amplified as well compared to negative tumours (53.3% vs. 23.7%, P=0.014, chi-square test). The same was observed for patients who received CROSS neoadjuvant therapy (83.3% vs. 34.8%, P<0.001, chi-square test).

There was no correlation observed between *MET* status and CD138 expression (data not shown).

Strong CD138 expression is an independent favourable prognostic marker. In the next step, CD138 expression was correlated with overall survival (OS). Survival data were available for 639 patients (88.4%); the minimal follow-up period included was 1 month. During the clinical follow-up, 360 patients died (56.3%). The median time of follow-up was 22.3 months (range 1-233 months).

Patients with CD138-positive tumours had a significantly longer OS than patients without CD138 expression (26.2 vs. 20.2 months, P=0.002) (Fig. 2A).

In univariate analysis, an expression of CD138 was a favourable prognostic factor (HR 0.73, 95% CI 0.59-0.92,

Table II. Histopathological parameters in CD138-expression subgroups.

Characteristic	CD138 negative	CD138 positive	CD138 strong expression
Sex, n (%)			
Male	426 (86.8)	206 (88.8)	84 (87.5)
Female	65 (13.2)	26 (11.2)	12 (12.5)
Median age at surgery, years (range)	64 (27-91)	63 (30-85)	62.5 (39-84)
pT/ypT, n (%)			
Submucosa-limited (pT1)	72 (14.7)	59 (25.4)	30 (31.3)
Extensive (pT2+)	419 (85.3)	173 (74.6)	66 (68.8)
pN/ypN, n (%)			
pN0	168 (34.2)	109 (47.0)	57 (59.4)
pN1+	322 (65.6)	122 (52.6)	39 (40.6)
UICC stage, n (%)			
I	55 (11.2)	42 (18.1)	21 (21.9)
II	52 (10.5)	37 (15.9)	17 (17.7)
III	226 (46.0)	99 (42.7)	39 (40.6)
IV	158 (32.2)	54 (23.3)	19 (19.8)
ERBB2 status, n (%)			
NA	35 (7.1)	30 (12.9)	15 (15.6)
Wild type	430 (87.6)	166 (71.6)	64 (66.7)
Amplified	26 (5.3)	36 (15.5)	17 (17.7)
MET status, n (%)			
Wild type	448 (91.2)	219 (94.4)	90 (93.8)
Amplified	43 (8.8)	13 (5.6)	6 (6.2)

TNM classification was performed following UICC 2020, 8th edition criteria. For all 723 patients, data were available as follows: Tumour stage (pT/ypT) in all 723 patients (100%), lymph node status (N) in 721 cases (99.7%). UICC staging was available for 722 patients (99.9%). The ERBB2 status (Her2/neu) was available in 658 cases (91.0%), the MET status in 723 cases (100%).

Table III. Extent of CD138 expression in treatment cohorts.

		Neoadjuvant treatment		
Intensity	Primary surgery, n (%)	CROSS, n (%)	FLOT/NOS, n (%)	Total, n (%)
Negative (0)	195 (72.2)	137 (60.6)	159 (70.0)	491 (67.9)
Weak (1+)	5 (1.9)	8 (3.5)	1 (0.4)	14 (1.9)
Moderate (2+)	38 (14.1)	44 (19.5)	40 (17.6)	122 (16.9)
Strong (3+)	32 (11.9)	37 (16.4)	27 (11.9)	96 (13.3)
Positive (1-3+)	75 (27.8)	89 (39.4)	68 (30.0)	232 (32.1)

Total cohort: n=723; primary surgery cohort (no neoadjuvant treatment) n=270; CROSS cohort n=226; FLOT/NOS cohort (FLOT chemotherapy or chemotherapy, not specified) n=227.

P=0.007). When other covariates were included (age, pT, pN, *ERBB2* status, *MET* status), CD138 expression did not remain a significant prognostic factor (HR 0.87, 95% CI 0.69-1.11, P=0.28).

When tumours with a strong CD138 expression were compared to tumours with no or weaker CD138 expression, a significant survival advantage was seen in tumours with strong CD138 expression (31.7 vs. 21.5 months, P=0.006), (Fig. 2B). In univariate analysis, strong CD138 expression was a prognostic favourable factor (HR 0.56, 95% CI 0.39-0.80, P=0.001). In multivariate Cox analysis, strong levels of CD138 were an independent favourable prognostic factor as well (HR 0.63, 95% CI 0.43-0.94, P=0.02) (Table IV).

When sub-divided by treatment, no significant advantage in OS was observed in the cohort with primary surgery or the FLOT/NOS cohort (data not shown). However, patients who

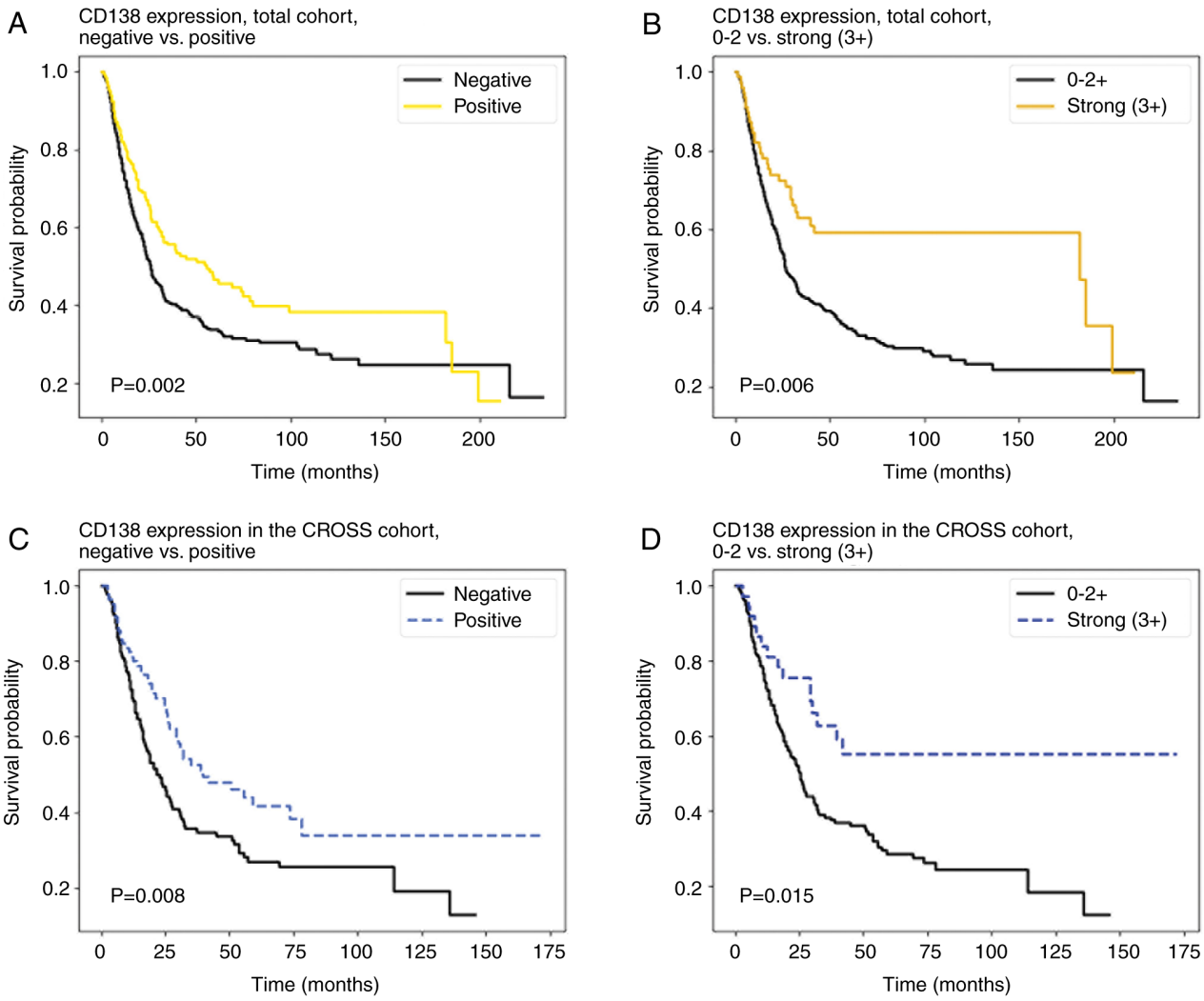

Figure 2. Kaplan-Meier survival curves of CD138 expression in oesophageal adenocarcinoma. (A) Patients with CD138-positive tumours survived significantly longer than patients with CD138-negative tumours; (B) the same effect was also observed for strong CD138 expression compared to patients with moderate, weak or absent expression of CD138. Positivity for and a strong expression of CD138 were also associated in the patient cohort which received neoadjuvant radiochemotherapy (CROSS). (C) Kaplan-Meier-curve comparing CD138 positivity with negativity. (D) Kaplan-Meier-curve showing the effect of highly CD138-positive tumours. For patients with no neoadjuvant treatment or FLOT chemotherapy/not specified chemotherapy, this correlation between CD138 and prolonged survival was not observed (data not shown). For the comparative analysis, a log-rank test was used; in cases (A and B) a Breslow test was used. P<0.05 was considered significant. CROSS, Chemoradiotherapy for Oesophageal Cancer followed by Surgery Study; FLOT, Fluorouracil, Leucorovin, Oxaliplatin, Docetaxel, followed by surgery.

received neoadjuvant CROSS therapy and had CD138-positive tumours lived significantly longer (30.75 vs. 20.75 months, P=0.008) (Fig. 2C). Patients with strong CD138 expression lived longer compared to patients with weaker intratumoural CD138 expression (33.2 vs. 23.1 months, P=0.015) (Fig. 2D). Positivity for CD138 was a favourable prognostic factor in this subgroup (HR 0.64, 95% CI 0.45-0.91, P=0.02), as well as strong expression (3+) of CD138 compared to weaker expression (HR 0.49, 95% CI 0.29-0.84, P=0.01).

CD138 is a favourable prognostic marker in tumours with higher tumour stage. In the next step, the patients were sub-divided into a cohort with mucosa/submucosa-limited tumours [(y)pT1] and patients with deeper infiltrating tumours [(y)pT2+]. For (y)pT2+ tumours, a statistical trend towards prolonged OS was observed for CD138-positive tumours and tumours with strong CD138 expression (median OS positive tumours: 21.8 months vs. 17.5 months, P=0.07; median OS tumours with strong CD138 expression: 29.1 months vs. 18.8 months, P=0.08). In multivariate analysis, strong CD138 expression was a significant favourable prognostic factor (HR 0.65, 95% CI 0.43-0.99, P=0.04).

CD138 is a favourable prognostic marker in tumours without lymph node involvement. The same effect was observed in patients without lymph node metastasis: patients with (y)pN0 tumours displayed a statistical trend for longer OS when the tumour was positive for CD138 (45.3 months vs. 38.7 months, P=0.07). When tumours had strong CD138 expression, a longer OS was observed as well (45.9 months vs. 39.9 months, P=0.08). In univariate analysis, a strong CD138 expression (3+) was a favourable prognostic factor (HR 0.52, 95% CI 0.28-0.95, P=0.03). In multivariate analysis, however, a statistical favourable trend was shown for strong expression of CD138 (HR 0.56, 95% CI 0.29-1.09, P=0.09) (Table IV).

Table IV. Multivariate analysis for CD138 expression in patient cohorts.

A, Total cohort

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.02 (1.01-1.03)	0.001	1.01 (1.01-02)	0.001
pT	1.27 (1.08-1.48)	0.003	1.23 (1.08-1.48)	0.003
pN	1.49 (1.34-1.65)	<0.001	1.48 (1.34-1.65)	<0.001
ERBB2 status	0.81 (0.53-1.23)	0.32	0.82 (0.54-1.24)	0.35
MET status	1.16 (0.80-1.67)	0.44	1.20 (0.83-1.72)	0.34
CD138	0.87 (0.68-1.11)	0.27	0.63 (0.43-0.94)	0.02

B, CROSS cohort

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.01 (0.98-1.03)	0.48	1.01 (0.99-1.03)	0.47
pT	1.26 (0.95-1.68)	0.1	1.29 (0.97-1.71)	0.08
pN	1.57 (1.33-1.84)	<0.001	1.54 (1.31-1.81)	<0.001
ERBB2 status	0.92 (0.43-1.96)	0.82	0.91 (0.43-1.93)	0.81
MET status	1.01 (0.58-1.76)	0.98	1.04 (0.60-1.81)	0.88
CD138	0.66 (0.44-0.97)	0.04	0.43 (0.24-0.79)	0.007

C, (y)pT2+ cohort

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.01 (1.00-1.02)	0.02	1.01 (1.00-1.02)	0.02
pN	1.45 (1.31-1.61)	<0.001	1.45 (1.31-1.61)	<0.001
ERBB2 status	0.65 (0.39-1.09)	0.1	0.67 (0.40-1.11)	0.12
MET status	1.15 (0.79-1.68)	0.46	1.19 (0.82-1.74)	0.36
CD138	0.85 (0.66-1.11)	0.24	0.61 (0.39-0.95)	0.03

D, (y)pN0 cohort

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.01 (1.00-1.02)	0.02	1.01 (1.00-1.02)	0.02
pT	1.43 (1.13-1.82)	0.003	1.44 (1.14-1.82)	0.002
ERBB2 status	0.68 (0.35-1.33)	0.26	0.71 (0.37-1.38)	0.31
MET status	2.34 (1.06-5.16)	0.04	2.33 (1.06-5.14)	0.04
CD138	0.93 (0.60-1.45)	0.75	0.56 (0.29-1.09)	0.09

E, *ERBB2*-not amplified

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.03)	<0.001

Table IV. Continued.

E, *ERBB2*-not amplified

	CD138 positive vs. negative		CD138 strong vs. negative/weak	
Covariate	HR (95% CI)	P-value	HR (95% CI)	P-value
pT	1.25 (1.08-1.45)	0.003	1.25 (1.08-1.45)	0.003
pN	1.55 (1.41-1.71)	<0.001	1.55 (1.40-1.71)	<0.001
MET status	1.15 (0.81-1.63)	0.45	1.18 (0.83-1.68)	0.36
CD138	0.85 (0.68-1.07)	0.16	0.66 (0.46-0.94)	0.02

P<0.05 was considered to be significant, P<0.1 a statistical trend.

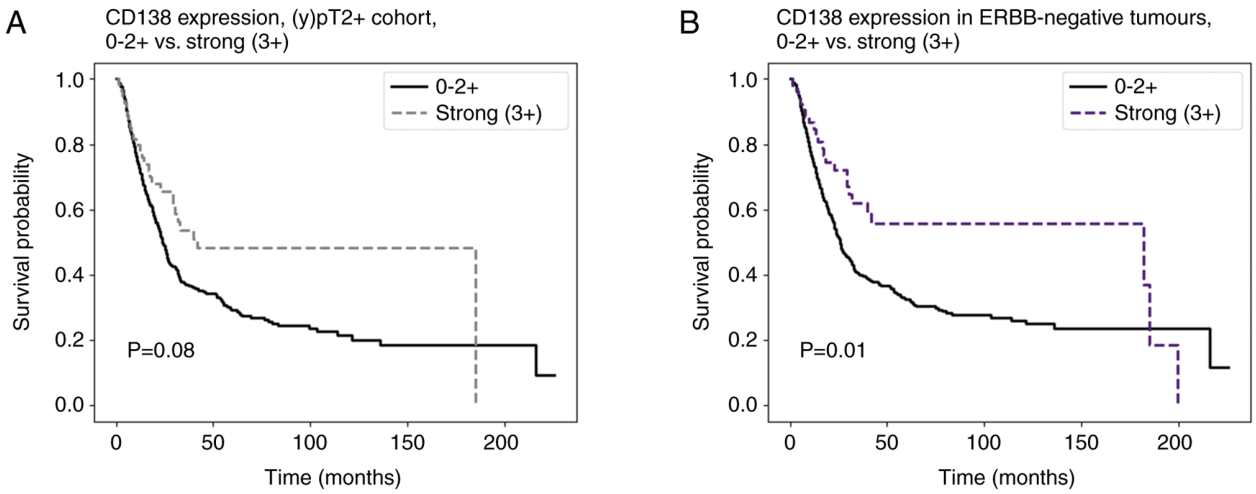

Figure 3. CD138 expression in extensive and ERBB2-negative tumours. (A) In tumours with a more extensive tumour stage ((y)pT2+), strong CD138 expression was associated with a statistical trend toward prolonged overall survival; in multivariate analysis, strong CD138 expression was observed to be an independent prognostic factor (P=0.04). (B) Patients without ERBB2-amplified tumours and strong CD138 expression survived significantly longer than patients with moderate, weak or absent CD138 expression; additionally, strong CD138 expression was a favourable prognostic factor (P=0.01). For survival comparison, a Breslow test was used, with P<0.05 being significant and P<0.1 interpreted as a statistical trend.

CD138 is a favourable prognostic marker in tumours without ERBB2 amplification. In tumours without Her2/neu amplification, positive expression of CD138 was significantly correlated to a longer OS (23.2 months vs. 18.9 months, P=0.03). The same was observed for strong expression of CD138 compared to weaker or no expression (29.7 vs. 19.6 months, P=0.01) (Fig. 3). In multivariate analysis, strong expression of CD138 remained an independent favourable prognostic marker (HR 0.61, 95% CI 0.40-0.93, P=0.02) (Table IV).

Discussion

In this study, the protein expression of CD138 and its clinical and molecular as well as prognostic significance were assessed in the largest cohort of EAC so far. We observed that CD138 expression was strong in 13.3% of the included cases. These tumours with high CD138 expression levels may be particularly amenable to targeted therapy with ADC such as indatuximab ravtansine. Whilst CD138 is best known for staining plasma cells and therefore is mainly used in the diagnostic assessment of plasma cell tumours (31), CD138 expression has been shown in up to 87% of different tumour entities, in which 71% had strong positive staining in at least one case (28). To the best of our knowledge, just one study before has evaluated the expression of CD138 in a large number of human tumours, but that included only a limited number of EACs (n=33). Based on this study, 30% of EACs (n=11) strongly expressed CD138 (28). There are several studies on CD138 expression in tumours and related prognosis: cell-surface CD138 expression has been demonstrated as favourable in mesothelioma, gastric cancer, hepatocellular carcinoma, cervical cancer and bladder cancer (32). Kusumoto *et al* (33) demonstrated that in ovarian cancers, epithelial CD138 expression is significantly lower in advanced disease. Additionally, in prostate adenocarcinomas, CD138 overexpression predicts early recurrence and is associated with a higher Gleason grade (34). Lendorf *et al* (35) found similar results in breast cancer cases, in which CD138 expression is associated with tumours of higher grade. There are several studies on CD138 expression and its prognostic significance in oesophageal squamous cell carcinomas showing CD138 expression to be higher in less invasive tumours (lower T stage) and in better differentiated tumours (36,37).

This study demonstrates for the first time that CD138 is also a favourable prognostic marker in specific patient subgroups (e.g., those with tumours resected after neoadjuvant therapy using the CROSS protocol). Since more than 90% of operable patients receive neoadjuvant therapy and the CROSS protocol is a very commonly used therapeutic regime in Europe, these findings have high clinical and therapeutical relevance. However, it must be noted that, according to the results of the Checkmate 577 study, all patients with vital tumour after surgery, who received neoadjuvant CROSS treatment, additionally receive 1 year of nivolumab; concluding prognostic statements on this specific group are not yet possible, since this therapy has only been used for a very short time (38).

Apart from CD138 being a possible prognostic indicator, therapeutical agents which specifically target CD138 could be a new treatment approach. Several of these agents have already shown promising results in some cancer types: indatuximab ravtansine (BT062) is the ADC comprising the anti-CD138 monoclonal antibody (nBT062) and the microtubule-binding cytotoxic agent maytansinoid DM4 (39); when used in multiple myeloma patients, 75% achieved a state of stable disease (40). In addition, it has been observed that in CD138-positive, triple-negative breast cell carcinoma cell lines, indatuximab ravtansine therapy is highly effective showing complete remission (41). This efficacy of indatuximab ravtansine in breast carcinoma correlated with the expression levels of CD138 on tumour cells, assessed with immunohistochemistry. This emphasizes the potential of CD138 as a predictive, therapeutically relevant biomarker for EAC as well.

The easy applicability and broad availability of a prognostic and predictive biomarker is a great advantage in practical and clinical everyday routine, as the implementation of PD-L1 and its immense therapeutical implications have demonstrated. Especially the assessment of a biomarker by immunohistochemistry, a routine procedure available in almost all pathology institutes, fulfils these requirements.

Due to the fact that CD138 is expressed in a variety of normal epithelial cells and plasma cells (28), treatment side effects could be potentially problematic; indatuximab ravtansine, however, as demonstrated by first trials, is tolerated well, with the most common adverse side effects being grade 1 or 2 (diarrhoea and fatigue) (40). Grade 3-4 adverse effects are neutropaenia, anaemia and thrombocytopaenia (42). Another newly developed agent is VIS832, a humanized IgG1-κ monoclonal antibody targeting human CD138, inducing immune cell-mediated cytotoxicity (26). The preclinical trial demonstrated a promising efficacy of VIS832 *in vitro* as well as *in vivo* (26).

Our study has some limitations. These include the retrospective nature of the analyses and the carcinomas were from a large single-tumour centre. We only studied operable patients. Future clinical studies should also determine the expression level of CD138 in endoscopic biopsies from the EAC in nonoperable or primarily hematogenously metastasized (palliative) patients. As an advantage, and this can also be understood as a prospect for future studies: We used a long-established, commercially available and well-established immunohistochemical antibody to determine CD138 on tumour cells. Analyses are thus readily reproducible. Also, clinical trials testing the efficacy of the ADC indatuximab ravtansine in EAC need to correlate therapeutic response with the expression level of CD138 on tumour cells. For this, a technique that is easy to use and also widely available in pathology institutes is helpful.

In conclusion, this is the largest and most comprehensive study on the significance of CD138 (syndecan-1) expression in EAC. We demonstrated that a significant proportion of EAC is strongly CD138-positive (13.3%). CD138 is already utilized by ADCs such as indatuximab ravtansine, whose effectiveness depends on the extent of CD138 on tumour cells. This makes CD138 an ideal predictive, therapeutically relevant biomarker. Future clinical trials now need to show how effective the corresponding ADCs are in CD138-positive EACs.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DA, AS, RB and AQ made substantial contributions to conception and design. DA, AS, CB and FG were responsible for analysis and interpretation of data. DA, AS, TZ and AQ wrote the main manuscript. CB, WS and TZ were responsible for the data collection. TZ, WS and RB have reviewed the text. All authors were involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript. AQ, DA and AS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The objective of the project is primarily in the field of diagnostics and quality assurance; approval was obtained from the University of Cologne Ethics Committee (approval nos. 20-1583 and 10-242). All authors confirm that methods used were carried out in accordance with relevant guidelines and regulations. The experimental protocols were approved by the licensing committees. We confirm that written informed consent was obtained from all subjects and/or their legal guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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