Morphological, clinical and molecular characteristics in ARID1a-deficient microsatellite-stable oesophageal adenocarcinoma

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Abstract. Publications describe the relevance of the AT-rich interactive domain-containing protein 1A (*ARID1a*) mutation in gastric adenocarcinoma, which occurs predominantly in the microsatellite instable (MSI)- and Epstein-Barr virus (EBV)-associated subtypes. It is unclear whether potential therapeutic, prognostic or morphologic descriptions are not epiphenomena of MSI (or EBV). Since personalised therapeutics are largely lacking for oesophageal adenocarcinoma (EAC), clinical trials investigating the efficacy of these therapeutics specifically in this subgroup are useful. To the best of our knowledge, this was the first study analysing the relevant tumour subset of microsatellite-stable (MSS) EAC with loss of function of ARID1a. A total of 875 patients with EAC and data from The Cancer Genome Atlas (TCGA) were analysed. Statistical analyses associating previously known molecular characteristics of the present tumour cohort, overall survival, morphological growth patterns and tumour heterogeneity issues were considered. Subsequently, 10% of EAC were *ARID1a*-deficient, the majority of which were MSS (7.5%). There was no characteristic growth pattern. Approximately 60% of tumours were PD-L1 positive to varying degrees. *TP53* mutations occurred together with ARID1a defective EAC in the present cohort and in the TCGA collective. The extent of 7.5% MSS-EAC with ARID1a loss was unaffected by neoadjuvant therapy. ARID1a loss was often detected to be homogeneous (92%). ARID1a loss is not an epiphenomenon of MSI in EAC. The high homogeneity of ARID1a loss tumour clones could be considered an argument for the effectiveness of potential therapeutics. Since the majority of genomic *ARID1a* alterations result in protein loss, immunohistochemistry is a useful screening technique, especially in the absence of morphological characteristics.

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Introduction

AT-rich interactive domain-containing protein 1a (*ARID1a*) is a functionally relevant component of the switch/sucrose non-fermentable (SWI-SNF) chromatin remodeller complex. Access to various genes is regulated via this complex (1,2). *ARID1a* is one of the most frequently mutated genes in carcinomas and is considered a tumour suppressor gene (3,4). Approximately 50% of clear cell ovarian cancers show *ARID1a* mutations and over 90% of *ARID1a* mutations that occur in ovarian cancers are nonsense or frame-shift mutations that result in loss of protein expression (5,6). Immunohistochemical analyses visualising ARID1a protein in tissue are therefore well suited to reveal underlying gene alterations. Several publications describe the relevance of the *ARID1a* mutation in adenocarcinomas of the stomach, which occur predominantly in the microsatellite unstable (MSI)- and Epstein-Barr virus (EBV)-associated subtypes (7-10). However, molecular alteration of *ARID1a* is likely to represent a biologically minor epiphenomenon of the already highly mutated or epigenetically altered tumours in these subgroups. Little data are available on the significance in oesophageal adenocarcinoma (EAC). Our group, as well as another, have shown that *ARID1a* alterations occur in ~10% of EAC, including MSI tumours (compare in more detail in 'Discussion') (11,12).

When Drage *et al* describe a clustering of the medullary phenotype in ARID1a loss EAC, this may merely describe the underlying MSI phenotype (11). The clinical and molecular significance of ARID1a loss in the non-MSI group of EAC is entirely unclear. However, this distinction is becoming

increasingly clinically relevant. There is mounting evidence that tumours with functional alteration of ARID1a qualify for various therapies. Discussed is the possibility of an increased response probability of immune checkpoint inhibitors (ICI) targeting PD-L1/PD-1 or PARP inhibitors (for further therapy options, see also 'Discussion') (13,14). However, if *ARID1a* alteration is an epiphenomenon in MSI tumours, the predictor of increased treatment response is the underlying high MSI and not the *ARID1a* alteration. MSI tumours also qualify for ICI therapy based on their high PD-L1 expression in the tumour, and the additional determination of ARID1a is probably of little value. Thus, separation of ARID1a-altered tumours independent of MSI is reasonable.

The relevant questions are i) how frequently is ARID1a alteration found in non-MSI-EAC? ii) what is the level of PD-L1 expression in this group? iii) what morphological, clinical, and additional molecular characteristics are found in this subgroup? iv) what is the impact of neoadjuvant therapy regimens used in EAC?

The present work is the first to describe the clinical, molecular, and morphological characteristics of therapy-relevant non-MSI ARID1a loss EAC. To this end, we examined a very large cohort of 875 patients with EAC and additional data from the TCGA cohort.

Materials and methods

Patients. We analysed formalin-fixed, paraffin embedded material from 875 patients with EAC who underwent primary surgical resection or resection after neoadjuvant therapy between 1999 and 2018 at the Department of General, Visceral and Cancer Surgery, University of Cologne, Germany (Table I, Fig. 1). The majority of patients were male (88.1%). The average age at surgery was 61.2 years (31-83 years). The standard surgical procedure was laparotomic or laparoscopic gastrolysis and right transthoracic en bloc esophagectomy including two-field lymphadenectomy of mediastinal and abdominal lymph nodes. Reconstruction was performed by high intrathoracic esophagogastrostomy as described previously (15).

Patients with advanced oesophageal cancer (cT3, cNx, M0) received preoperative chemoradiation (5-FU, cisplatin, 40 Gy as treated in the area prior the CROSS trial) or chemotherapy alone. All patients were followed up according to a standardised protocol. During the first 2 years, patients were followed up clinically in the hospital every 3 months. Afterwards, annual exams were carried out. Follow-up examinations included a detailed history, clinical evaluation, abdominal ultrasound, chest X-ray, and additional diagnostic procedures as required. Follow-up data were available for all patients. Patient characteristics are given in Table I. Depending on the effect of neoadjuvant chemo- or radio-chemotherapy, there is a preponderance of minor responders in the tissue microarrays (TMAs), defined as histopathological residual tumour of ≥10% (16).

Immunohistochemistry. All tumours were analysed for protein expression using appropriate immunohistochemical antibodies: ARID1a (clone EPR 13501, rabbit, EDTA buffer 1:1,000, on automated Leica Bond stainer) and PD-L1 (clone E1L3N, rabbit, EDTA buffer 1:400, on automated Leica Bond stainer). Inflammatory cells and fibroblasts served as internal controls. Only complete loss of ARID1a with concomitant positive expression of the proteins in peritumoral tissue was scored. An appropriate in situ technique (EBER, Leica PB0589, ready-to-use on automated Leica Bond stainer) against EBV RNA was used, which showed no EBV-positive EAC in our cohort.

TMA as a screening method. Tissue samples of 875 EACs were converted to a TMA format as previously described (17,18). In brief, tissue cylinders with a diameter of 1.2 mm each were punched from selected tumour tissue blocks using a self-constructed semi-automated precision instrument and embedded in empty recipient paraffin blocks. 4 μm sections of the resulting TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ) for immunohistochemistry.

Tumour whole slide analysis. All tumours with a loss of ARID1a in the tumour cell nuclei at the TMA were examined for their ARID1a loss on tumour whole slides. Possible heterogeneous protein loss within the tumour or their corresponding lymph node metastasis could thus also be determined.

On whole tumour slides, the combined positive score (CPS) was used for the PD-L1 expression in tumour tissue. The CPS was also applied in all relevant recent studies (e.g., checkmate 649 study; see 'Discussion') and considers PD-L1 expression on tumour cells, as well as on specific inflammatory cells (e.g., macrophages). Tumours were classified into four different PD-L1 expression groups (CPS <1 (negative), CPS 1-5, CPS 5-10, and CPS >10).

The histomorphological growth patterns were described (according to WHO 2019): a) tubular and papillary, b) solid, c) mucinous, d) poorly cohesive (including signet ring cell tumours), e) others (including rhabdoid-like features as described before) (19). If a tumour had multiple growth patterns, the individual patterns were considered from a proportion of 10% of the total tumour (e.g., tubular and mucinous).

Mismatch-repair-protein status/MSI. We have analysed all tumours for their mismatch-repair-protein status/MSI for a previous publication [compare (20)]. In brief we screened for the mismatch-repair-protein-Status using proper immunohistochemical antibodies for MLH1 (clone: M1 Ventana), MSH2 (G219-1129), PMS2 (EPR3947) and MSH6 (Clone44, Ventana) on Ventana Benchmark stainers. Microsatellite status was determined using an in-house PCR protocol with primers for the Bethesda markers, including the mononucleotide markers BAT25 and BAT26 or the dinucleotide markers D5S346, D2S123, D17S250, D10S197, D18S58, and D13S153 and the tetranucleotide marker MYC. The methods used are also listed in detail in this publication.

TP53 status of the tumours. The TP53 status of the tumours was carried out as already described in detail (21). In brief, for the p53-status immunohistochemistry (IHC) was performed using the primary antibody specific for TP53 (DAKO, clone DO-7). The intensity of the TP53 staining was scored manually by two pathologists (A.Q. and H.L.) according to a 3-tier

Table I. Patient's characteristics.

Characteristic	Overall collective		ARID1a loss		ARID1a intact		P-value
Total	843	100%	63	7.5%	780	92.5%	
Sex							0.070
Male	743	88.1%	60	8.1%	683	91.9%	
Female	100	11.9%	3	3.0%	37	97.0%	
Age, years							0.076
≤65	460	54.6%	28	5.7%	432	94.3%	
>65	383	45.4%	35	9.1%	348	90.9%	
Neoadjuvant therapy							1.000
No	320	37.9%	26	8.1%	294	91.9%	
Yes	523	62.1%	37	7.1%	487	92.9%	
Tumour stage							0.819
pT1	154	18.4%	11	7.1%	143	92.9%	
pT2	155	18.5%	11	7.1%	144	92.9%	
pT3	500	59.7%	40	8.0%	460	92.0%	
pT4	29	3.5%	1	3.4%	28	96.6%	
Lymph node metastasis							0.265
pN0	331	39.3%	31	9.4%	300	90.6%	
pN1	267	31.7%	14	5.2%	253	94.8%	
pN2	120	14.2%	10	8.3%	110	91.7%	
pN3	125	14.8%	8	6.4%	117	96.6%	
UICC							0.736
1	110	13.1%	11	10.0%	99	90.0%	
2	101	12.1%	8	7.9%	9	92.1%	
3	383	45.7%	26	6.8%	357	93.2%	
4	244	29.1%	18	7.4%	226	92.6%	

scoring system. Discrepant results were resolved by consensus review. For a smaller proportion of tumours, we additionally used next-generation sequencing for *TP53*, exons 5-8.

Analysis of the TCGA collective. TCGA data were obtained from the GDC Data Portal website (22). For mutation analyses, we used open-access Mutect2 data. The MSI status was determined by quantifying frameshift mutations in the form of short insertions and deletions (indels) in mononucleotide repeats (MNRs). Only indels with a length of one base were considered. MNR were analysed for indels above a length of three bases. Cases with more than 100 indels were classified as MSI tumours. In the assessment of *ARID1a* and *TP53* mutation status, nonsense, missense, nonstop, and frameshift mutations were considered. If corresponding mutations were detected, the respective cases were classified as mutated (Fig. 2).

Statistical analysis. Patient data were prospectively collected. Overall survival was evaluated from the date of surgery until death. Kaplan-Meier curves were generated and compared using a log-rank test. Patient data with no events or lost follow up were censored at the last known date. A two-sided P-value <0.05 was considered as statistically significant. SPSS package version 25 (IBM, Armonk, New York) was used for all statistical analyses.

Results

Patient baseline characteristics. Thirty-one patients (n=31; 3.5%) showed MSI and were excluded from further analysis. Twenty-one MSI tumours (67.7%) showed concurrent ARID1a loss. In the subgroup of 844 microsatellite-stable (MSS) EACs, we detected loss of ARID1a in 63 cases (7.5%; P<0.001). This distribution was also true in both subgroups of neoadjuvant and primary surgery patients (Table I, Fig. 3).

It was already known from previous analyses of the tumour cohort that there was no case of MSH2/MSH6 failure, and clinically there was no known case of Lynch syndrome (12,20).

Clinicopathological data is depicted in Table I. Patients were predominantly men (n=744, 88.2%; women n=101, 11.8%). The median age of the proficient-Mismatch-Repair/Microsatellite-stability (MMR-p/MSS)-patient cohort at the time of diagnosis was 63.4 years (range 27.8-87.8 years). In 524 patients (62.0%), a neoadjuvant treatment (chemo- or radio-chemotherapy) was performed before surgery.

Loss of ARID1a in MSS-EAC. Loss of ARID1a was detectable in 63 patients (7.5%) (Fig. 1). In cross table analysis for the entire patient cohort, a correlation between ARID1a loss and clinical parameter could not be revealed (Table I). Subgroup analyses were performed for patients after neoadjuvant

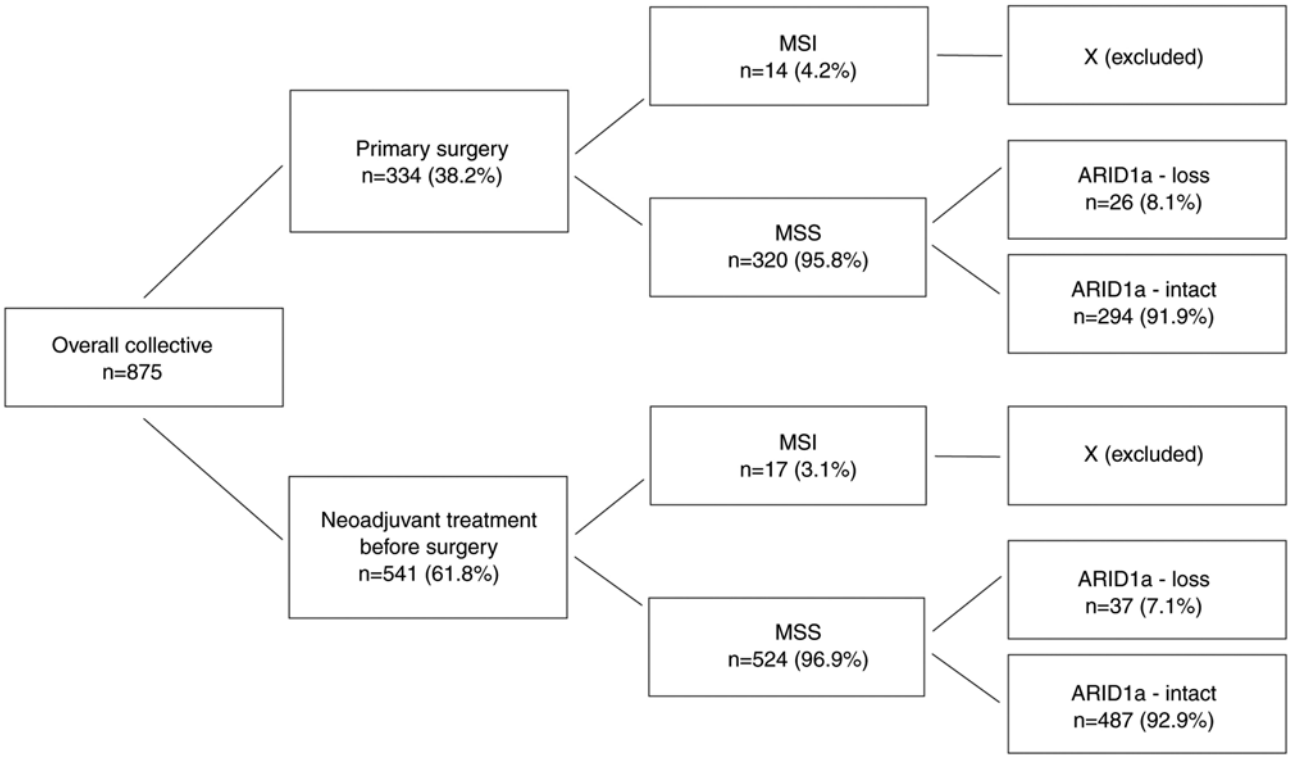

Figure 1. Tumour cohort composition. A total of 875 adenocarcinomas of the oesophagus were available for analysis. Tumours that underwent primary surgery were considered separately from tumours that underwent surgery after neoadjuvant therapy. All tumours were evaluated for their MLH1 status. All tumours that showed loss of MLH1 expression in tumour cell nuclei (with internal control by lymphocytes or fibroblasts) were classified as deficient MMR/MSI and were not analysed further. Only mismatch-repair proficient tumours separated by their ARID1a expression status were considered. MLH, mismatch repair protein; MSI, microsatellite instable; MMR, mismatch-repair; MSS, microsatellite stability; ARID1a, AT-rich interactive domain-containing protein 1A.

therapy and patients after primary surgery without preoperative therapy. Loss of ARID1a was not associated with any of the analysed clinical parameter, neither in the neoadjuvant group nor in the primary surgery group.

Molecular characteristics. The co-occurrence of ARID1a loss and TP53 mutations is a rare event. Within the group after neoadjuvant treatment, AIRD1a loss *TP53* wild-type tumours were observed in 15 patients (14.2%), whereas ARID1a loss in *TP53*-mutated tumours were seen in five patients (5.3%; P<0.057). In patients without neoadjuvant treatment, a similar distribution was observed. ARID1a loss in *TP53* wild-type tumours was seen in 13 patients (19.7%) and in *TP53*-mutated tumours in three patients (4.0%; P=0.003).

AIRD1a loss and prognosis. Loss of ARID1a is not associated with a shortened overall survival (OS) (P=0.568). Median OS for the entire patient cohort is 30.9 months (95% confidence interval (95%CI) 27.2-34.7 months) in patients with intact ARID1a and 23.7 months (95%CI 10.8-36.6 months) in patients with ARID1a loss tumours. A survival difference is also not detectable when stratifying patients according to neoadjuvant therapy or primary surgery and ARID1a loss (P=0.237 and P=0.505, respectively).

Neither in *TP53* wild-type tumours nor in *TP53*-mutated tumours did ARID1a loss show significant impact on the OS, though a trend towards shortened OS in the group of *TP53* wild-type tumours could be observed in the Kaplan-Meier survival analysis (P=0.209; Fig. 4, Kaplan-Meier-Curve).

Morphological subtypes of ARID1a loss MSS-EAC. Within the subcohort of MSS-EACs with ARID1a loss, the following distribution regarding morphological patterns was observed: 51.6% (32/63) tubular/papillary, 11.3% (7/63) solid growth, 11.3% (7/63) poorly cohesive, and mixed 25.8% (16/63). Tumours with mixed pattern harboured at least 10% of two different growth patterns. No predominant mucinous pattern was seen.

In this cohort MSI-like features (medullary phenotype, increased tumour-infiltrating lymphocytes, peritumoral lymphoid follicle formation) were seen in 9.7% (6/63) cases (see discussion).

Heterogeneity of ARID1a loss in MSS-EAC. In most cases of MSS-EACs with ARID1a loss, the tumours showed homogenous loss of ARID1a expression (58/63, 92%). A heterogeneous loss of ARID1a expression was seen in only five cases (5/63, 8%).

PD-L1 expression CPS. In the overall cohort, ~40% of ARID1a loss MSS tumours showed no PD-L1 expression (CPS <1). This number is not relevantly affected by neoadjuvant therapy (for details, see Table II). The six tumours with MSI-like phenotype were PD-L1 positive. They showed a CPS from a minimum of 5 up to 100.

Analysis of the TCGA collective. For the analysis of TCGA cases, subtyping and mutation data of the GDC Data Portal were available for 184 EACs. These were subclassified into

Table II. Expression of PD-L1.

PD-L1 combined positive score	Total cohort (n=50)	Primary surgery (n=21)	Neoadjuvant treatment (n=29)
0	20 (40%)	9 (43%)	11 (38%)
1-5	16 (32%)	7 (33%)	9 (31%)
5-10	10 (20%)	2 (10%)	8 (28%)
>10	4 (8%)	3 (14%)	1 (3%)

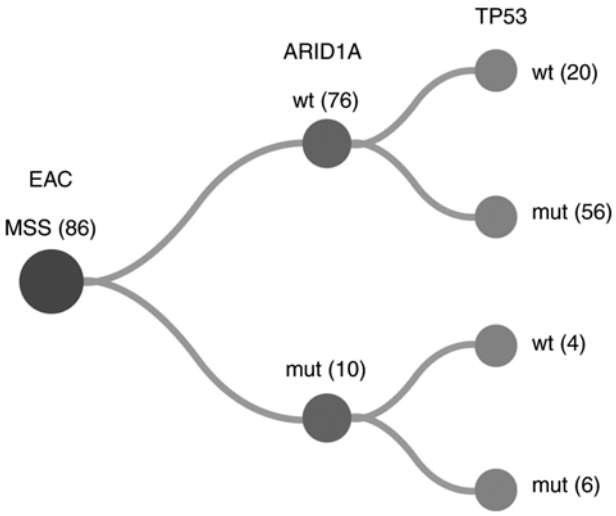

Figure 2. Composition of the TCGA cohort. In the analysis of TCGA data, 10 adenocarcinomas of the oesophagus with MSS and mutated *ARID1a* were found. Overall, six of the 10 tumours show a concurrent *TP53* mutation in the tumour. *TP53* mutant and *ARID1a*-deficient tumours were defined in the current study. TCGA, The Cancer Genome Atlas; MSS, microsatellite stability; ARID1a, AT-rich interactive domain-containing protein 1A.

96 squamous cell and 88 adenocarcinomas. In agreement with the results of previous papers, two adenocarcinomas and one squamous cell carcinoma exhibited MSI (see 'Methods') (23). Analysing the mutation status, among the MSS adenocarcinomas, we detected *TP53* mutations in 72% and *ARID1a* mutations in 12% of the cases. A simultaneous occurrence of *ARID1a* and *TP53* mutations was observed in 7% of all tumours (Fig. 2). In the subgroup of 10 *ARID1a*-mutated EACs, six cases also harboured mutations in *TP53*.

Discussion

Since ARID1a loss tumours in the GI tract occur frequently in the context of MSI (and additionally in gastric carcinoma associated with EBV), many consider prognostic or morphologic aspects are overlaid by the characteristics of MSI or EBV. In our cohort of 875 EAC, only 31 tumours were proven to be dMMR/MSI (3.5%). It was already known from previous analyses of the tumour cohort that there was no case of MSH2/MSH6 failure and clinically there was no known case of Lynch syndrome (20), so MLH1 was only analysed for the detection of defective mismatch-repair protein status (20). This is in line with the literature reporting MSI in EAC of 1-5%. There is no EBV-associated EAC consistent with previous publications (24,25).

We detected loss of ARID1a expression in ~10% of EAC, in accordance with the previous work of Drage *et al* (11). This finding is also in line with further studies and data by the TCGA describing ARID1a alteration in 10-13% (26-28).

Drage *et al* only considered primary operated EAC. This also explains the long period of time considered in this paper, ranging from 1989 to 2011. Today, the majority of EAC are treated with neoadjuvant therapy. Knowledge of the frequency and characteristics of neoadjuvantly treated EAC with concurrent ARID1a loss is discussed here for the first time.

The extent of 7.5% MSS-EAC with ARID1a loss is unaffected by neoadjuvant therapy. This suggests that in an operable patient population, ARID1a loss tumours are not strikingly more chemo-sensitive, as we would otherwise find them in a significantly lower volume after neoadjuvant treatment has occurred.

According to the TCGA data, all *ARID1a* alterations are either deep deletions or truncating mutations. This fact and the high percentage of ARID1a deficient tumours in the upper GI tract (10-17%) can be taken as a good indication that the loss of ARID1a is important for tumour biology. Furthermore, it also explains well that immunohistochemistry is indeed able to reliably represent an underlying genomic alteration of the *ARID1a* gene via the lack of protein detection in tumour cell nuclei. Since the extent of mutation and protein loss is comparable in different collectives, other possibilities, such as epigenetic downregulation of *ARID1a* at least do not seem to play a major role. As histopathologists, we strive to define morphological characteristics in the same molecular subgroups. This works well, for example, in MSI tumours that show clustered tumours with so-called medullary features or highly inflamed tumours in which lymphocytes show close spatial adjacency to tumour cells. The latter is also found in Epstein-Barr virus-associated carcinomas-appropriately referred to in WHO as carcinomas with lymphoid stroma. One paper claimed to find characteristics of the medullary phenotype also clustered in ARID1a-deficient carcinomas of the upper GI tract. We cannot actuate this in our collective. Since the previous publication [Drage *et al* (11)] did not distinguish between MSI and MSS-ARID1a deficient tumours, we evaluate the accumulation of medullary features in their collective as an expression of tumour microsatellite instability only. Thus, the following statement is relevant: ARID1a-deficient tumours are not predictable by morphological criteria. If this subgroup is indeed therapeutically relevant in the future, immunohistochemical or molecular testing must be performed to detect ARID1a alteration. According to our data, histomorphology is not able to perform a reliable preselection.

We did not find rhabdoid-like features as considered in a study of gastrointestinal tract carcinomas with SWI/SNF loss (19).

In agreement with Drage *et al* we see no prognostic relevance of ARID1a loss in EAC, even when considering the overall collective. This applies to primary operated and neoadjuvant pre-treated tumours (11).

The notion that an *ARID1a* mutation occurs mutation-exclusively and does not occur concomitantly with a *TP53* mutation has been described mainly in carcinomas of the

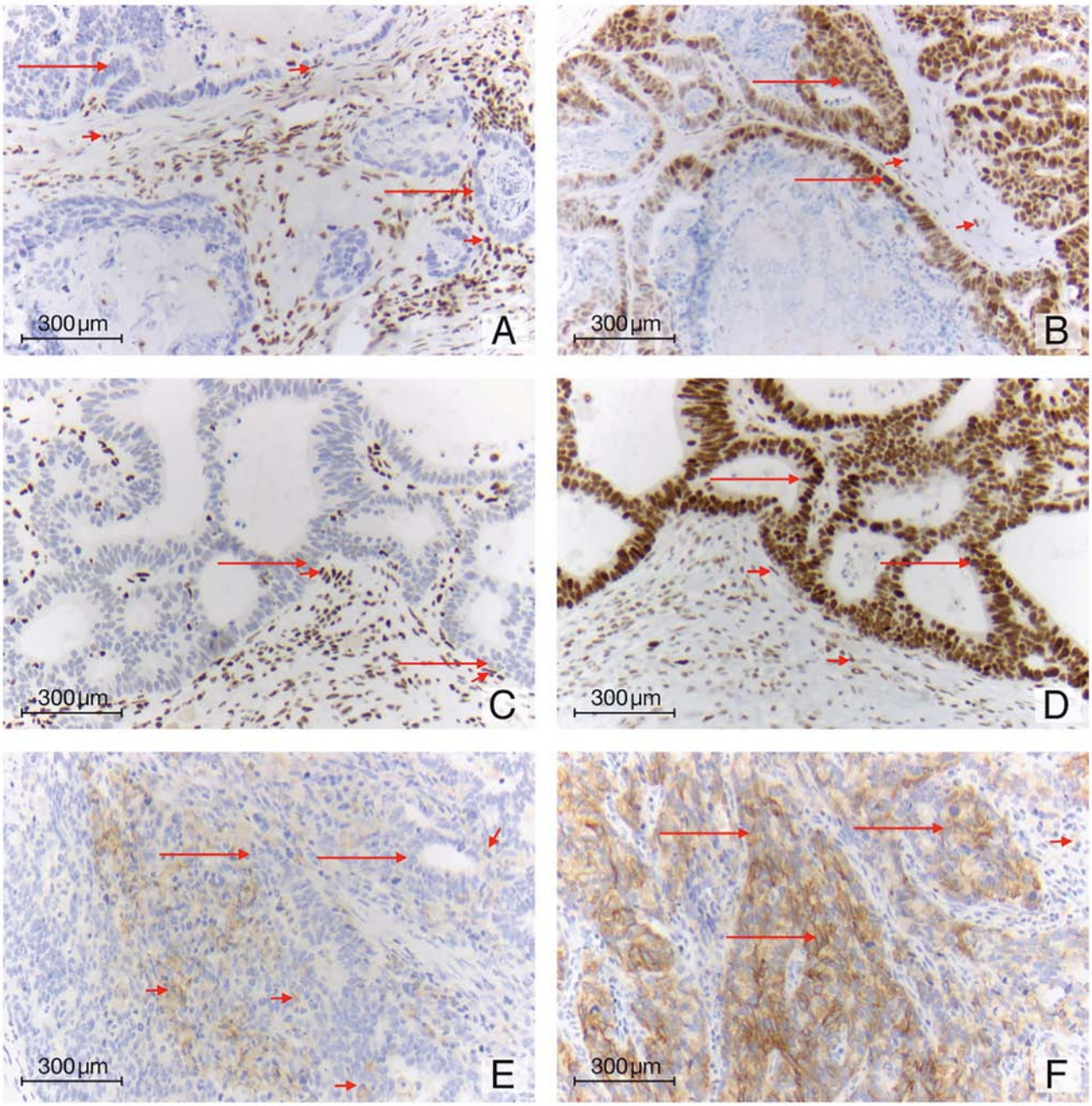

Figure 3. Immunohistochemical analysis. The long arrows show the tumour cells in each case, the short arrows show internal controls in the form of lymphocytes or fibroblasts. (A) Loss of ARID1a in tumour cell nuclei in a tubulo-cribriform oesophageal adenocarcinoma (long arrow), lymphocytes and fibroblasts show preserved expression of ARID1a in the tumour (short arrows). (B) Same tumour as in (A) and shows preserved expression of MLH1 in tumour cell nuclei (long arrow) as well as in adjacent fibroblasts (short arrows) (proficient mismatch-repair/MSS). (C and D) Examples of tubular adenocarcinoma with (C) loss of ARID1a and (D) preserved expression of MLH1. (E and F) Examples of PD-L1-positive ARID1a-deficient and MSS adenocarcinoma. Different areas of the same PD-L1-positive tumour are shown: (E) Predominant PD-L1 labelled inflammatory cells (short arrows) between carcinoma cells (long arrows) and (F) predominant positive carcinoma cells with clear membranous tumour cell labelling. Magnification, x200. ARID1a, AT-rich interactive domain-containing protein 1A; MLH, mismatch repair protein; MMR, mismatch-repair; MSS, microsatellite stable.

internal genitalia. This is not true for adenocarcinoma of the oesophagus. (29,30). While in endometrioid endometrial carcinomas ARID1a loss and mutations in *TP53* are almost mutually exclusive, this is not the case in EAC (31). In our collective, as well as in the TCGA-cohort we analysed, mutations in *TP53* are found to be simultaneously manifest. Interestingly, in the subgroup of TP53 wild-type EAC we find a tendency towards an unfavourable prognosis (but even there without statistical significance, P=0.209).

A heterogeneous distribution of ARID1a-deficient and ARID1a-proficient tumour clones in the same tumour is the exception in EAC. Homogeneity also applies to their lymph node metastases. The homogeneous occurrence of ARID1a loss clones within the tumour and its metastases is particularly significant for effective therapeutic intervention. The more homogeneous a therapeutically relevant change occurs in the tumour, the more likely it can be assumed that the therapy will be effective.

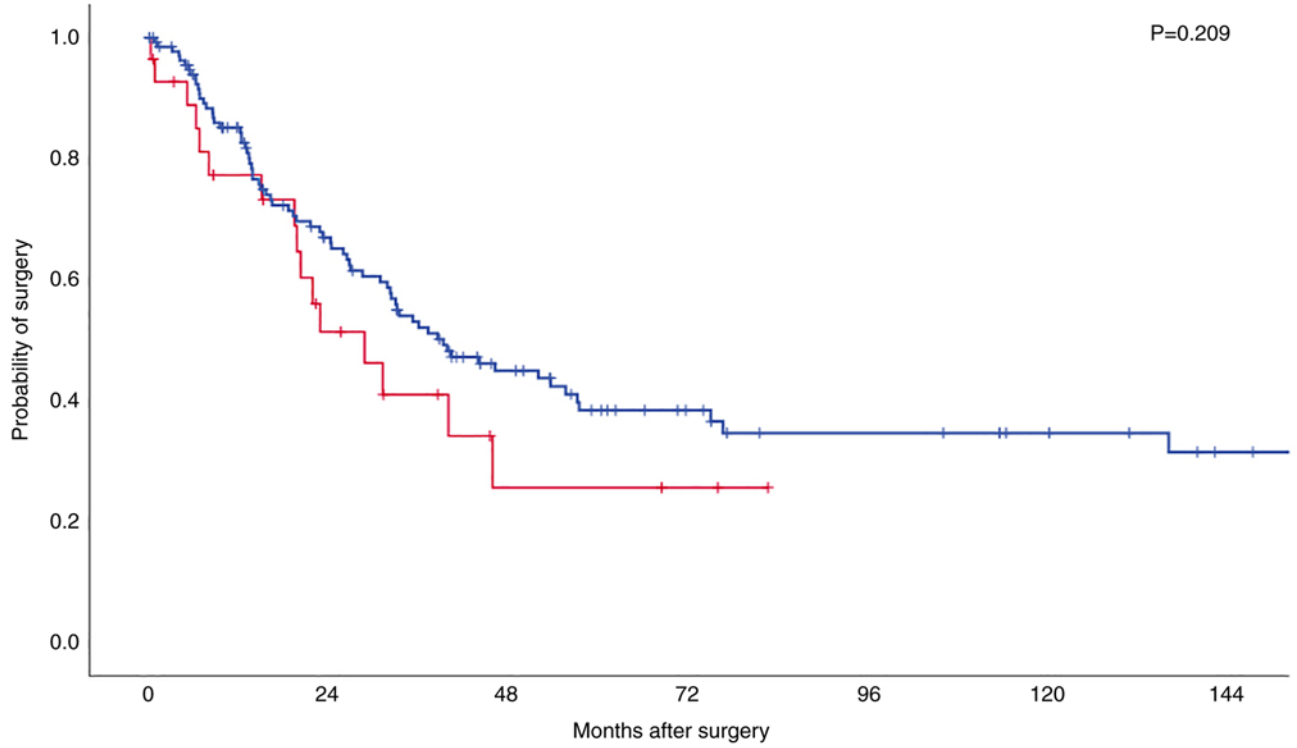

Figure 4. Kaplan-Meier curve. *TP53* wild-type AT-rich interactive domain-containing protein 1A-loss microsatellite stable oesophageal adenocarcinoma had a tendency towards an unfavourable prognosis (P=0.209).

For example, Her2/neu is more often not homogeneously expressed in EAC, in contrast to breast carcinoma. The lack of homogeneity is likely one reason for the only moderate benefit of trastuzumab in OS of just under 3 months in EAC and a major reason for the failure of the GATSBY study (32,33).

MSI carcinomas of the colon, stomach, and oesophagus would be effectively treated with ICIs directed against PD-1 or PD-L1 in most cases. From a therapeutic perspective, concurrent ARID1a failure in the MSI subgroup is probably irrelevant. Thus, at ~7.5% ARID1a loss, MSS-EAC represent a relevant tumour subgroup. In ARID1a-altered tumours of different entities, different agents have been described as (potentially) effective (EZH2 inhibition, HDAC6 inhibition, PARP inhibition, PIK3CA pathway inhibitor, and PD-1/PD-L1 inhibitors). In malignant extrarenal rhabdoid tumours, which typically show a failure of the SMARCB1 (INI-1) subunit of the SWI/SNF complex, phase 2 clinical trials are ongoing to investigate the efficacy of inhibition of EZH2 methyltransferase as a catalytic subunit of the Polycomb complex (34). Bitler *et al* then also describe the synthetic lethality of EZH2 methyltransferase inhibition in *ARID1a* mutant tumours (35). Shen *et al* have been able to demonstrate the efficacy of PARP inhibition (e.g., Olaparib) in ARID1a-deficient tumours *in vitro* and *in vivo* (36).

There is evidence that ARID1a is involved in the repair of DNA double-strand breaks (similar to BRCA1 and BRCA2). The enzyme PARP works in the same way. Loss of function of ARID1a with concomitant therapeutic blockade of PARP could be lethal to the tumour cell (as has been successfully used therapeutically in BRCA-deficient ovarian cancers). Specific HDAC6 inhibitory small molecules are in clinical trials in haematologic tumours (37).

In clear cell ovarian cancer with ARID1a loss, cell culture experiments and mouse models have also demonstrated the efficacy of this class of compounds. Furthermore, cell culture analyses have shown that loss of ARID1a protein renders tumour cells highly sensitive to inhibition for PI3K and AKT inhibitors (38,39).

Some work has also discussed the relevance of PD-L1 expression in the context of ARID1A deficiency and the effectiveness of the corresponding checkpoint inhibitors (14).

According to our results, ARID1a-altered EAC are not disproportionately frequent or particularly marked PD-L1 positive tumours. Approximately 60% of tumours in our collective are PD-L1 positive (CPS >1), 32% show a CPS of 1-5, 20% of 5-10, and 8% of >10. Thus, slightly fewer PD-L1 positive tumours are found compared to the Checkmate 649 study, which looked at a molecularly unselected collective of gastric carcinomas and gastroesophageal transition carcinomas. The Checkmate 649 study also measured PD-L1 using the CPS-Score and showed the efficacy of nivolumab in PD-L1 positive upper GI-tract tumours (40). Whether ARID1a-deficient EAC could nevertheless particularly benefit from PD-1/PD-L1 blockade therapy will have to be shown by future studies or retrospective subgroup analyses of already completed studies.

We have investigated the significance of different altered SWI/SNF proteins, including ARID1a, on over 600 EACs in a previous two-year-old study (12). In that study, we found that ARID1a can fail in MSI tumours but also independently of MSI in oesophageal cancer. This publication was the basis

of the present work. Similarly to Drage *et al* (11) we had not clearly distinguished between characteristics of MSS and MSI carcinomas in the previous publication, an inaccuracy that we resolve with this work. Here we focus exclusively on alteration of ARID1a and MSS-EAC in the current manuscript.

A limitation of our current study is that we surveyed ARID1a status only at the protein level. It may be that some mutations induce a functionless ARID1a protein that is still recognised by the antibody used. The proportion of ARID1a-deficient tumours would then be somewhat higher than we have described. This may be supported by studies reporting *ARID1a* mutation status at 13% (rather than 10%).

However, this once again highlights the importance of this subgroup in EAC. Another limitation is the retrospective nature and single-centre analysis. However, we think that the number of the EACs considered (N=875) may provide relevant information despite these limitations. We have deliberately refrained from digitally supported image analyses, as image analyses are not helpful in these cases from our point of view. Today, the PD-L1 TPS score can already be determined well by image analysis; this does not work comparably well for the CPS score. However, histopathologists are able to determine both growth patterns and the CPS score in the tumour with high concordance. This was also done in all studies (e.g., PD-L1 determination in the Checkmate 649 study). For our work, we believe it made sense to provide morphologists with comparative analytics.

In conclusion, according to analysis of a very large tumour collective (N=875), at least 10% of EAC are ARID1a-deficient, the majority of which are MSS (7.5%, MSS/MMR-p). A specific morphologic phenotype is absent (e.g., there is no clustering of tumours with mucinous or rhabdoid differentiation or so-called medullary features). However, there is strong evidence that this tumour subgroup is particularly sensitive to some agents (such as PARP or anti-PD-L1 checkpoint inhibitors). Since personalised therapeutics are largely lacking in EAC, clinical trials investigating the efficacy of these therapeutics, specifically in this subgroup, are useful (biomarker-based trials).

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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

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

Ethics approval and consent to participate

Approval was obtained from the University of Cologne Ethics Committee (approval nos. 20-1583 and 10-242). We confirm that informed consent was obtained from all subjects and/or their legal guardians.

Patient consent for publication

Patients gave their written consent to usage of their tumor specimens.

Competing interests

All authors declare that they had no competing interests.

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