The immune environment in ovarian cancer

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Chapter 6

Claudin-6 is a target for therapy in serous ovarian cancer and serous and clear cell endometrial cancer

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Abstract

Purpose Gynecological malignancies represent 12% of new cases of cancer in women each year. Since current treatment strategies show limited responses, there is an urgent need for new therapeutic targets in these cancer types. Claudins are tight junction proteins that can be aberrantly expressed in human tumors. Claudin-6 (CLDN6) has been found to be upregulated in various solid tumors, while expression in healthy tissue is restricted to embryonic development. With a CLDN6-targeting antibody in clinical trial phase, it is of high interest to determine the expression levels of this surface protein on gynecological tumors.

Methods We assessed the expression of CLDN6 on tumor cells by immunohistochemistry on tissue microarrays containing tumor samples of patients with epithelial ovarian, endometrial, and cervical cancer.

Results Membranous expression of CLDN6 was present in 79.3% of serous ovarian tumors as well as 83.3% of serous and 30.8% of clear cell endometrial tumors, while being absent from cervical cancer. 70.5% of tumors showed a moderate to high staining intensity and expression was related to higher tumor grade. The serous ovarian cancer patient dataset consisted of two separate cohorts with various primary treatment regimens. One cohort of patients was treated with primary surgery, followed by adjuvant platinum-based chemotherapy, and the other cohort consisted of patients that had received neo-adjuvant platinum-based chemotherapy. Analyzed tissue was obtained at time of primary or interval surgery. We found comparable levels of expression as well as percentage of patients showing any expression of CLDN6 in the two cohorts. This suggests that chemotherapeutic treatment may not affect expression of CLDN6 on these tumors and therapeutic strategies targeting CLDN6 can be considered in combination or after chemotherapy treatment.

Conclusion CLDN6 is an interesting target for immunotherapy (e.g. antibody-based strategies) in serous ovarian as well as in serous and clear cell endometrial cancer.

Introduction

Gynecological malignancies represent 12% of new cases of cancer in women, with endometrial cancer (EC) representing the most common form (1). Endometrial and cervical cancers are often diagnosed at early stage disease with a favorable prognosis. Epithelial ovarian cancer (EOC) on the other hand, is the least common but most deadly form, which is in large part due to diagnosis at advanced stage (1,2). These malignancies comprise several histological subtypes, representing major differences in clinical course and prognosis (2,3).

In the past decade, it has become evident that these gynecological malignancies represent immunogenic tumors that can be recognized by the host's immune response. The infiltration of CD3+ T cells and, in particular, CD8+ cytotoxic T cells (CTLs) are associated with a better prognosis in all three cancer types (4–9). These findings have provided rationale for the development of immunotherapeutic strategies towards these tumors to further boost the immune response in order to eradicate tumors. One strategy is the use of therapeutic monoclonal antibodies targeting tumor-associated antigens (TAA) that specifically recognize tumor cells. For a successful targeting of TAA, it is essential that the target is abundantly and, ideally, homogeneously expressed on the cell surface of tumor cells, while having no or low expression on normal tissues.

Members of the claudin (CLDN) family of tight junction proteins may represent such targets for monoclonal antibody therapy. CLDNs are important components of tight junctions, which regulate the permeability, barrier function, and polarity of epithelial cell layers (10,11). There are 27 CLDN family members (12), and most are abundantly expressed in both embryonic development and adult tissues. CLDN coding genes show a very restricted expression pattern (13) and expression is regulated by transcription factors as well as epigenetic mechanisms (14), which both may be dysregulated in cancer. Indeed, various CLDNs were found to be up- or downregulated in cancer, which might be related to metastatic properties (15–17).

IMAB027 is an immune-activating chimeric therapeutic antibody binding CLDN6 specifically, which is in phase I/II clinical trial (clinicaltrials.gov; NCT02054351). While CLDN6 expression is normally restricted to embryonic development (13,18,19), overexpression was found in various cancer types such as gastric adenocarcinoma and non-small-cell lung cancer (19–22). Therefore, it is of high interest to analyze the expression pattern of this CLDN in gynecological tumors and determine which of those might serve as therapeutic targets and determine what are the criteria for stratification of patients for such strategies.

In this study, we performed an extensive analysis on the expression of CLDN6 in large, well-defined cohorts of cervical, endometrial, and epithelial ovarian carcinomas. We show that in the serous subtype of both endometrial and ovarian cancer high percentages of patients express CLDN6 on the tumor cell membrane and that this expression is correlated with higher-grade disease.

Methods

Patient cohorts

4 cohorts of patients with cervical, endometrial, and ovarian cancer were analyzed for the expression of CLDN6 on archived formalin-fixed paraffin-embedded (FFPE) tumor samples by use of immunohistochemistry. All tumors were classified by a gynecologic pathologist and subsequently graded and staged according to World Health Organization (WHO) criteria and International Federation of Gynecology and Obstetrics (FIGO) guidelines.

Serous ovarian cancer (sOC) cohorts

The serous ovarian cancer cohort was split in two separate cohorts, one that consisted of patients that had received cytoreductive surgery (Primary Surgery (PS) cohort) and one in which patients received neo-adjuvant chemotherapy (NACT cohort) as start of their primary treatment strategy. All patients were diagnosed at the University Medical Center Groningen (UMCG) between January 2000 and December 2012. Patients that underwent PS subsequently received six cycles of platinum-based chemotherapy, often supplemented with paclitaxel. Patients that were selected for NACT first received three cycles of chemotherapy, followed by interval cytoreductive surgery and three more cycles of chemotherapy. Follow-up was calculated from date of initial treatment and was last updated April 2014. FFPE tumor samples were obtained during primary (PS cohort) or interval cytoreductive surgery (NACT cohort) and were used to construct tissue microarrays (TMA).

Mucinous ovarian cancer (mOC) cohort

The mucinous ovarian carcinoma cohort was created on basis of inclusion of those patients diagnosed with mucinous ovarian carcinoma at the UMCG between January 1985 and December 2010. All 43 patients received primary surgery, in 40.9% followed by platinum-based chemotherapy, which was supplemented with taxanes from 1995 onwards. Follow-up was calculated from date of initial treatment and was last updated April 2014.

Endometrial cancer (EC) cohort

The endometrial cancer cohort consisted of 359 patients diagnosed at the UMCG between January 1980 and December 2004. Follow-up was last updated in May 2010. A gynecologic pathologist determined the histological subtype post-operatively. Next to surgical treatment, most patients (55.4%) received radiotherapy and 2.5% received adjuvant chemotherapy.

Cervical cancer (CXCA) cohort

The cervical cancer cohort consisted of 318 patients diagnosed with early stage cervical carcinomas in the UMCG between January 1981 and December 2006.

Most patients received surgery as primary treatment, which was in 50% of patients supplemented with radio- and/or chemotherapy.

Ethical review

For all four cohorts, patient data were retrieved from the institutional database into a new anonymous database, in which patient's identity was protected by unique patient codes. According to Dutch law no approval from our institutional review board was needed.

Tissue microarrays (TMA)

TMAs were constructed as described previously (5,8,23,24). In brief, each patient was selected for inclusion on the TMA if enough FFPE tumor tissue was available. A gynecologic pathologist selected representative areas from each tissue block on basis of H&E staining and cores of either 0.6 mm (mOC, EC, CXCA) or 1.0 mm (sOC) diameter were taken from selected regions of each paraffin block and placed into a recipient block using a microarrayer (Beecher Instruments).

Tissue staining for CLDN6

From all TMA blocks 4 µm sections were cut and stained for CLDN6 by use of immunohistochemistry. Slides were deparaffinized in xylene and rehydrated through a series of alcohol gradients, and subsequently subjected to heat-induced antigen retrieval in a Tris-EDTA buffer (pH=9.0) for 15 min. After cooling down, endogenous peroxidase was quenched in a 3.0% H_2O_2 solution containing 15 mM NaN_3 for 5 minutes. Subsequently, the slides were incubated with an anti-human CLDN6 monoclonal antibody (clone 58-42, Ganymed Pharmaceuticals, Germany) for 60 minutes at room temperature. Binding of the antibody was visualized by use of the visualization reagent CLAUDETCT18.2 and subsequent incubation with Vector Red alkaline phosphatase substrate solution (Vector Laboratories, USA). Counterstaining was done by using Mayer's hematoxylin. Slides were mounted and scanned with Aperio ScanScope XT (Aperio Technologies, Vista, USA).

Scoring

Two individuals without prior knowledge of patient characteristics scored all tissue samples. For each patient, the TMA core showing highest expression was scored for percentage of tumor cells being positive and the intensity of the staining categorized as 0 (no staining), 1+ (weak staining), 2+ (intermediate staining), or 3+ (strong staining). Patients were considered to have a CLDN6 positive tumor when at least 1% of tumor cells presented a 1+ staining intensity.

Statistical analysis

All statistical analyses were performed using IBM SPSS version 22 (SPSS Inc., USA) or Graphpad Prism. Comparison between patients with or without expression

of CLDN6 and clinicopathological parameters was performed by χ^2 tests. P-values <0.05 were considered significant, and all tests were performed two-sided.

For survival analysis, the Kaplan Meier method was used in combination with the log rank test to estimate differences between groups. Disease-specific survival (DSS) was determined in the sOC cohort, which was defined as the time period from date of surgery until death due to ovarian cancer or last follow-up. Variables that were significantly associated with DSS in univariate analyses were entered into a multivariate analysis using the Cox proportional hazards model. For analysis of disease-free survival (DFS) in the EC cohort, all patients that were in complete remission three months after primary treatment were included (90.0%), the time until recurrence or the time until dead due to disease was used for analysis or, for patients without recurrences, date of last follow-up.

Results

Serous ovarian cancer patient characteristics

The serous OC dataset was split into two separate cohorts, consisting of patients primarily treated with surgery (PS cohort) or neo-adjuvant chemotherapy (NACT cohort) (**Table 1**). The PS cohort consisted of 107 patients with advanced stage disease (FIGO> IIB), of which the majority (63.6%) of patients had stage IIIC disease. Most tumors (76.6%) were of high grade (G3). All patients received 6 courses of platinum-based chemotherapy after surgery, except for 7 patients that did not receive any chemotherapy; these patients either died before receiving any treatment or received palliative treatment. Surgical cytoreduction was complete, leaving no residual tumor tissue, in 40.2% of patients.

The NACT cohort consisted of 115 patients (**Table 1**) of whom all tumors were clinically staged as advanced stage. All patients received 3 cycles of platinum-based chemotherapy before and 3 cycles after interval surgery. Determining the differentiation grade of tumors after chemotherapeutic treatment is considered difficult and not directly comparable to grading of primary tumor samples. As a result, from 16 patients grade could not be determined; of the remaining most tumors (67.8%) were considered to be of high grade (G3). Interval surgical cytoreduction had resulted in a complete removal of the tumor in 38.3% of patients.

Table 1 : patient characteristics serous ovarian cancer dataset

	PS cohort		NACT cohort	
	All patients	**CLDN6+**	**All patients**	**CLDN6+**
	N=107	N=105	N=115	N=114
	N (%)	**N (%)**	**N (%)**	**N (%)**
Grade				
1	10 (9.3)	1 (11.1)	9 (7.8)	3 (33.3)
2	15 (14.0)	14 (93.3)	12 (10.4)	11 (92.7)
3	82 (76.6)	70 (82.4)	78 (67.8)	661 (79.2)
missing	0 (0)	-	16 (13.9)	-
FIGO stage				
IIB	1 (0.9)	1 (100)	0 (0)	-
IIC	9 (8.4)	4 (50.0)	0 (0)	-
IIIA	2 (1.9)	2 (100)	0 (0)	-
IIIB	8 (7.5)	57 (85.1)	3 (2.6)	2 (66.7)
IIIC	68 (63.6)	57 (85.1)	89 (77.4)	69 (77.5)
IV	19 (17.8	15 (78.9)	23 (20.0)	18 (81.8)
surgical debulking				
complete (no residual tissue)	43 (40.2)	27 (64.3)	44 (38.3)	37 (84.1)
optimal (<1cm residual tissue)	26 (24.3)	23 (92.0)	48 (41.7)	34 (72.3)
incomplete (>1cm residual tissue)	38 (35.5)	35 (92.1)	23 (20.0)	18 (78.3)

PS: primary surgery. NACT: neo-adjuvant chemotherapy

Expression of CLDN6 in serous ovarian cancer

CLDN6 proved to be abundantly expressed in serous ovarian cancer, **Fig. 1A** shows a representative example of a sOC tumor with CLDN6 expression. A total of 105 and 114 patients could be analyzed in the PS cohort and NACT cohort, respectively (**Table 1**). In the PS cohort, 85 patients (81.0%) expressed any positivity of CLDN6 on the tumors, and in the NACT 89 patients (84.8%) were found to be positive, showing no difference between the two cohorts (p=0.552). Staining intensity was not variable when comparing patients with expression from the PS and NACT cohort (p=0.493, p=0.802, p=0.518, for percentage of cells with intensity 1, 2, or 3, respectively, **Fig. 1B**), suggesting expression of CLDN6 remains similar upon chemotherapeutic treatment.

In both cohorts, a significantly lower number of patients had expression of CLDN6 in tumors with grade 1 ($p<0.001$, p=0.001 for PS and NACT, respectively), while no difference was found in the expression between grade 2 and high-grade tumors (p=0.457, p=0.300), suggesting CLDN6 is upregulated upon grading (**Fig. 1C, Table 1**). No differences in expression were found between the different FIGO stages included in this study (p=0.248, p=0.809, **Table 1**). Expression of CLDN6 was associated with a difference in disease-specific survival in the PS cohort; patients having no expression of CLDN6 on the tumor (N=20; p=0.045) had a longer survival. To correct for difference in expression that was detected for differentiation grade, a multivariate analysis was performed in which stage and grade were also taken into account. Here, no significant survival difference was detected (p=0.121, HR: 1.826; 95%CI:0.853-3.906) for CLDN6 expression. In the NACT cohort no difference in survival was found (p=0.566).

Mucinous ovarian cancer cohort

The mucinous ovarian cancer cohort consisted of 43 patients of which most presented with early stage disease (56.8%, FIGO stage IA), and 39.5% of patients had a low-grade tumor (**Table 2**). After primary surgery, half of the patients received chemotherapy, which was in most cases platinum-containing. Expression of CLDN6 was detected in 9 patients (20.5%), of which most tumors (66.7%) showed a staining intensity that was restricted to intensity 1 or 2. CLDN6 expression correlated with higher-grade tumors (p=0.010, **Table 2**).

Endometrial cancer cohort

The cohort of endometrial patients consisted of 359 patients (**Table 3**) of whom 71.9% had early stage disease and 27.9% of patients presented with high-grade (G3) disease. Most patients had a tumor of the adenocarcinoma subtype (84.1%), 18 patients had a serous tumor and 26 a tumor of clear cell subtype.

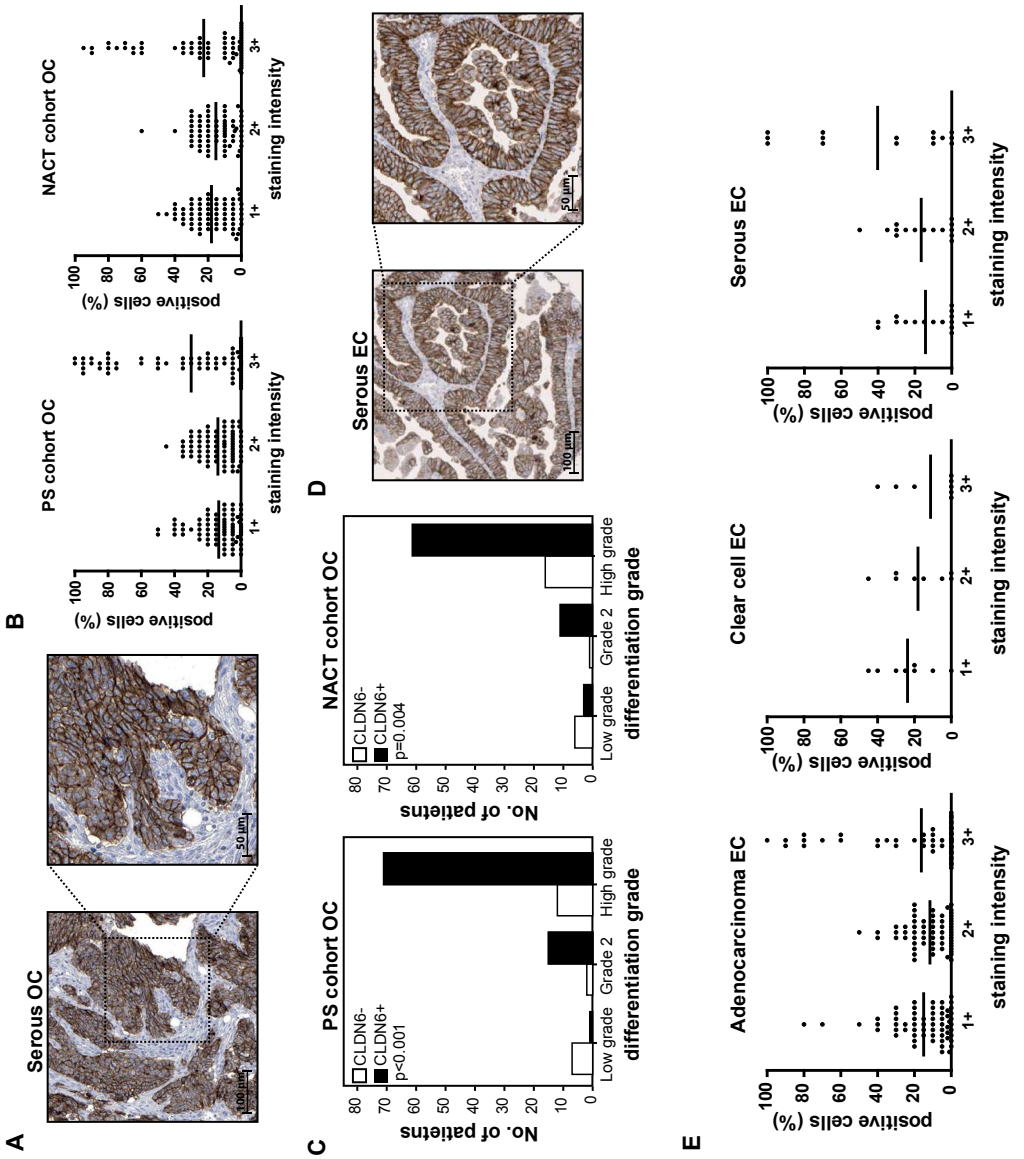

Figure 1: Claudin-6 expression in ovarian and endometrial cancer subtypes. **A)** Representative image of immunohistochemical staining of a serous ovarian cancer tumor sample. **B)** Staining intensity percentages of all positive samples in the primary surgery (PS) and neo-adjuvant chemotherapy (NACT) cohort of serous ovarian cancer patients. **C)** Bar graph depicting number of patients with CLDN6-positive tumor split on differentiation grade for both the PS and NACT cohort. **D)** Representative image of immunohistochemical staining of a serous endometrial cancer tumor sample. **E)** Staining intensity percentages of all positive samples for the adenocarcinoma, clear cell, and serous subtype of endometrial cancer.

Table 2: patient characteristics mucinous tumors

	All patients N (%)	CLDN6+ N (%)
Grade		
1	17 (39.5)	1 (5.9)
2	16 (37.3)	3 (18.8)
3	8 (18.6)	5 (62.5)
missing	2 (4.7)	-
FIGO stage		
IA	24 (55.8)	3 (12.5)
IC	8 (18.6)	1 (12.5)
IIC	1 (2.3)	1 (100)
IIIC	9 (20.9)	4 (44.4)
IV	1 (2.3)	0 (0)
Chemotherapy		
platinum-containing	18 (41.9	5 (27.8)
other	2 (4.7)	1 (50.0)
no chemotherapy	21 (48.8)	2 (9.5)
missing	2 (4.7)	-

Table 3: patient characteristics endometrial dataset

	all patients N=359 N (%)	CLDN6+ N=359 N (%)
FIGO stage		
IA	147 (40.9)	24 (16.3)
IB	53 (14.8)	14 (26.4)
II	58 (16.2)	18 (31.0)
IIIA	36 (10.0)	10 (27.8)
IIIB	4 (1.1)	1 (25.0)
IIIC	37 (10.3)	10 (27.0)
IVA	5 (1.4)	2 (40.0)
IVB	19 (5.7)	8 (42.1)
Differentiation grade		
Grade 1	159 (44.3)	21 (13.2)
Grade 2	92 (25.6)	22 (23.9)
Grade 3	100 (27.9)	43 (43.0)
Undifferentiated	2 (0.6)	0 (0)
missing	6 (1.7)	-
Histological subtype		
adenocarcinoma	302 (84.10	62 (20.5)
adenocanthoma	6 (1.7)	0 (0)
adenosquamous	7 (1.9)	2 (28.6)
clear cell	26 (7.2)	8 (30.8)
serous	18 (5.0)	15 (83.3)
age		
<60	120 (33.4)	26 (21.7)
>60	239 (66.6)	61 (25.5)
recurrence		
yes	82 (22.8)	22 (26.8)
no	277 (87.2)	65 (23.5)

CLDN6 expression in endometrial carcinomas

In the endometrial cohort large differences were found in the expression of CLDN6 between different histological tumor subtypes. A total of 87 patients showed any expression (24.2%), of which 20.5% of the adenocarcinomas, 30.8% of the clear cell, and 83.3% of serous carcinoma patients had CLDN6-positive tumors. A representative image of a positive serous EC (sEC) tumor is shown in **Fig. 1D**. When comparing those patients with CLDN6 expression, the amount of cells scored with staining intensity 3 was significantly higher in the serous group as compared to the positive adenocarcinoma subtype samples (**Fig. 1E**, p=0.005).

No differences in CLDN6 expression were found between FIGO stages (p=0.116 overall). Within the adenocarcinomas a significant increase in positive samples was found when comparing the G1 to G2 (p=0.045) and G3 (p<0.001), suggesting that, like in serous ovarian cancer, CLDN6 expression is related to differentiation of the tumors.

We studied the disease-free survival (DFS) of these patients in order to determine whether expression of CLDN6 may affect the chance of recurrences. No significant difference was found in DFS for patients with or without any expression of CLDN6 (p=0.105), also no difference was found when patients were split based on histological subtype.

Early stage cervical cancer dataset

The cervical cancer cohort consisted of 309 patients with early stage disease (**Table 4**). 64.4% of patients had a squamous cell carcinoma and 27.7% presented with adenocarcinoma. 33.0% of tumors had a differentiation grade 3. Of the 309 patients, 10 showed expression of CLDN6 (3.2%), with none of these tumors showing cells with a high staining intensity.

Table 4: Patient characteristics cervical cancer dataset

	All patients N=309
	N (%)
Stage	
IA1	2 (0.6)
IA2	1(0.3)
IB1	195 (63.1)
IB2	62 (20.1)
IIA	49 (15.9)
Histology	
Planocellular carcinoma	199 (64.4)
Adenocarcinoma	86 (27.7)
Small cell carcinoma	19 (6.1)
Large cell carcinoma	2 (0.6)
Other	3 (1.0)
Differentiation grade	
Grade 1	28 (15.5)
Grade 2	136 (44.0)
Grade 3	102 (33.0)
Undifferentiated	16 (5.2)
Missing	7 (2.3)
Recurrence	
Yes	69 (22.3)
No	240 (77.7)
Treatment	
Primary surgery	184 (59.5)
Primary surgery + radiotherapy	102 (33.)
Primary surgery + radio/chemotherapy	14 (4.5)
Primary radiotherapy	5 (1.6)
Unknown	4 (1.3)

Discussion

Claudins, tight junction proteins expressed on cell membranes that regulate the permeability, barrier function, and polarity of epithelial cell layers, are found to be dysregulated in many different solid cancer types. Here, we performed comprehensive analyses on CLDN6 expression in gynecological malignancies. We show that advanced stage serous ovarian cancer, as well as serous and clear cell endometrial cancer often upregulate CLDN6.

A high percentage of serous ovarian cancer (79.3%) and serous endometrial cancer patients (83.3%) expressed CLDN6 on their tumors. Normal uterus and ovary tissues do not express CLDN6 (19), and therefore upregulation seems correlated with malignant potential. Low-grade tumors were significantly more often negative for CLDN6, suggesting CLDN6 is correlated to differentiation of the tumor. Thus, we detected expression of CLDN6 on those tumor subtypes that are most aggressive and therefore account for most deaths within both OC and EC. Alternative treatment strategies are urgently warranted to improve the poor prognosis associated with these subtypes, and CLDN6 represents a good target for this.

In serous ovarian cancer we compared tumor samples that were either taken at primary surgery or after neo-adjuvant chemotherapy. The two groups did not differ in CLDN6 expression, suggesting expression is not downregulated after treatment with chemotherapy. This is a crucial finding for treatment options, since these patients will likely receive standard treatment of chemotherapy before they will be eligible for immunotherapeutic strategies. Furthermore, combination of chemotherapy with immunotherapy might lead to synergistic effects. The high expression levels also provide rationale for tracer-labeled targeting of this protein for diagnostic purposes.

Importantly, the different subtypes of ovarian and endometrial cancers show various levels of positive cases, demonstrating the importance of analyzing these groups separately. In this study, endometrioid and clear cell ovarian cancer were not included. It will be of interest to determine the expression levels of CLDN6 in a large cohort containing these tumor types to confirm if these patients are also eligible for CLDN6-targeted therapy. Previous work on ovarian and endometrial cancer showed a high expression of CLDN6 (19,25), with no expression in mucinous ovarian (0%), and high expression in high grade serous ovarian cancer (58%). Furthermore, endometrioid endometrial cancer was analyzed in 24 patients with 21% positivity. Here, we analyzed the expression on large homogeneous well-defined cohorts, and show similar percentages of positive cases. Serous ovarian cancer and serous endometrial cancer are the subtypes of these malignancies with an overall worse prognosis, in which current treatment strategies are in most cases not curative. Therefore, a specific new target for antibody treatment that is present on a large proportion of these tumors has the potential to improve prognosis in these patients.

Antibody-based therapies have been developed and are in use for several solid and hematological cancers. Examples of those include Bevacizumab (targeting VEGF), Cetuximab (targeting EGFR), and Trastuzumab (targeting HER2). Other options include bispecific antibodies such as Catumaxomab, which targets both EpCAM, expressed on tumor cells, and CD3, expressed on T cells. For functionality of treatment with these antibodies, the target needs to be abundantly expressed on the tumor cells. Function may either be blocking of signaling crucial for survival of targeted tumor cells, or induction of an immune response due to binding of the antibody to the target. The bispecific function of Catumaxomab leads to recruitment and activation of CD3+ T cells to the tumor environment, where epithelial tumor cells express EpCAM. Furthermore, antibody treatment may induce tumor killing via antibody-dependent, cell-mediated cell toxicity (ADCC) through retention of a functional Fc domain on the antibody bound to the tumor cell. So far, clinical successes have been reported for these strategies (26–30), but the high variability of expression of the target on tumor cells hinders clinical efficacy.

The high percentages of CLDN6-expressing tumors in ovarian and endometrial cancer patients provide rationale for targeted antibody-treatment directed against CLDN6. Currently, a monoclonal antibody directed against CLDN6 is tested in clinical setting for serous ovarian cancer (ClinicalTrials.gov; NCT02054351). This strategy induces ADCC or complement-dependent cytotoxicity (CDC), boosting the immune response towards these tumors. Since expression of CLDN6 is restricted to embryonic development, the selective expression on tumor cells provides for targeting with a low risk of off-target effects. Next to determining efficacy, another question is how clinical efficacy relates to extent of tumoral expression of CLDN6, in order to be able to determine eligibility of patients for this treatment strategy.

Expression of CLDN6 was not found in many grade 1 tumors, but was present in a large percentage of tumors with a higher differentiation grade, suggesting CLDN6 is upregulated upon tumor progression. This suggests that CLDN6 may not only be a potential specific target, but might also have a function in tumor growth or aggressiveness. Previous *in vitro* work has shown that overexpression of CLDN6 in gastric tumor cells is related to an increase in proliferation, enhanced migration and invasive potential (21). This would mean targeting of this protein potentially could lead to inhibition of tumor growth directly. Future studies focusing on the function of CLDN6 and the effect of overexpression on tumorigenesis may provide new leads in this regard.

In conclusion, we showed here in large, well-defined cohorts of gynecological malignancies that CLDN6 is an interesting therapeutic target in multiple histological subtypes of ovarian and endometrial cancer. The finding that tumors show high expression levels of CLDN6, while expression is not present in healthy tissues, makes this CLDN a very suitable target for immunotherapeutic strategies.

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