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Therapy-relevant *MDM2* amplification in cholangiocarcinomas in Caucasian patients

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Abstract

Background: Cholangiocarcinomas (CCA) are a group of aggressive malignancies with poor prognosis. The distinct subtypes are related to different etiologies and genetic aberrations that are subject to targeted therapies. Mouse double minute 2 homolog (MDM2) is a potent inhibitor of tumor suppressor p53 and is proven to be altered in certain carcinomas. Novel targeted drugs, such as the MDM2-p53 antagonist Brigimadlin, have shown promising results for therapeutic efficacy in patients with *MDM2* amplification and wild-type *TP53*.

Objectives: This study therefore aimed to characterize CCAs regarding their MDM2 status, compare the concordance between fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) methods, and elucidate the role of *MDM2* amplification in prognosis and other clinicopathological characteristics.

Design: Retrospective cohort study.

Methods: All patients ($n = 52$) were diagnosed with CCA and received surgical resection with curative intention at the University Hospital of Cologne. Samples were analyzed retrospectively for *MDM2* amplification with FISH and IHC. We correlated results with pre-existing molecular as well as clinical data.

Results: We included 52 patients with primary CCA, three of which showed positive *MDM2* amplification (5.8%). *MDM2* amplification was present only in the intrahepatic CCA type and all patients with positive *MDM2* amplification exhibited normal p53 status. Among the large-duct subtypes of intrahepatic CCAs, patients with positive *MDM2* amplification demonstrated better survival than patients with negative *MDM2* amplification ($p = 0.041$). Of the patients with MDM2 amplification, two underwent adjuvant therapy post-surgery (66.7%). There was a strong correlation between *MDM2* amplification and positive protein expression in IHC. There were no identifiable molecular co-alterations of *MDM2* with *FGFR2* or SWI/SNF complex alterations.

Conclusion: Real-world evidence in our Caucasian patient population confirmed that a significant number of intrahepatic CCAs showcase *MDM2* amplification, qualifying for a personalized therapy option with Brigimadlin. *MDM2* amplification must therefore be considered in the context of personalized molecular testing in CCA.

Keywords: amplification, Brigimadlin, cholangiocarcinoma, *MDM2*, treatment

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Introduction

Cholangiocarcinomas (CCA) represent a group of highly aggressive malignancies originating from the biliary tree with incidence and mortality rates that have been increasing during the past decade.[1] CCAs are a rare malignancy with a global incidence rate of 0.3–6/100,000 that can vary strongly among certain regions endemic for hepatobiliary flukes, and a 5-year survival rate that remains at <10% despite therapeutic

advances.[1–3] Furthermore, most CCAs are diagnosed late in the disease course, leading to limited treatment options and worse prognosis.[1]

General classification of CCAs occurs anatomically into intrahepatic, perihilar, and distal CCA, depending on the site of origin.[1,4] Intrahepatic CCAs (iCCA) are further classified into small- and large-duct types whereas small-duct iCCAs show a mass-forming appearance with mucus-poor duct-like histology and are associated with chronic parenchymal liver diseases such as cirrhosis.[5,6] Large-duct iCCAs demonstrate infiltrative duct-forming growth with fibrotic stroma and are frequently associated with chronic cholangiopathies such as liver flukes and primary sclerosing cholangitis.[5] Each of these subtypes exhibits distinct etiologies and genetic aberrations that need to be understood to improve therapeutic options and patient outcomes.[7]

Known molecular targets of iCCA include *fibroblast growth factor receptor 2* (*FGFR2*) gene fusions and variants of the genes encoding isocitrate dehydrogenase (IDH).[8] Both FGFR2 and IDH inhibitors such as Futibatinib and Ivosidenib have rapidly emerged and were approved for the targeted treatment of CCA.[8–11] Another possible target in iCCAs is SWI/SNF complexes. SWI/SNF complexes are ATP-dependent nucleosome remodeling complexes that modulate gene expression and play a role as tumor suppressors in various human malignancies, including CCA.[3,12] Wagner et al. showed that protein loss of SWI/SNF core subunits ARID1A-, BRG1-, BRM-, PBRM-1 and INI1 occur in 35% of cases and are associated with worse survival among small-duct and large-duct iCCA, exposing them as an interesting future therapeutic target.[3]

TP53 is recognized as the most frequently mutated tumor suppressor gene among all human cancers and plays a critical role in tumorigenesis.[13] TP53 mutations are highly prevalent across various cancer types and exhibit variability in prevalence among different ethnic groups. In Caucasian populations, the prevalence of TP53 mutations is relatively high, often occurring in 30%–50% of cases depending on the specific type of cancer.[14] These mutations are most frequently seen in cancers such as breast, lung, and colorectal cancers. In other ethnic groups, the prevalence can vary. For instance, studies have shown that East Asian populations have a lower prevalence of TP53 mutations compared to Caucasians, with significant differences in the types and frequencies of specific mutations.[15]

Mouse double minute 2 homolog (MDM2) is a natural binding partner of this tumor suppressor protein and leads to its inhibition.[13,16,17] It was shown to be mutated in various cancers, such as sarcoma, glioblastoma multiforme, bladder urothelial carcinoma, and CCA.[13,16,17] While detailed data on ethnic differences in *MDM2* amplification are less prevalent, it is understood that, similar to *TP53* mutations, the prevalence and impact of *MDM2* amplification can vary among different populations. However, specific comparative prevalence rates across ethnic groups are not as well-documented.[18] MDM2, a key regulator of the p53 tumor suppressor protein, typically binds to and inhibits p53, leading to its degradation. When *TP53* is mutated, MDM2 can still bind to it, but the consequences of this interaction depend on the nature of the *TP53* mutation. In many cases, even if MDM2 binds to mutant p53, it may not lead to the degradation of the mutated protein, allowing the aberrant p53 to accumulate in the cell. This accumulation can either fail to perform normal p53 functions (loss of function) or actively promote oncogenic pathways (gain of function), contributing to cancer progression.[19]

Recent studies on *MDM2* amplification in CCA showed an association with the large-duct iCCA subtype and indicated that *MDM2* amplification could lead to poor clinicopathological characteristics such as high histological grade, lymph node metastasis, and worse overall survival.[5,20,21] Therefore, MDM2 is being considered as a new therapeutic target in selected patients.

The novel MDM2-p53 antagonist Brigimadlin (BI 907828) has shown promising results in early-phase clinical trials and is currently being further investigated in the phase IIa/IIb *Brightline-2* trial.[22,23] The trial investigates Brigimadlin as a new therapeutic strategy in selected unresectable or metastatic *MDM2*-amplified, *TP53*-wild-type tumors, including advanced biliary tract cancer.[22]

The goal of this study is to assess the MDM2 status in CCAs, examine the concordance between fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) techniques, and explore how MDM2 amplification relates to prognosis and other clinicopathological features.

Materials and methods

Patients and tumor samples

All patients with the diagnosis of a primary CCA of the current analysis underwent surgical treatment with curative intent at the University Hospital of Cologne (Cologne, Germany) between 2000 and 2019. Data was retrospectively collected from the clinical database, follow-up visits, and contact with the local registration offices for all 52 patients of the cohort. The study was conducted with the approval of the Ethics Committee of the University of Cologne (application 18-269). Patients who received chemotherapy prior to surgery and patients with survival of less than 14 days were excluded from the study to avoid survival bias. Tissue microarray analysis (TMA) was performed as previously described, using tissue cylinders of 1.2 mm in diameter, that were punched out with a semiautomated precision instrument, four cylinders per patient to cover possible tumor heterogeneity.[24] These cylinders were then embedded in empty recipient paraffin blocks and four-micrometer sections of these TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc.) for further staining.

The reporting of this study follows the STROBE guidelines for cohort studies,[25] and the completed checklist is provided as a supplementary file (Supplemental File 1).

Fluorescence in situ hybridization and analysis

FISH was performed on TMAs using established methods as previously published.[26,27] For the analysis, the ZytoLight SPEC *MDM2/CEN 12* Dual Color Probe (ZytoVision GmbH, Bremerhaven, Germany) was used. Analyses were performed with the immunofluorescence microscope Leica DM5500B (Leica Biosystems, Germany) at 63× by two independent pathologists (A.Q. and S.I.L.). Amplification of *MDM2* was considered positive in case of an *MDM2* centromere 12 (CEN12) ratio ⩾2.0 or an average number of *MDM2* signals per tumor cell nucleus ⩾6 or large clusters of *MDM2* signals ⩾10%, which complies with previously used thresholds.[26]

Immunohistochemical study

For the verification of the FISH results, we have additionally performed IHC against the MDM2-protein. Stainings were conducted using the mouse monoclonal antibody Ab.1/IF2 (Calbiochem, 1:50, EDTA) via the fully automated Leica Bond stainer (Wetzlar, Germany).

To assess the immunohistochemical stainings, the nuclear staining intensity was evaluated semi-quantitatively on a scale of 0 to 3. Moreover, the percentage of positive tumor cells within the cores was examined. A combined score was computed based on both the staining intensity and the proportion of positive tumor cells. The staining intensity was categorized as follows: 0 for no staining; >0 to 50 for low staining level; >50 to 100 for intermediate staining level; and >100 for strong staining.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 29.0.1.1; Armonk, USA). *p*-Values below 0.05 were considered statistically significant. Qualitative values were assessed using the chi-square test. Survival analyses were conducted with Kaplan–Meier curves and log-rank tests. The concordance between FISH and IHC methods was accessed using the percent agreement.

Results

In total, 52 patients with primary CCA were included in this study. The age ranged from 49 to 82 years (median: 70.66 years). 59.6% of the recruited patients were male and 46.2% received adjuvant therapy after surgical resection. Table 1 summarizes the cohort in more detail. We performed FISH for *MDM2* as mentioned above and divided the cohort into patients with positive amplification status for *MDM2* ($n=3$; 5,8%) and negative amplification status for *MDM2* ($n=49$; 94,2%).

Furthermore, the results of the FISH analyses were correlated with the immunohistochemical protein expression status.

Among the patients identified with a positive *MDM2* amplification status, two exhibited a homogeneous cluster amplification throughout all tumor cells, as illustrated in Figure 1. Remarkably, these cases also displayed a homogenous strong immunohistochemical staining pattern. In contrast, one case with *MDM2* amplification revealed a heterogeneous cluster amplification. Correspondingly, immunohisto-

Table 1. General clinicopathological characteristics of the total study population and patients with low or high MDM2 expression.

Characteristic	Total	MDM2 amplification		*p*
		negative	positive	
	n (%)	*n* (%)	*n* (%)	
No. patients	52 (100)	49 (100)	3 (100)	
Sex				0.798
Male	31 (59.6)	29 (59.2)	2 (66.7)	
Female	21 (40.4)	20 (40.8)	1 (33.3)	
Adjuvant therapy				0.463
No	29 (53.8)	27 (55.1)	1 (33.3)	
Yes	24 (46.2)	22 (44.9)	2 (66.7)	
Type				0.234
Intrahepatic	36 (69.2)	33 (67.3)	3 (100)	
Extrahepatic	16 (30.8)	16 (32.7)	0 (0)	
Gross features				0.122
Mass-forming	23(44.2)	22 (44.9)	1 (33.3)	
Periductal	9 (17.3)	7 (14.3)	2 (66.7)	
Intraductal	3 (5.8)	3 (6.1)	0 (0)	
Extraductal	17 (32.7)	17 (0.35)	0 (0)	
Histological type				**0.019**
Small-duct type iCCA	29 (55.8)	28 (57.1)	1 (33.3)	
Large-duct type iCCA	7 (13.5)	5 (10.2)	2 (66.7)	
eCCA	16 (30.8)	16 (32.7)	0 (0)	
pT				0.335
1	11 (21.2)	10 (20.4)	1 (33.3)	
2	25 (48.1)	25 (51.0)	0 (0)	
3	10 (19.2)	9 (18.4)	1 (33.3)	
4	6 (11.5)	5 (10.2)	1 (33.3)	
pN				0.695
0	29 (55.8)	27 (55.1)	2 (66.7)	
1	23 (44.2)	22 (44.9)	1 (33.3)	
R				0.228
0	38 (73.1)	37 (75.5)	1 (33.3)	

(Continued)

Table 1. (Continued)

Characteristic	Total	MDM2 amplification		*p*
		negative	positive	
	n (%)	*n* (%)	*n* (%)	
1	13 (25.0)	11 (22.4)	2 (66.7)	
2	1 (1.9)	1 (2.0)	0 (0)	
L				0.375
0	8 (15.4)	7 (14.3)	1 (33.3)	
1	44 (84.6)	42 (85.7)	2 (66.7)	
V				0.746
0	30 (57.7)	28 (57.1)	2 (66.7)	
1	22 (42.3)	21 (42.9)	1 (33.3)	
Pn				0.962
0	18 (34.6)	17 (34.7)	1 (33.3)	
1	34 (65.4)	32 (65.3)	2 (66.7)	
M				0.659
0	49 (94.2)	46 (93.9)	3 (100)	
1	3 (5.8)	3 (6.1)	0 (0)	
G				0.979
1	1 (1.9)	1 (2.0)	0 (0)	
2	30 (57.7)	28 (57.1)	2 (66.7)	
3	20 (38.5)	19 (38.8)	1 (33.3)	
4	1 (1.9)	1 (2.0)	0 (0)	
UICC stage				0.259
I	10 (19.2)	9 (18.4)	1 (33.3)	
II	14 (26.9)	14 (28.6)	0 (0)	
III	4 (7.7)	3 (6.1)	1 (33.3)	
IVa	24 (46.2)	23 (46.9)	1 (33.3)	
Inflammation				0.519
Low	9 (17.3)	8 (16.3)	1 (33.3)	
Medium	30 (57.7)	28 (57.1)	2 (66.7)	
High	13 (25.0)	13 (26.5)	0 (0)	

(Continued)

Table 1. (Continued)

Characteristic	Total	MDM2 amplification		*p*
		negative	positive	
	n (%)	*n* (%)	*n* (%)	
Precursor lesions				0.953
No	37 (71.2)	35 (71.4)	2 (66.7)	
BLIN	12 (23.1)	11 (22.4)	1 (33.3)	
IPNBD	2 (3.8)	2 (4.1)	0 (0)	
Caroli syndrome	1 (1.9)	1 (2.0)	0 (0)	
Liver disease				0.396
Normal	26 (50.0)	25 (51.0)	1 (33.3)	
Pathological	17 (32.7)	15 (30.1)	2 (66.7)	
Not assessable	9 (17.3)	9 (18.4)	0 (0)	
Steatohepatitis				0.620
Negative	40 (76.9)	37 (75.5)	3 (100)	
Positive	8 (15.4)	8 (16.3)	0 (0)	
Not assessable	4 (7.7)	4 (8.2)	0 (0)	
Cirrhosis				0.296
Negative	38 (73.1)	36 (73.5)	2 (66.7)	
Positive	5 (10.2)	4 (8.2)	1 (33.3)	
Not assessable	9 (17.3)	9 (18.4)	0 (0)	

Bold print marks *p*-values below 0.05.
BLIN, biliary intraepithelial neoplasia; IPNBD, intraductal papillary neoplasm of the bile duct; UICC, union for international cancer control.

chemistry in this instance exhibited a heterogeneous, yet strongly positive pattern.

The immunohistochemical expression of the MDM2 protein was assessed and compared with the results obtained from FISH analysis. Notably, all *MDM2*-amplified CCAs exhibited strong staining on immunohistochemistry ($n = 3$; 5.8%). Intriguingly, one case of a small-duct type iCCA displayed an intermediate staining level despite lacking *MDM2* amplification on FISH analysis ($n = 1$; 1.9%). Here, *MDM2* FISH analysis has shown a polysomy. The remaining patients demonstrated a negative IHC staining ($n = 48$; 92.3%).

The concordance between FISH and IHC methods was analyzed using the percent agreement metric. Thus, the percent agreement between the FISH and IHC methods in this study is 98.08% (with True Positives = 3; True Negatives = 48; False Positives = 0; False Negatives = 1).

We then compared the general clinicopathological characteristics of these subgroups as outlined in Table 1. *MDM2* amplification was present only in the iCCA subtype (two men and one female patient). Furthermore, positive *MDM2* was only found in the histological large-duct type iCCA and small-duct type iCCA and not in the extrahepatic type ($p = 0.019$).

In terms of treatment, two patients with *MDM2* amplification received adjuvant therapy, while one did not. Gross morphological examination

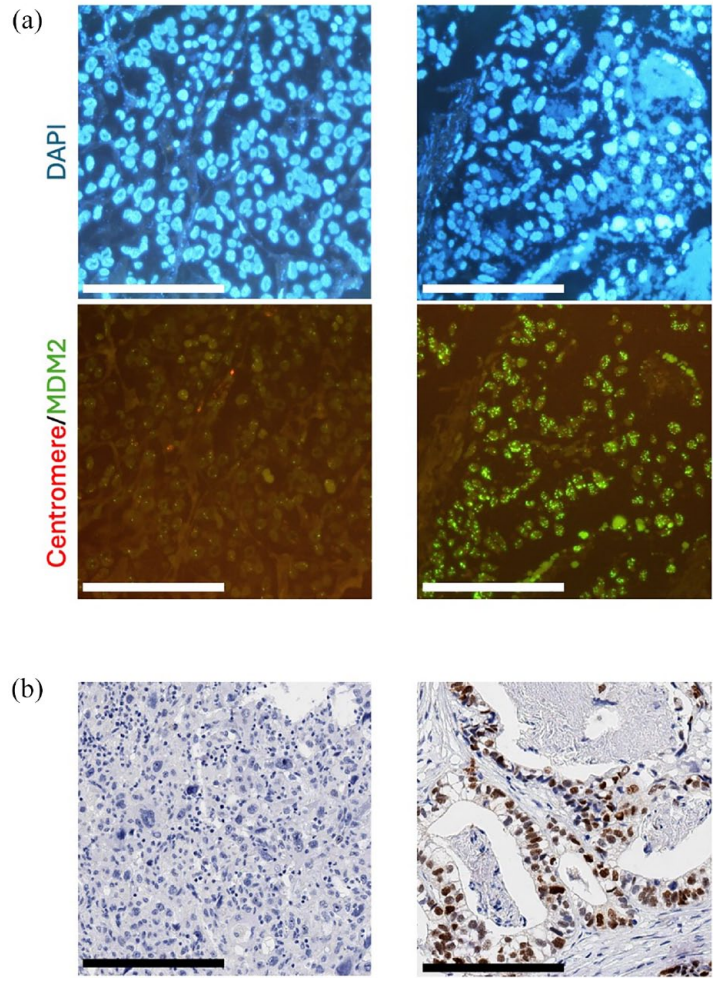

Figure 1. (a) Representative pictures of an *MDM2*-amplified (right) and not-amplified tumor (left) in FISH analysis. (b) MDM2 immunohistochemistry (left: no staining, right: strong nuclear staining). Scale bar: 50 µm.
FISH, fluorescence in situ hybridization; MDM2, mouse double minute 2 homolog.

revealed that one patient presented with a mass-forming type, whereas two displayed periductal involvement. Further clinical staging demonstrated that the tumor staging (pT) varied among these patients, with one patient each presenting with stages pT1, pT3, and pT4. Lymph node involvement (pN) was seen in one patient (pN1), while two patients had no lymph node metastasis (pN0). Regarding surgical margins, one patient had clear margins (R0), whereas two had residual disease (R1).

The presence of lymphatic invasion (L) was noted in two patients (L1), with one case negative for lymphatic spread (L0). Similarly, vascular invasion (V) was present in one patient but absent in the other two. Perineural invasion (Pn) was observed in two patients, while one patient showed no such involvement. Importantly, none of the patients exhibited distant metastasis (M0).

Grading of the tumors showed that two patients had moderately differentiated tumors (G2), and one had a poorly differentiated tumor (G3).

The overall staging according to the UICC classification placed one patient in Stage I, another in Stage III, and the third in Stage IVa.

Inflammatory response varied, with one patient showing low inflammation and two demonstrating medium levels.

Additionally, precursor lesions were identified in one patient as biliary intraepithelial neoplasia (BLIN), while the other two had no precursor

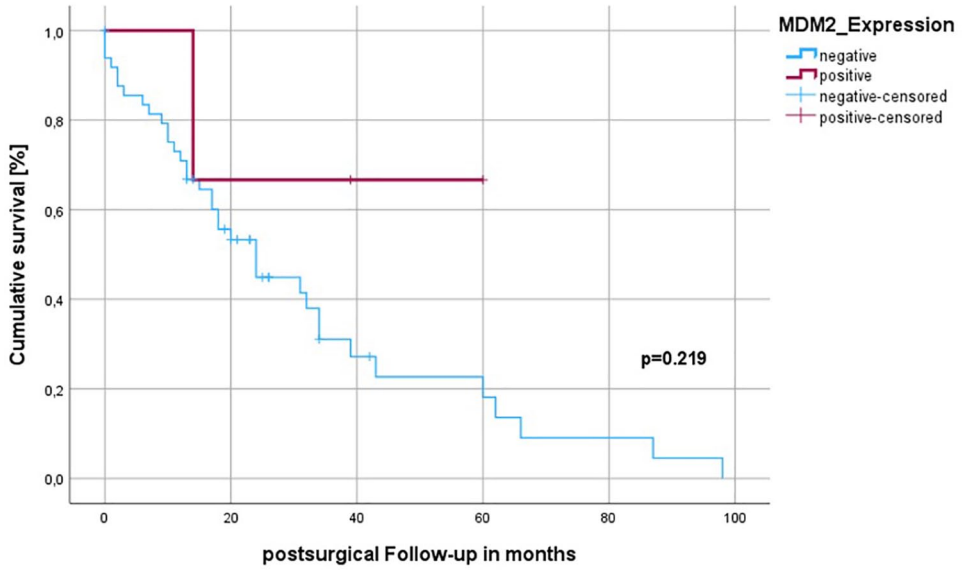

Figure 2. Kaplan–Meier survival analysis of the patient's postsurgical prognosis depending on the MDM2 status of the tumor. MDM2-positive patients showed a favorable prognosis after surgical resection in the current cohort ($p = 0.219$).
MDM2, mouse double minute 2 homolog.

lesions. No patients with MDM2 amplification presented with steatohepatitis, and cirrhosis was observed in only one patient.

Moreover, all patients with *MDM2* amplification exhibited wild-type p53 status. *FGFR2* translocations or alterations of the SWI/SNF complex were not observed in any of the *MDM2* amplified tumors.

In the next step, we performed survival analyses considering putative correlation between the patients` MDM2 status concerning the postsurgical prognosis. Here, we could demonstrate better postsurgical survival for MDM2-positive patients, however, the differences were not statistically significant ($p = 0.219$; see Figure 2). Additional subgroup analysis, including pT-category, pN-category, gross features, grading, M-status, UICC stage intra- versus extrahepatic CCA and occurrence of precursor lesions, steatohepatitis or cirrhosis did not reveal further aspects (data not shown). Among large-duct type iCCA, patients with positive *MDM2* amplification ($n = 2$) showed better survival than those patients with absent *MDM2* amplification ($n = 5$; $p = 0.041$). Only one patient with small-duct iCCA was MDM2-positive while 28 patients within this subgroup had no *MDM2* expression and had a decreased prognosis ($p = 0.160$). Since all patients with extrahepatic CCA expressed no *MDM2*, a survival analysis depending on this factor was not possible.

Notably, our study population exhibited only a sparse number of positive *MDM2* amplifications ($n = 3$), wherefore these results should be reviewed critically.

Discussion

We conducted a retrospective, single-center cohort study that included a total of 52 therapy-naïve Caucasian patients with primary CCA. Prevalence of *MDM2* amplification could be detected in 5.8% which roughly corresponds with previously reported prevalences of 2.7%–5.7%.[13,28] Furthermore, *MDM2* amplification could be found only in iCCA and each of the amplified samples presented p53 wild-type status. Previously conducted studies found that *MDM2* amplification was significantly more frequent in iCCA, particularly in the large-duct type, which also corresponds with our results.[20] Among the three detected samples with positive *MDM2* amplification, two fulfilled criteria to be considered large-duct type iCCA with bile-duct-like histology and periductal gross features, while one fulfilled criteria to be considered small-duct-type with mass-forming features and cholangiolar morphology.[3,6] Among the large-duct-type CCAs,

patients with *MDM2* amplification status showed significantly better survival than patients with negative amplification status ($p = 0.041$). Due to the small sample size of patients with *MDM2* amplification in the study ($n = 3$), this result should be interpreted with caution and may not be generalizable. Kim et al. initially found a worse prognosis for *MDM2* amplification which was revised after subgroup analysis for large-duct iCCA.[20] Therefore, the predictive value of *MDM2* amplification for patient survival remains unclear. Moreover, we could not confirm results from a previous publication claiming that *MDM2* amplification was associated with poor clinicopathological characteristics, such as high lymph node metastasis or distant metastases.[21]

However, prognostic questions are of secondary clinical relevance, as *MDM2* amplification primarily functions as a therapeutically relevant biomarker. *MDM2* amplified, *TP53*-non-mutated CCAs can be treated with modern MDM2-inhibitors.

For deeper analysis, we assessed previously described commonly mutated genes among *MDM2* amplified samples.[3] Here, we did not find any molecular co-altered patterns of *MDM2* and *FGFR2-translocations* or *MDM2* and SWI/SNF complex alterations (such as ARID1A-, BRG1-, BRM-, PBRM-1, and INI1) despite their common occurrence in iCCA.

Brigimadlin is a novel therapeutic agent that antagonizes MDM2-p53 and therefore regulates tumor growth.[22] It is currently under investigation among patients with amplified *MDM2* status, wild-type p53, and unresectable biliary tract cancers.[22] All three of our tumor samples with *MDM2* amplification are *TP53* wild-type CCAs and therefore would have been potential candidates for treatment with Brigimadlin.

Based on our data, strong immunohistochemical staining correlates closely with the results of FISH analysis. Additionally, the absence of MDM2 IHC positivity is likely indicative of the lack of polysomy or amplification. These findings suggest that MDM2 immunohistochemistry could serve as a valuable screening tool in routine diagnostics, particularly for identifying potential candidates for Brigimadlin treatment.

Limitations of the study entail the small population, especially among the *MDM2* amplified samples. In this study, we focused on *MDM2* amplification as the drug Brigimadlin is currently only under investigation among *MDM2*-amplified tumors.[22] Due to the small number of recruited patients our study's power is limited. As our cohort comprises solely Caucasian patients, another limitation is the inability to assess the prevalence of *MDM2* amplification across other ethnic groups. The available literature indicates variability in the prevalence of genetic mutations and amplifications among different populations, which may also apply to MDM2 amplification. However, comprehensive comparative data across diverse ethnicities are scarce. To address this gap and reliably confirm the role of *MDM2* amplification in prognosis, future studies should include a larger and more diverse patient population. Such multicentric studies are crucial to understand the full spectrum of *MDM2* amplification's epidemiology and its implications across different populations, especially given the reported low prevalence of this genetic aberration (2.7%–5.8%), and to confirm the role of *MDM2* amplification regarding prognosis.[13,28]

Conclusion

Real-world evidence in our Caucasian patient population confirmed that a significant number of iCCAs exhibit *MDM2* amplification, making them eligible for a personalized therapy option with Brigimadlin. *MDM2* amplification should therefore be considered in the context of personalized molecular testing in CCA.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the University of Cologne (protocol code 18-269). Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Author contributions

Su Ir Lyu: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Patrick Sven Plum: Formal analysis; Investigation; Writing – review & editing.

Caroline Fretter: Formal analysis; Writing – original draft.

Adrian Georg Simon: Methodology; Writing – review & editing.

Tillmann Bedau: Formal analysis; Writing – review & editing.

Karl Knipper: Investigation; Writing – review & editing.

Michael N. Thomas: Investigation; Writing – review & editing.

Dirk Stippel: Investigation; Writing – review & editing.

Britta Janina Wagner: Conceptualization; Writing – review & editing.

Christiane Bruns: Conceptualization; Writing – review & editing.

Dirk Waldschmidt: Methodology; Writing – review & editing.

Reinhard Büttner: Conceptualization; Writing – review & editing.

Uta Drebber: Conceptualization; Writing – review & editing.

Alexander Quaas: Conceptualization; Investigation; Methodology; Supervision; Writing – review & editing.

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Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Supplemental material
Supplemental material for this article is available online.

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