

# Mesothelin expression in esophageal adenocarcinoma and squamous cell carcinoma and its possible impact on future treatment strategies

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## Abstract

**Background:** Mesothelin is expressed at very low levels by normal mesothelial cells but is overexpressed in several human cancers. This makes mesothelin a promising target for immunotherapy. Limited data exist about mesothelin expression in esophageal carcinoma. In a current clinical trial, the highly potent anti-mesothelin antibody anatumab ravtansine is used in patients with mesothelin-positive tumors. Response rates are correlated with mesothelin expression (using the Ventana antibody) in tumor cells. No data are available on expression levels using the Ventana antibody. Most data have been generated using the Novocastra antibody. As patients are selected for clinical trials based on antibody staining of tumor samples, a comparison of these two available antibodies is crucial.

**Methods:** We analyzed 481 esophageal carcinomas [373 esophageal adenocarcinomas (EACs), 108 esophageal squamous cell carcinomas (ESCCs)] using two different monoclonal antibodies (Novocastra and Ventana) for mesothelin expression (low-mid and high-level expression, as used in one clinical trial). We also checked for the correlation of these results with clinical and molecular data.

**Results:** We revealed different staining results for both antibodies in EACs: Ventana: 53.6% (low expression: 25.3%; high expression: 28.3%) and Novocastra: 35.7% (low expression: 21.2%; high expression 14.5%). In ESCC we found comparable staining results: Ventana: 13.3% (low expression: 9.5%; high expression: 3.8%) and Novocastra: 13% (low expression: 11.1%; high expression: 1.9%). ARID1a-deficient EAC patients demonstrated significantly higher rates of mesothelin-positive tumors than ARID1a intact EAC patients. No correlations were found with other molecular alterations (*TP53* mutation, *ERBB2* amplification) or survival rates.

**Conclusion:** To the best of our knowledge, this is the largest study analyzing the importance of mesothelin expression in esophageal carcinoma. This study revealed a significant number of mesothelin-positive esophageal carcinomas, especially adenocarcinomas. New therapeutic targets are urgently required to improve the outcome of patients with locally advanced or metastasized esophageal carcinoma. The inhibition of mesothelin can be a new attractive target.

**Keywords:** esophageal carcinoma, mesothelin expression, targeted therapy

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## Introduction

The incidence of esophageal adenocarcinoma has increased rapidly over the past few decades, mainly in the western world, both by period and birth cohort.<sup>1</sup> Despite improvements in perioperative treatments, the overall survival of patients with esophageal carcinoma remains low. The relative 5-year survival rate remains poor, with 21% in both sexes.<sup>2</sup>

Mesothelin is a 40-kDa cell surface glycoprotein expressed at very low levels by normal mesothelial cells lining the pleura, peritoneum, and pericardium.<sup>3</sup> It was initially identified in 1992 by Chang *et al.*<sup>4</sup> The function of mesothelin is not yet completely clear, but in normal mesothelial cells, it seems to be involved in cell adhesion, cell differentiation, and signal transduction.<sup>5</sup> Normal esophageal mucosa does not express mesothelin.<sup>6,7</sup> In cancer cells, mesothelin seems to be involved in the promotion of proliferation and cell migration, resistance to chemotherapy, and inhibition of apoptosis.<sup>8–10</sup> Mesothelin overexpression has been described in several human cancers, including virtually all mesotheliomas and pancreatic adenocarcinoma, approximately 70% of ovarian and extra bile duct carcinomas, and 50% of lung and gastric adenocarcinomas.<sup>3,11–13</sup>

Limited data on mesothelin expression in esophageal carcinoma exist so far. Alvarez *et al.* analyzed 84 esophageal adenocarcinomas and found that approximately one third were positive for mesothelin using the Novocastra antibody.<sup>14</sup> In a study by Chang *et al.* 86% of 13 analyzed esophageal squamous cell carcinomas (ESCCs) showed mesothelin expression using another antibody that is not commercially available.<sup>15</sup> The very limited expression of mesothelin in normal tissue and the high expression in malignant tumors offers an opportunity for mesothelin-targeted therapy.

One aim of our study was to compare the level of mesothelin expression in a large number of esophageal carcinomas using two different commercially available monoclonal immunohistochemical antibodies: Novocastra (mesothelin mouse monoclonal antibody clone 5B2) and Ventana [MSLN (clone SP74), a ready-to-use monoclonal rabbit antibody currently being used in a clinical trial].<sup>16</sup> Most previous data about mesothelin expression have been generated using antibodies like the Novocastra antibody, and there are no available data on mesothelin expression levels using the Ventana antibody. As patients for clinical trials and potential future treatment are selected by

antibody staining of tumor samples, comparison of these two available antibodies is crucial.

## Materials and methods

### *Patients and tumor samples*

We analyzed formalin-fixed and paraffin-embedded material from 481 patients with esophageal carcinomas (including 373 adenocarcinomas and 108 squamous cell carcinomas) who underwent primary surgical resection or resection after neoadjuvant therapy between 1999 and 2012 at the Department of General, Visceral and Cancer Surgery, University of Cologne, Germany. The standard surgical procedure was laparotomic or laparoscopic gastrotomy and right transthoracic en-bloc esophagectomy, including two-field lymphadenectomy of mediastinal and abdominal lymph nodes. Reconstruction was performed by intrathoracic esophagogastronomy, as described previously.<sup>17</sup>

Patients with advanced esophageal cancer (cT3, cNx, M0) received preoperative chemoradiation (5-FU, cisplatin, 40 Gy according to the CROSS protocol) or chemotherapy alone (according to the FLOT protocol). Follow-up data were available for all patients. Patient characteristics are shown in Tables 1–4. Depending on the effect of neoadjuvant chemotherapy or chemoradiotherapy, there is a preponderance of minor responders, defined as histopathological residual tumor of  $\geq 10\%$ .<sup>18</sup>

For tissue microarrays (TMAs), one tissue core from each tumor was punched out and transferred into a TMA recipient block. TMA construction was performed as previously described.<sup>19,20</sup> In brief, tissue cylinders with a diameter of 1.2 mm each were punched from selected tumor tissue blocks using a self-constructed semi-automated precision instrument and embedded in empty recipient paraffin blocks. Four-micrometer sections of the resulting TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ, USA) for immunohistochemistry.

### *Immunohistochemistry*

Immunohistochemistry (IHC) was performed on single-spot TMAs. In order to answer the question of the heterogeneous distribution of mesothelin expression within the tumor, we additionally examined 38 tumors using a multi-spot TMA considering 12 tumor spots from different tumor areas (for details, refer to a previous study)<sup>21</sup> as

**Table 1.** Univariate analysis of clinical parameters – Esophageal adenocarcinoma – Mesothelin Ventana.

Clinical parameters		Mesothelin Ventana								p value
		Total		Expression score						
				0		1		2		
n	%	n	%	n	%	n	%			
Total number		371	100%	172	46.4%	105	28.3%	94	25.3%	
Sex	Female	38	10.2%	21	55.3%	7	18.4%	10	26.3%	0.333
	Male	333	89.8%	151	45.3%	98	29.4%	84	25.2%	
Age group	0	193	52.0%	84	46.9%	46	25.7%	49	27.4%	0.635
	1	178	48.0%	73	44.2%	50	30.3%	42	25.5%	
pT	1	43	11.6%	22	51.2%	14	32.6%	7	16.3%	0.838
	2	33	8.9%	15	45.5%	9	27.3%	9	27.3%	
	3	283	76.5%	129	45.6%	78	27.6%	76	26.9%	
	4	11	3.0%	5	45.5%	4	36.4%	2	18.2%	
pN	0	144	38.9%	78	54.2%	39	27.1%	27	18.8%	0.061
	1	140	37.8%	61	43.6%	41	29.3%	38	27.1%	
	2	44	11.9%	16	36.4%	16	36.4%	12	27.3%	
	3	42	11.4%	16	38.1%	9	21.4%	17	40.5%	
UICC	1	69	18.7%	35	50.9%	21	30.9%	13	18.2%	0.279
	2	67	18.0%	40	60.4%	13	18.9%	14	20.8%	
	3	148	39.8%	57	38.5%	45	30.8%	45	30.8%	
	4	69	23.5%	29	42.0%	22	31.9%	18	26.1%	

Sum of patients does not add to 371 patients due to missing clinical data or missing tumor spots on the TMA.  
pN, pathological classification of the degree of spread to regional lymph nodes; pT, pathological classification of the size or direct extent of the primary tumor; TMA, tissue microarray; UICC, Union internationale contre le cancer stage.

well as ten large-scale whole tumor slides using two antibodies. These ten cases are considered in the multi-spot TMA and the 38 tumors of the multi-spot TMA are considered in the single spot TMA. The structure of our multi-Spot TMA is able to reflect the expression situation of mesothelin on the large tumor surface.

Ventana antibody: MSLN, clone SP74, rabbit monoclonal antibody, ready to use (Ethylene-diamine tetraacetic acid (EDTA) buffer) on Ventana Benchmark stainer.

Novocastra antibody: mesothelin, clone 5B2, mouse monoclonal using a dilution of 1:50 with EDTA buffer on BOND stainer.

The mesothelin staining intensity was scored manually by two pathologists (AQ and HL) according to a two-tier scoring system (low–mid level and high level) used in a phase Ib study by Bayer [A phase Ib multi-indication study of anatumab ravtansine (BAY 94-9343) in patients with mesothelin-expressing advanced or recurrent malignancies]. In this study protocol, Score 2 (high expression) means  $\geq 30\%$  positive tumor cells with 2+ or 3+ membrane staining intensity and Score 1 (low–mid expression) means  $\geq 5\%$  all intensities and  $< 30\%$  positive tumor cells with 2+ or 3+ membrane staining intensity.<sup>16</sup>

In addition, we used a well-known analysis algorithm of IHC (H-Score) and applied two

**Table 2.** Univariate analysis of clinical parameters – Esophageal adenocarcinoma – Mesothelin Novocastra.

Clinical parameters		Mesothelin Ventana								p value
		Total		Expression score						
				0		1		2		
n	%	n	%	n	%	n	%			
Total number		373	100%	240	64.3%	79	21.2%	54	14.5%	
Sex	Female	39	10.5%	26	66.7%	9	23.1%	4	11.2%	0.724
	Male	334	89.5%	214	64.1%	70	21.0%	50	15.0%	
Age group	0	192	51.6%	113	63.1%	38	21.2%	28	15.6%	0.994
	1	181	48.4%	107	63.7%	35	20.8%	26	15.5%	
pT	1	41	11.0%	29	70.7%	11	26.8%	1	2.4%	0.285
	2	33	8.9%	20	60.6%	9	27.3%	4	12.1%	
	3	287	77.2%	182	63.4%	57	19.9%	48	16.7%	
	4	11	3.0%	8	72.7%	2	18.2%	1	9.1%	
pN	0	140	37.6%	98	70.0%	31	22.1%	11	7.9%	0.09
	1	146	39.2%	93	63.7%	30	20.5%	23	15.8%	
	2	44	11.8%	24	54.5%	9	20.5%	11	25.0%	
	3	42	11.3%	24	57.1%	9	21.4%	9	21.4%	
UICC	1	66	17.7%	46	69.8%	16	24.5%	4	5.7%	0.144
	2	68	18.1%	49	72.2%	12	18.5%	6	9.3%	
	3	151	40.5%	89	58.7%	30	19.8%	32	21.5%	
	4	88	23.7%	56	63.4%	17	19.7%	15	16.9%	

Sum of patients does not add to 373 patients due to missing clinical data or missing tumor spots on the TMA.  
pN, pathological classification of the degree of spread to regional lymph nodes; pT, pathological classification of the size or direct extent of the primary tumor; TMA, tissue microarray; UICC, Union internationale contre le cancer stage.

established interpretation pathways [two-tier expression behavior in low and high (H-Score 2) and three-tier in low, intermediate, and high (H-Score 1)]. The H-Score is obtained using the following formula:  $3 \times$  percentage of strongly stained cells +  $2 \times$  percentage of moderately stained cells and percentage of weakly stained tumor cells. The H-Score ranges from 0 to 300. We analyzed both scoring systems (study-related scoring protocol *versus* H-Scores) in a blinded manner and from clinical and molecular data. Discrepant results were resolved by a consensus review.

Procedures were followed as outlined in accordance with ethical standards formulated in the

Helsinki Declaration 1995 (and revised in 2000). Patients provided their written consent with respect to the use of their tumor specimens; an approval was obtained from the University of Cologne Ethics Committee (reference number: 13-091).

#### Statistical analyses

Clinical data were collected prospectively according to a standardized protocol. For statistical analysis, SPSS Statistics for Mac (IBM Corp. Released 2012. IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY: IBM Corp.) was used. Interdependence between staining and clinical data was calculated using the chi-squared and Fisher's

**Table 3.** Univariate analysis of clinical parameters – Esophageal squamous cell carcinoma – Mesothelin Ventana.

Clinical parameters		Mesothelin Ventana								p value
		Total		Expression score						
		n	%	0		1		2		
n	%			n	%	n	%	n	%	
Total number		105	100%	91	86.7%	10	9.5%	4	3.7%	
Sex	Female	27	25.7%	25	92.6%	2	7.4%	0	0.0%	0.427
	Male	78	74.3%	66	84.6%	8	10.3%	4	5.1%	
Age group	0	55	52.0%	50	91.7%	5	8.3%	49	0.0%	0.354
	1	50	48.0%	42	83.6%	6	11.3%	3	5.1%	
pT	1	13	12.7%	10	76.9%	0	0.0%	3	23.1%	<0.001
	2	4	3.9%	4	100.0%	0	0.0%	0	0.0%	
	3	84	82.4%	75	89.3%	8	9.5%	1	1.2%	
	4	1	1.0%	0	0.0%	1	100.0%	0	0.0%	
pN	0	36	35.0%	32	88.9%	2	5.6%	2	5.6%	0.895
	1	63	61.2%	53	84.1%	8	12.7%	2	3.2%	
	2	2	1.9%	2	100.0%	0	0.0%	0	0.0%	
	3	2	1.9%	2	100.0%	0	0.0%	0	0.0%	
UICC	1	12	11.3%	9	74.3%	0	0.0%	3	25.7%	0.003
	2	7	6.7%	7	100.0%	0	0.0%	0	0.0%	
	3	83	79.5%	75	89.6%	7	7.9%	2	2.5%	
	4	3	2.5%	1	25.0%	2	75.0%	0	0.0%	

Sum of patients does not add to 105 patients due to missing clinical data or missing tumor spots on the TMA.

pN, pathological classification of the degree of spread to regional lymph nodes; pT, pathological classification of the size or direct extent of the primary tumor; TMA, tissue microarray; UICC, Union internationale contre le cancer stage.

exact tests and displayed by cross-tables. The interdependence of mesothelin expression by Ventana and Novocastra antibodies was additionally tested by kappa and Goodman–Kruskal tau test. Survival curves were plotted using the Kaplan–Meier method and analyzed using the log-rank test.

## Results

In cross-table analysis (chi-square test), a strong correlation was observed between Ventana and Novocastra ( $p < 0.001$ ). The kappa value for the correlation was 0.447 ( $p < 0.001$ ), and the Goodman–Kruskal tau correlation was 0.372 ( $p < 0.001$ ).

### Esophageal adenocarcinoma

**Ventana antibody.** A total of 371 esophageal adenocarcinomas were analyzable; 53.6% showed a mesothelin expression. Of these, 25.3% showed a low mesothelin expression (Score 1) and 28.3% a high expression (Score 2; Table 1 and Figures 1, 3, and 5). Only tumor cells showed mesothelin expression (Figures 7 and 8).

**Novocastra antibody.** A total of 373 esophageal adenocarcinomas were analyzable. The detection rate of mesothelin-positive tumors was lower with the Novocastra antibody; 35.7% demonstrated mesothelin expression. Of these, 21.2% showed a low mesothelin expression (Score 1) and 14.5% a high

**Table 4.** Univariate analysis of clinical parameters – Esophageal squamous cell carcinoma – Mesothelin Novocastra.

Clinical parameters		Mesothelin Ventana								p value
		Total		Expression score						
		n	%	0		1		2		
n	%			n	%	n	%	n	%	
Total number		108	100%	94	87.0%	12	11.1%	3	1.9%	
Sex	Female	28	25.9%	26	92.9%	2	7.1%	0	0%	0.501
	Male	80	74.1%	68	85.0%	10	12.5%	2	2.5%	
Age group	0	56	51.6%	113	92.9%	38	7.1%	28	0,0%	0.654
	1	52	48.4%	107	85.0%	35	12.5%	26	2.5%	
pT	1	15	14.3%	12	80.0%	2	13.3%	1	6.7%	0.815
	2	4	3.8%	4	100.0%	0	0.0%	0	0%	
	3	85	81.0%	75	88.2%	9	10.6%	1	1.2%	
	4	1	1.0%	1	100.0%	0	0%	0	0%	
pN	0	37	34.9%	33	89.2%	3	8.1%	1	2.7%	0.941
	1	64	60.4%	54	84.4%	9	14.1%	1	1.6%	
	2	3	2.8%	3	100.0%	0	0%	0	0%	
	3	2	1.9%	2	100.0%	0	0%	0	0%	
UICC	1	14	13,0%	11	78.0%	2	15.3%	1	6.7%	0.905
	2	4	3.8%	3	75.0%	1	25.0%	0	0%	
	3	89	82.0%	75	85.2%	12	13.5%	1	1.3%	
	4	1	1.2%	1	100.0%	0	0%	0	0%	

Sum of patients does not add to 108 patients due to missing clinical data or missing tumor spots on the TMA.

pN, pathological classification of the degree of spread to regional lymph nodes; pT, pathological classification of the size or direct extent of the primary tumor; TMA, tissue microarray; UICC, Union internationale contre le cancer stage.

expression (Score 2; Table 2 and Figures 2, 4, and 6). Only tumor cells showed mesothelin expression.

#### *Esophageal squamous cell carcinoma*

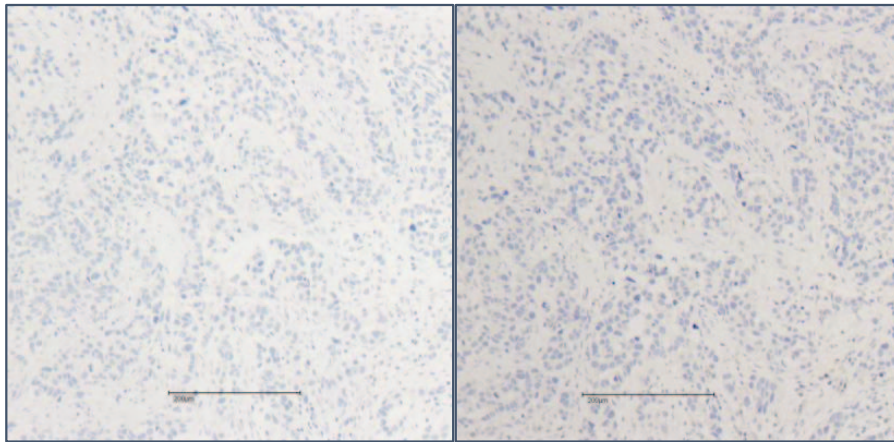
Mesothelin expression in ESCC is lower compared with that in adenocarcinoma.

*Ventana antibody.* A total of 105 ESCCs were analyzable; 13.3% of these in our cohort were mesothelin positive. Of these, 9.5% showed a low expression (Score 1) and 3.8% a high mesothelin expression (Score 2; Table 3 and Figures 9 and 10). Only tumor cells showed mesothelin expression.

*Novocastra antibody.* A total of 108 ESCCs were analyzable; 13% of these in our cohort were mesothelin- positive using the Novocastra antibody. Of these, 11.1% showed a low expression (Score 1) and 1.9% a high mesothelin expression (Score 2; Table 4 and Figures 9 and 10). Only tumor cells showed mesothelin expression.

#### *Mesothelin expression and correlation to clinical parameters*

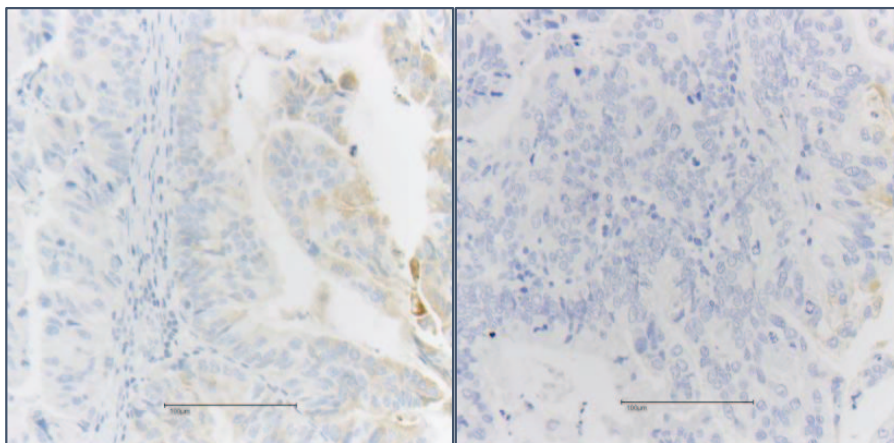
*Esophageal adenocarcinoma.* We could not find any positive correlations between mesothelin expression and tumor stages [Union internationale



**A: Ventana, negative, magnification 100x, scale bar 200  $\mu$ m**

**B: Novocastra, negative, magnification 100x, scale bar 200  $\mu$ m**

**Figures 1 and 2.** The same esophageal adenocarcinoma completely negative for mesothelin using both antibodies.



**C: Ventana, Score 1, magnification 200x, scale bar 100  $\mu$ m**

**D: Novocastra, Score 1, magnification 200x, scale bar 100  $\mu$ m**

**Figures 3 and 4.** The same esophageal adenocarcinoma with low staining intensity (Score 1). Ventana antibody detects few more tumor cells. Both antibodies show a more pronounced cytoplasmatic staining pattern.

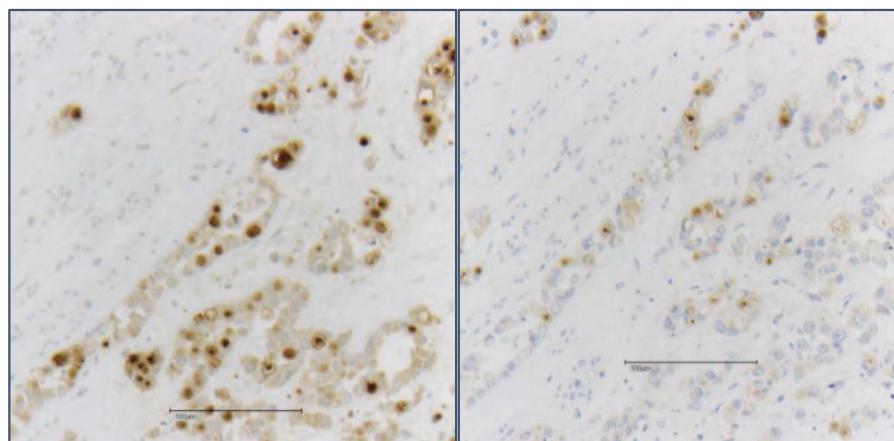
contre le cancer (UICC) stages (Ventana:  $p=0.279$ ; Novocastra:  $p=0.144$ ), sex (Ventana:  $p=0.333$ ; Novocastra:  $p=0.724$ ) or survival data (Tables 1 and 2)].

*Esophageal squamous cell carcinoma.* In ESCCs, there are contradictory results for the UICC stages when using different antibodies. The Ventana antibody-related staining results revealed statistically significant correlations of mesothelin expression and advanced tumor stages (Ventana:  $<0.001$ ), whereas the staining results found by Novocastra

showed no such correlations:  $p=0.815$ . Furthermore, no correlations were found when considering the sex (Ventana:  $p=0.427$ ; Novocastra:  $p=0.501$ ; Tables 3 and 4).

#### *Mesothelin expression and correlation to molecular tumor parameters*

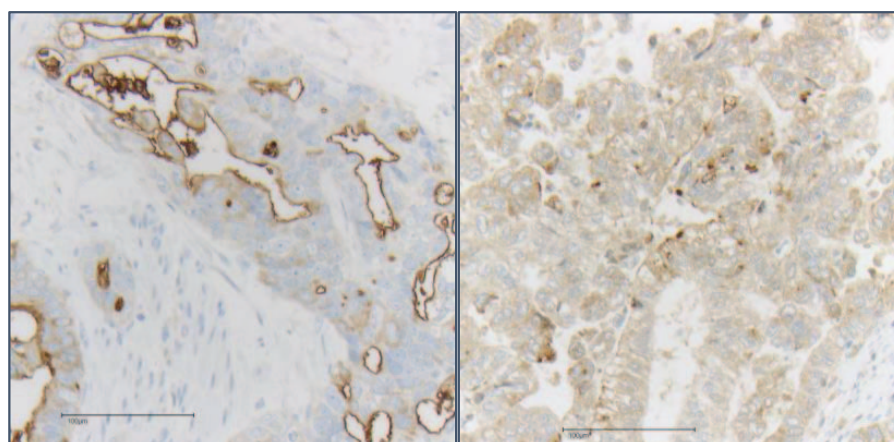
A correlation between molecular data and mesothelin expression was observed for EAC alone. However, a correlation between mesothelin expression and TP53 mutations (Ventana:  $p=0.619$ ,



**E: Ventana, Score 2, magnification 200x, scale bar 100  $\mu$ m**

**F: Novocastra, Score 2, magnification 200x, scale bar 100  $\mu$ m**

**Figures 5 and 6.** The same esophageal adenocarcinoma with high staining intensity (Score 2). Ventana antibody detects few more tumor cells and shows a combined drop-like and cytoplasmatic staining pattern. Novocastra antibody shows a drop-like pattern.



**G: Drop-like (short arrow) and membranous (long arrow) staining pattern, magnification 200x, scale bar 100  $\mu$ m**

**H: Membranous (long arrow) and cytoplasmic (short arrow) staining pattern; magnification 200x, scale bar 100  $\mu$ m**

**Figures 7 and 8.** Different positive staining pattern for mesothelin in esophageal adenocarcinoma.

Novocastra:  $p=0.592$ ) and Her-2 amplification (Ventana:  $p=0.905$ , Novocastra:  $p=0.102$ ) was not found, respectively. Mesothelin expression was correlated with loss of the chromatin remodeling protein ARID1A (Ventana:  $p<0.001$ , Novocastra:  $p=0.003$ ).

*Mesothelin expression and correlation to overall survival*

We could not find any positive correlation between mesothelin expression and overall

survival (Ventana:  $p=0.327$ ; Novocastra:  $p=0.323$ ) (see Supplemental material).

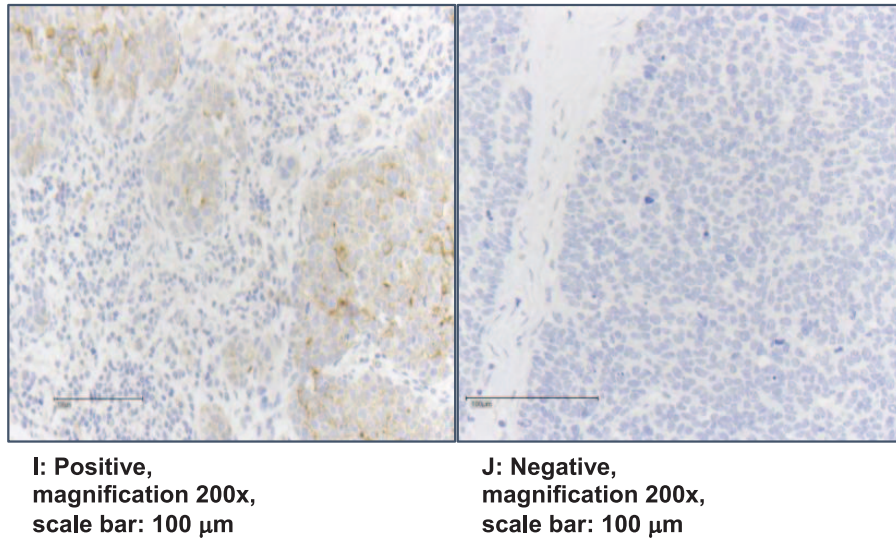
*H-Score*

The additionally applied H-Score showed a high concordance of the evaluation results with the study protocol used in parallel (Table 5).

*Heterogeneity of mesothelin expression*

In order to answer the question of the heterogeneous distribution of mesothelin expression





**Figures 9 and 10.** Esophageal squamous cell carcinoma using the Novocastra antibody (identical staining pattern using the Ventana antibody).

within the tumor, we additionally examined 38 tumors. We analyzed multi-spot TMAs (12 tumor biopsies) and large tumor areas.

Two cases showed a significant heterogeneity in the expression behavior of mesothelin. One primary tumor was negative for mesothelin but positive in lymph node metastasis, and one additional tumor showed mesothelin expression of its tumor cells only in the invasion zone, while the near-surface tumor portions were negative. The remaining 36 tumors showed a homogeneous expression distribution within the tumor even when using the H-Score with only minor variations.

## Discussion

This is the largest and, to the best of our knowledge, the first comparative description of mesothelin protein expression in esophageal carcinomas considering different immunohistochemical antibodies.

Currently, very little data exist about mesothelin expression in esophageal carcinomas. The expression data of mesothelin in gastric cancers cannot be transferred to esophageal carcinomas<sup>22,23</sup> since comparative molecular analyses of gastric adenocarcinomas revealed important differences between gastric and esophageal adenocarcinomas (e.g. absence of Epstein–Barr virus and near absence of microsatellite instability subtypes in esophageal adenocarcinoma).<sup>24</sup> This further

emphasizes the importance of our study that places large-scale focus on esophageal carcinoma.

Most data about mesothelin expression were generated using monoclonal antibodies like the Novocastra antibody.<sup>7,14,25</sup> There are presently no data available on the expression levels of the Ventana antibody. As patients for clinical trials and potential future use are selected by antibody staining of tumor samples, the comparison of these two antibodies is crucial. Our study revealed significant differences in the staining results of the two antibodies used, indicating a more sensitive detection rate of mesothelin expression for the Ventana kit, at least in esophageal adenocarcinomas, but comparable results for ESCC.

The results of our study are consistent with those obtained by Alvarez *et al.* who showed that approximately one-third of esophageal adenocarcinomas express mesothelin using the Novocastra antibody (in our study the Novocastra antibody showed mesothelin expression in 35.7% of esophageal adenocarcinomas) even though they used a lower number of tumor samples ( $n = 84$ ).<sup>14</sup>

Furthermore, we could demonstrate that mesothelin expression is much lower in ESCCs with 13.3% using the Ventana antibody *versus* 13% using the Novocastra antibody.

These findings contradict the results of Chang *et al.*, who described expression of mesothelin in

**Table 5.** The additionally applied H-Score showed a high concordance of the evaluation results with the study protocol used in parallel.

	Total number	Mesothelin H1						Mesothelin H2										
		0		1		2		3		0		1		2				
		n	%	n	%	n	%	n	%	n	%	n	%	n	%			
Novocastra antibody	352	156	45.1%	97	27.6%	53	15.1%	46	13.1%	<0.0001	156	44.3%	120	34.1%	76	21.6%	<0.0001	
Mesothelin	Total number	0	223	151	67.7%	59	26.5%	8	3.6%	5	2.2%	151	67.7%	65	29.1%	7	3.1%	
	1	76	5	6.6%	34	44.7%	30	39.5%	7	9.2%	5	6.6%	48	63.2%	23	30.3%		
	2	53	0	0.0%	4	7.5%	15	28.3%	34	64.2%	0	0.0%	7	13.2%	46	64.2%		
Ventana antibody	359	162	45.1%	98	27.3%	53	14.8%	46	12.8%	<0.0001	162	45.1%	121	33.7%	76	21.2%	<0.0001	
Mesothelin	Total number	0	164	150	91.5%	13	7.9%	0	0.0%	1	0.6%	150	91.5%	13	7.9%	1	0.60%	
	1	102	12	11.8%	77	75.5%	12	11.8%	1	1.0%	12	11.0%	87	85.3%	3	2.9%		
	2	93	0	0.0%	8	8.6%	41	44.1%	44	47.3%	0	0.0%	21	22.6%	72	77.4%		

Mesothelin, Results according to study protocol; Mesothelin H1, Results according to the H-Score [3-tier scoring algorithm]; Mesothelin H2, Results according to the H-Score [2-tier scoring algorithm].

86% of ESCCs,<sup>15</sup> and emphasize the importance of our study further. The significantly higher expression rate might be explained by the very small number of tumor samples they analyzed ( $n=13$ ) and the self-constructed, and not commercially available antibody used in the study (MAb K1).<sup>26</sup>

However, some limitations of our study should be noted. As previously described, there are no available data describing expression levels of mesothelin using the Ventana antibody. Our results indicate that the Ventana antibody is more sensitive in terms of mesothelin detection in esophageal adenocarcinoma. We do not have an explanation for this fact. Therefore, this should be addressed in future studies on this topic.

The fact that only two previous studies with a small number of cases on mesothelin expression in esophageal carcinoma exist so far and the limited tumor sampling are other limitations that should be mentioned. Both should be addressed in future studies with a higher number of tumor samples to detect the expression of mesothelin in esophageal carcinoma.

Regarding mesothelin as a potential target for immunotherapy, these findings may have an impact on screening procedures and possible future therapeutic strategies. The fact that it is only expressed at very low levels by normal mesothelial cells lining the pleura, peritoneum, and pericardium,<sup>3</sup> and its overexpression in several human cancers, including virtually all mesothelioma and pancreatic adenocarcinomas as well as approximately 70% of ovarian and extrahepatic bile duct carcinomas and 50% of lung and gastric adenocarcinomas,<sup>3,11-13</sup> make it such an appealing target. We did not observe any staining in the endothelia or nerves.

Different novel antibody-based therapeutics targeting mesothelin for solid tumors are in various stages of preclinical and clinical development and have shown anti-tumor activity.<sup>16,27-31</sup> In a current phase Ib study anetumab ravtansine is being investigated in different mesothelin-expressing advanced or recurrent malignancies [non-small cell lung cancer (NSCLC), TNBC (triple-negative breast cancer), gastric adenocarcinoma including gastro-esophageal-junction (GEJ) cancer, thymic carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma] to evaluate safety and efficacy in the respective indications in patients

with either of two mesothelin expression levels (the same expression levels we used in our study).<sup>16</sup> The results are not available yet, but might predict a response rate according to the level of mesothelin expression.<sup>16</sup>

How homogeneously a protein relevant for therapy is distributed within the tumor is important from both a therapeutic and a diagnostic point of view. The probability of the efficacy of a therapy increases with the homogeneity of protein distribution. Tumor segments close to the surface, usually achieved with endoscopically obtained tumor biopsies, showing a different expression behavior compared with tumor segments from the invasion zone are also important from a diagnostic point of view, since the entire tumor situation can no longer be deduced from the endoscopically obtained material. We have devoted ourselves to this question and found a relevant homogeneity of mesothelin expression in different tumor areas. It can be assumed that endoscopic biopsy material is likely to provide representative results for the residual tumor.

Due to the extremely aggressive nature and poor survival rates of locally advanced or metastasized esophageal carcinoma, new targets to improve the outcome of patients suffering from these diseases are crucial. The inhibition of mesothelin could be such a novel attractive target. This study revealed a significant number of mesothelin-positive esophageal carcinomas, especially adenocarcinomas.

#### Availability of data and materials

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

#### Conflict of interest

The authors declare that there is no conflict of interest.

#### Consent for publication

Not applicable.

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#### Research ethics and patient consent

Procedures were followed as outlined in accordance with ethical standards formulated in the Helsinki Declaration 1995 (and revised in 2000).

Patients gave their written consent for usage of their tumor specimens, and approval was obtained from the University of Cologne Ethics Committee (reference number: 13-091).

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#### Supplemental material

Supplemental material for this article is available online.

#### References

1. Lepage C, Rachtel B, Jooste V, *et al.* Continuing rapid increase in esophageal adenocarcinoma in England and Wales. *Am J Gastroenterol* 2008; 103: 2694–2699.
2. Robert Koch-Institut (Hrsg) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg). Berlin. Krebs in Deutschland für 2013/2014. In Vol 11. Ausgabe 2017.
3. Chang K and Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci U S A* 1996; 93: 136–140.
4. Chang K, Pai LH, Batra JK, *et al.* Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. *Cancer Res* 1992; 52: 181–186.
5. Rump A, Morikawa Y, Tanaka M, *et al.* Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J Biol Chem* 2004; 279: 9190–9198.
6. Rizk NP, Servais EL, Tang LH, *et al.* Tissue and serum mesothelin are potential markers of neoplastic progression in Barrett's associated esophageal adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2012; 21: 482–486.
7. Ordonez NG. Application of mesothelin immunostaining in tumor diagnosis. *Am J Surg Pathol* 2003; 27: 1418–1428.
8. Kaneko O, Gong L, Zhang J, *et al.* A binding domain on mesothelin for CA125/MUC16. *J Biol Chem* 2009; 284: 3739–3749.
9. Uehara N, Matsuoka Y and Tsubura A. Mesothelin promotes anchorage-independent growth and prevents anoikis via extracellular signal-regulated kinase signaling pathway in

- human breast cancer cells. *Mol Cancer Res* 2008; 6: 186–193.
10. Cheng WF, Huang CY, Chang MC, *et al.* High mesothelin correlates with chemoresistance and poor survival in epithelial ovarian carcinoma. *Br J Cancer* 2009; 100: 1144–1153.
  11. Argani P, Iacobuzio-Donahue C, Ryu B, *et al.* Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* 2001; 7: 3862–3868.
  12. Tchou J, Wang LC, Selven B, *et al.* Mesothelin, a novel immunotherapy target for triple negative breast cancer. *Breast Cancer Res Treat* 2012; 133: 799–804.
  13. Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. *Am J Surg Pathol* 2003; 27: 1031–1051.
  14. Alvarez H, Rojas PL, Yong KT, *et al.* Mesothelin is a specific biomarker of invasive cancer in the Barrett-associated adenocarcinoma progression model: translational implications for diagnosis and therapy. *Nanomedicine* 2008; 4: 295–301.
  15. Chang K, Pastan I and Willingham MC. Frequent expression of the tumor antigen CAK1 in squamous-cell carcinomas. *Int J Cancer* 1992; 51: 548–554.
  16. Adjei A. Phase 1b multi-indication study of the antibody drug conjugate anatumab ravtansine in patients with mesothelin-expressing advanced or recurrent malignancies. *J Clin Oncol* 2018; 36 (Suppl. 15): abstract TPS2607.
  17. Holscher AH, Schneider PM, Gutschow C, *et al.* Laparoscopic ischemic conditioning of the stomach for esophageal replacement. *Ann Surg* 2007; 245: 241–246.
  18. Schneider PM, Metzger R, Schaefer H, *et al.* Response evaluation by endoscopy, rebiopsy, and endoscopic ultrasound does not accurately predict histopathologic regression after neoadjuvant chemoradiation for esophageal cancer. *Ann Surg* 2008; 248: 902–908.
  19. Simon R, Mirlacher M and Sauter G. Tissue microarrays. *Methods Mol Med* 2005; 114: 257–268.
  20. Helbig D, Ihle MA, Putz K, *et al.* Oncogene and therapeutic target analyses in atypical fibroxanthomas and pleomorphic dermal sarcomas. *Oncotarget* 2016; 7: 21763–21774.
  21. Loeser H, Kraemer M, Gebauer F, *et al.* The expression of the immune checkpoint regulator VISTA correlates with improved overall survival in pT1/2 tumor stages in esophageal adenocarcinoma. *Oncoimmunology* 2019; 8: e1581546.
  22. Fukayama M and Ushiku T. Epstein–Barr virus-associated gastric carcinoma. *Pathol Res Pract* 2011; 207: 529–537.
  23. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; 513: 202–209.
  24. Hewitt LC, Inam IZ, Saito Y, *et al.* Epstein–Barr virus and mismatch repair deficiency status differ between oesophageal and gastric cancer: a large multi-centre study. *Eur J Cancer* 2018; 94: 104–114.
  25. Frierson HF Jr, Moskaluk CA, Powell SM, *et al.* Large-scale molecular and tissue microarray analysis of mesothelin expression in common human carcinomas. *Hum Pathol* 2003; 34: 605–609.
  26. Chang K, Pastan I and Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer* 1992; 50: 373–381.
  27. Mizukami T. The anti-mesothelin monoclonal antibody amatuximab enhances the anti-tumor effect of gemcitabine against mesothelin-high expressing pancreatic cancer cells in a peritoneal metastasis mouse model. *Oncotarget* 2018; 9: 33844–33852.
  28. Morphotek. *An efficacy study of MORAb-009 in subjects with pancreatic cancer.* <https://clinicaltrials.gov/ct2/show/results/NCT00570713> (2018, accessed 24 March 2020).
  29. Vogelzang NJ, Rusthoven JJ, Symanowski J, *et al.* Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003; 21: 2636–2644.
  30. Hassan R. A randomized, placebo-controlled study of amatuximab in combination with pemetrexed and cisplatin (P/C) as front-line therapy for subjects with malignant pleural mesothelioma (MPM). *J Clin Oncol* 2016; 34 (Suppl. 15): TPS8577.
  31. Golfier S, Kopitz C, Kahnert A, *et al.* Anatumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. *Mol Cancer Ther* 2014; 13: 1537–1548.