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Prognostic immunohistochemical biomarkers of chemotherapy efficacy in biliary tract cancer: A systematic review and meta-analysis

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ABSTRACT

Introduction: Chemotherapy is the mainstay of systemic treatment of biliary tract cancer (BTC). However, the treatment response to chemotherapy varies between patients. Currently, no prognostic biomarkers for chemotherapy efficacy have been considered for use in clinical practice. A systematic review was conducted to evaluate the prognostic value of immunohistochemical biomarkers for chemotherapy in patients with resected as well as with advanced BTC.

Method: Medline and EMBASE databases were searched up to March 2017 for studies that evaluated biomarker expression by immunohistochemistry in resected or advanced BTC patients treated with chemotherapy. The primary endpoints were overall survival (OS) and disease or progression free survival (DFS or PFS).

Result: Twenty-six studies, including a total of 1348 patients and 26 different biomarkers, met the inclusion criteria and were included in this review. The most frequently studied prognostic biomarkers in BTC were the human Equilibrative Nucleoside Transporter 1 (hENT1), Ribonucleotide Reductase M1 (RRM1), and excision repair cross-complementation 1 (ERCC1). In the meta-analysis of patients treated with gemcitabine-based chemotherapy, high hENT1 expression was associated with longer OS (HR 0.43, 95% CI: 0.28 to 0.64) and DFS/PFS (HR 0.45, 95% CI: 0.33 to 0.61).

Conclusion: hENT1 is a promising prognostic biomarker for gemcitabine-based chemotherapy in resected as well as in advanced BTC and should be further validated for the selection of patients for chemotherapy.

1. Introduction

Biliary tract cancer (BTC) is a heterogeneous group of malignancies with a poor prognosis. The majority of patients present with metastatic or locally advanced and therefore unresectable disease (Khan et al., 2002). Only a minority of patients with localized disease are considered as candidates for surgical resection with curative intent (Tan et al., 2008). However, recurrence and progression to metastatic disease is common within 2 years after resection (Jarnagin et al., 2003; Zhang et al., 2017; Groot Koerkamp et al., 2015).

Chemotherapy is the cornerstone of systemic treatment in unresectable BTC. A number of chemotherapy regimens, including gemcitabine-, platinum- and fluoropyrimidines-based chemotherapy, have been studied in BTC patients (Lamarca et al., 2014; Marino et al., 2013). Since 2010, the combination gemcitabine and cisplatin is considered the standard first-line treatment in advanced BTC, based on the results of the ABC-02 trial (Valle et al., 2014). Targeted therapy agents, mostly in combination with gemcitabine-based chemotherapy, have been

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evaluated in various phase II studies, but did not demonstrate any significant added value compared to chemotherapy alone (Verlingue et al., 2017). The efficacy of chemotherapy in the adjuvant setting is under investigation (Stein et al., 2015; Ebata et al., 2018; Edeline et al., 2017; Nakachi et al., 2018; Primrose et al., 2017). The peer reviewed results of the BILCAP study and other ongoing phase III clinical trials are awaited before adjuvant chemotherapy will be implemented in clinical practice (Stein et al., 2015; Ebata et al., 2018; Edeline et al., 2017; Nakachi et al., 2018; Primrose et al., 2017). The survival benefit of chemotherapy, if any, is limited, but its toxicity may be significant.

Better patient selection for chemotherapy in BTC could result in less exposure for nonresponding patients and therefore low toxicities and less costs. Currently, patient selection for chemotherapy is based on clinical characteristics including, age, performance status, comorbidity and disease stage. Recently, a retrospective analysis of 410 patients (from the ABC-02 clinical trial) and 753 patients from international studies has shown a weak association between clinical parameters, such as performance status and disease status, and survival (Bridgewater et al., 2016). Although several studies have evaluated prognostic biomarkers for chemotherapy efficacy in BTC, none of them have been implemented in clinical practice. Therefore, there is an urgent need to identify potential biomarkers to guide treatment choices in daily practice.

Immunohistochemistry (IHC) is a frequently used method to assess biomarker expression in tumour tissue because it is a widely applicable and cost-effective technique (Besse et al., 2013). This review aims to evaluate the prognostic value of IHC biomarkers for chemotherapy efficacy in resected as well as in advanced BTC.

2. Materials and methods

In order to identify IHC biomarkers of interest, we performed a systematic review. This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) and was registered at PROSPERO International prospective register of systematic reviews (CRD42016033660).

2.1. Search strategy and selection criteria

Medline and EMBASE databases were searched up to March 1, 2017 with a combination of the following search terms: 'biliary tract neoplasms', 'cholangiocarcinoma', 'gallbladder cancer', 'biomarker', 'biological marker', 'survival', 'response', 'chemotherapy' and 'systemic treatment'. Medical Subject Heading (MeSH) terms were used if available. Relevant reference lists were screened for additional publications.

The inclusion criteria were based on the REporting recommendations for tumour MARKer prognostic studies criteria (REMARK) for reporting IHC-based tumour marker studies (McShane et al., 2005). The inclusion criteria were: retrospective or prospective studies of resected or advanced BTC patients treated with adjuvant or palliative chemotherapy; IHC methodology is clearly described; IHC biomarkers had to be correlated, either in univariate or multivariate analyses, with chemotherapy outcomes including overall survival (OS), disease free survival (DFS) or progression free survival (PFS).

We excluded studies that included only patients with periampullary carcinomas and studies that investigated biomarkers associated with outcome of targeted therapy agents. Conference abstracts, case reports and studies not written in English or without full-text were excluded. Also small studies with less than 10 patients were excluded.

2.2. Data extraction

Two reviewers (A.B. and T.L.) have independently screened and assessed the identified study titles, abstracts and full-text publications for eligibility. In case of disagreements in the eligibility of studies, a discussion was conducted in order to achieve consensus. The following data were extracted from each study: total number of patients with a biomarker, number of positive cases of a biomarker, disease stage, chemotherapy regimen, staining pattern, internal reference cells, origin of antibodies, cut-offs for high versus low expression, OS, DFS or PFS (as shown in Table 1 and 2). Hazard ratios (HRs) of OS and DFS or PFS with corresponding 95% confidence interval (CI) were obtained from each study or estimated using the spreadsheet provided by Tierney et al. (2007). In case of unpublished relevant data, authors were asked to provide us with these results to estimate the HRs of OS and DFS or PFS.

2.3. Prognostic versus predictive biomarker

A biomarker is defined as prognostic if it can stratify patients in terms of their clinical outcome independent of the received treatment (Ballman, 2015). A biomarker is predictive if the treatment effect differs significantly in biomarker-positive patients compared to biomarker-negative patients. This can only be assessed if at least 2 comparison groups (i.e. chemotherapy vs. best-supportive care) are available (Ballman, 2015). This systematic review discuses only prognostic biomarkers in BTC patients treated with chemotherapy.

2.4. Quality assessment of eligible studies

The modified Newcastle-Ottawa Scale (mNOS) for cohort studies was used to assess the quality of included studies in this review (Wells et al., 2000). This scale consists of 3 categories with a total of 8 items: patient selection (4 items, 1 point per item), comparability of cohorts (1 item, 0–2 points), and assessment of outcome (3 items, 1 point per item). The total score for each study ranged between 0 and 9 points. Studies with 6 or more points were considered as of high quality as previously described by Xue et al. (2017).

2.5. Statistical analysis

The primary endpoints were OS and DFS or PFS. The meta-analysis was performed in Review Manager 5.3.5. The heterogeneity between studies in the meta-analysis was assessed by the I^2 statistic. I^2 is widely used to assess the total variance in the pooled results that is caused by heterogeneity between the studies in a meta-analyse (Higgins et al., 2003). An I^2 below 50% was considered to show non-significant heterogeneity among studies and a fixed-effects model was used. The publication bias was evaluated by funnel plots.

Biomarkers were pooled in the meta-analysis if the HRs of at least 4 studies per biomarker were available or could be estimated and the patients received comparable chemotherapy regimens. Only univariate HRs of OS and DFS or PFS were used in the meta-analysis.

3. Results

3.1. Study selection

The search in PubMed and Embase yielded 3428 potentially relevant publications of which 175 duplicates were excluded. By screening the titles and abstracts of these records, 2956 irrelevant studies were excluded. Subsequently, a total of 298 articles were assessed for eligibility by full-text analysis. Finally, a total of 26 articles were included in this systematic review (da Costa Miranda et al., 2014; Harder et al., 2013; Hwang et al., 2011; Kim et al., 2016; Nakamura et al., 2010; Santini et al., 2011; Sekine et al., 2012; Woo et al., 2017; Park et al., 2016; Deng et al., 2014; Kim et al., 2013a; Mian et al., 2016; Murata et al., 2013; Won et al., 2010; Chen et al., 2016; Chiang et al., 2016; Borbath et al., 2012; Brandi et al., 2016; Kobayashi et al., 2012; Sasaki et al., 2014; Ogo et al., 2006). Fig. 1 illustrates the article selection process of this systematic review.

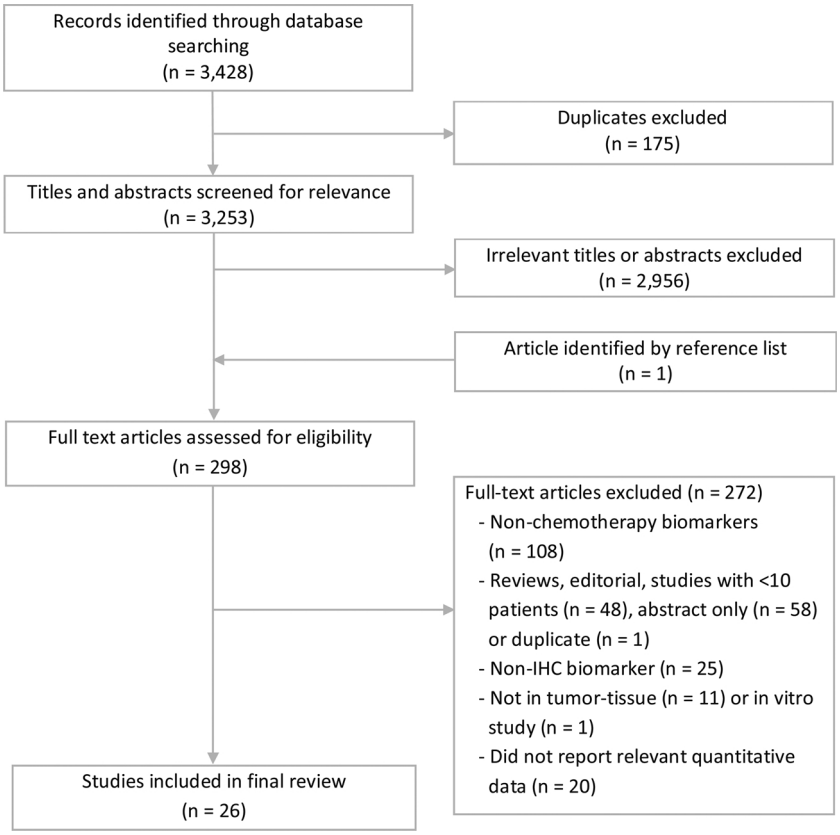

Fig. 1. PRISMA Flowchart of article selection process.

3.2. Study characteristics

Table 1 summarizes the main characteristics of the identified studies. Of the 26 eligible studies, 2 were post-hoc analyses of phase-II studies (Woo et al., 2017; Chiang et al., 2016) and the remaining 24 studies had a retrospective design. Eighteen studies were from East-Asia and 8 studies from Europe or America. The sample size of included studies varied from 10 to 99 patients with a total of 1348 patients. Included BTC subtypes were: all subtypes (8 studies), extrahepatic cholangiocarcinoma (EHCC) and gallbladder cancer (GBC) (6 studies), EHCC and intrahepatic cholangiocarcinoma (IHCC) (4 studies), only patients with EHCC (4 studies), only patients with IHCC (3 studies), and only patients with GBC (1 study). Fifteen studies included patients with resected BTC, 10 studies included patients with advanced BTC and 1 study included resected as well as advanced BTC patients treated with chemotherapy. The treatment consisted of gemcitabine, fluorouracil, S-1, platinum or uracil and tegafur-based chemotherapy in 15, 5, 1, 1 and 1 studies, respectively. Three studies included patients treated with gemcitabine as well as with fluorouracil-based chemotherapy (Table 1).

Twenty-six biomarkers were evaluated immunohistochemically in a total of 1348 patients with BTC. Most frequently evaluated biomarkers in BTC are human Equilibrative Nucleoside Transporter 1 (hENT1), Ribonucleotide Reductase M1 (RRM1), and Excision Repair Cross-Complementation 1 (ERCC1) and were reported in 8, 5 and 4 studies, respectively (Table 1). The remaining 23 biomarkers were evaluated in less than 3 studies and include aldehyde dehydrogenase (ALDH1A3), aquaporin 5 (AQP-5), c-erbB-2 protein (HER-2), cluster of differentiation 24 (CD24), cytidine deaminase (CDA), mesenchymal–epithelial transition factor (c-Met), cluster of differentiation 8/163 (CD8/CD163), cluster of differentiation 24 (CD24), Parkinson protein-7 (DJ-1), deoxycytidine Kinase (dCK), dihydropyrimidine (DPD), epidermal growth factor receptor (EGFR or HER-1), heat shock protein-60 (HSP60), inhibitor of differentiation 1–4 (ID 1–4), p53, programmed cell death 1 ligand 1 protein (PD-1 L), programmed cell death protein 1 /CD8 tumor-infiltrating lymphocytes (PD-1/CD8 TILs), Progranulin (PGRN), c-ros oncogene 1 (ROS), anaplastic lymphoma kinase (ALK) and c-Met (RAM), thymidine phosphorylase (TP), thymidylate synthase (TS), T-box transcription factor-4 (TBX4), vascular endothelial growth factor (VEGF) and x-ray repair cross-complementing group (XRCC1) (Table 1).

The results of most frequently studied biomarkers (hENT1, RRM1 and ERCC1) will be discussed below. The results of other biomarkers are summarized in Table 2, but will not discussed in details because of limited number of studies with low number of patients.

3.3. hENT1

The major transmembrane transporter for nucleoside analogues, including gemcitabine, is formed by the protein hENT1 (Pastor-Anglada and Perez-Torras, 2015). This transporter is mainly located on the cell membrane of different epithelial cells, including the biliary tract (Pastor-Anglada and Perez-Torras, 2015). Eight studies evaluated the prognostic value of hENT1 by IHC in a total of 334 BTC patients treated with gemcitabine monotherapy or gemcitabine-based chemotherapy. Four studies assessed hENT1 status in a total of 205 patients with resected BTC who received adjuvant gemcitabine monotherapy (137 patients from 2 studies) or gemcitabine-based chemotherapy (68 patients from 2 studies). The remaining 4 studies included a total of 129 patients with advanced BTC treated with gemcitabine monotherapy (in 2 studies) or gemcitabine-based chemotherapy. Of these 8 studies, 6 were of high quality (mNOS $\geq$ 6) and the other 2 studies had a mNOS of 5 points (Table 1).

95% CI, 95% confidence interval; ALDH1A3, aldehyde dehydrogenase; AQP-5, aquaporins 5; BTC, biliary tract cancer; CD24, cluster of differentiation 24; CD8/CD163, cluster of differentiation 8/163; CDA, cytidine deaminase; c-Met, mesenchymal-epithelial transition factor; dCK, deoxycytidine Kinase; DFS, disease-free survival; DJ-1,

Table 1
Main characteristics of studies with prognostic biomarkers of chemotherapy efficacy in BTC.

Biomarker	Ref.	Country	N.	Inclusion period	BTC subtype	Disease stage[a]	Chemotherapy	High expression n (%)	Staining pattern	Reference cells	Cut-off for positivity (staining intensity score)	Origin of antibody	Survival analysis	Median follow-up, mo. (range)	mNOS scale
hENT1	(Brandi et al., 2016)	Italy	71	2002 - 2011	IHCC and EHCC	resectable	gemcitabine	26 (36.6)	membrane/ cytoplasm	lymphocytes	strong staining (2+)[b]	polyclonal rabbit	DFS	18.1 (9.1 - 36.2)	6
	(Sasaki et al., 2014) [c]	Japan	68	1989 - 2012	IHCC and EHCC	resectable	gemcitabine-based	45 (66.2)	NR	lymphocytes	≥ moderate staining (2+ or 3+) in > 50% of cells	polyclonal rabbit	OS/DFS	81 (9 - 294)	6
	(Woo et al., 2017)	Korea	66	2010 - 2014	all subtypes	resectable	gemcitabine	31(47.0)	membrane	lymphocytes	NR	polyclonal rabbit	DFS	38.07 (3.68 - 68.25)	6
	(Kobayashi et al., 2012)	Japan	51	1989 - 2010	IHCC and EHCC	resectable	gemcitabine or gemcitabine-based	36 (70.6)	NR	lymphocytes	≥ moderate staining (2+ or 3+) in > 50% of cells	polyclonal rabbit	OS	76 (9 - 266)	6
	(Deng et al., 2014)	China	44	2006 - 2013	EHCC and GBC	advanced	gemcitabine or gemcitabine-based	26 (59.1)	cytoplasm/ nucleus	NR	H-score ≥ 6	polyclonal rabbit	OS/PFS	NR (14.9 - 99)[d]	6
	(Santini et al., 2011)	Italy	31	2001 - 2008	all subtypes	advanced	gemcitabine or gemcitabine-based	21 (67.7)	membrane/ cytoplasm	lymphocytes	≥ weak staining (1+ or 2+) in > 50% cells	monoclonal mouse	OS/PFS	13	6
	(Murata et al., 2013)	Japan	28	2006 - 2011	EHCC and GBC	advanced	gemcitabine	17 (60.7)	membrane/ cytoplasm	lymphocytes	≥ weak staining in > 50% of cells	polyclonal rabbit	OS/PFS	NR	5
	(Borbath et al., 2012)	Belgium	26	1998 - 2007	IHCC and EHCC	advanced	gemcitabine	17 (65.4)	NR	lymphocytes	≥ weak staining in > 50% of cells	monoclonal mouse[e]	OS/PFS	9.5 (1 - 120)	5
RRM1	(Sasaki et al., 2014) [c]	Japan	68	1989 - 2012	IHCC and EHCC	resectable	gemcitabine-based	35 (51.5)	NR	Plasma and stromal cells	≥ moderate staining (2+ or 3+) in > 50% of cells	polyclonal rabbit	OS/DFS	81 (9 - 294)	6
	(Woo et al., 2017)	Korea	66	2010 - 2014	all subtypes	resectable	gemcitabine	42 (63.6)	nucleus	NR	NR	polyclonal rabbit	DFS	38.07 (3.68 - 68.25)	6
	(Deng et al., 2014)	China	44	2006 - 2013	EHCC and GBC	advanced	gemcitabine or gemcitabine-based	22 (50.0)	membrane/ cytoplasm	NR	H-score ≥ 6	monoclonal rabbit	OS/PFS	NR (14.9 - 99)[d]	6
	(Murata et al., 2013)	Japan	28	2006 - 2011	EHCC and GBC	advanced	gemcitabine	17 (60.7)	cytoplasm	Plasma and stromal cells	≥ weak staining in > 50% of cells	monoclonal mouse	OS/PFS	NR	5
	(Nakamura et al., 2010)	Japan	10	2005 - 2008	all subtypes	advanced	gemcitabine or gemcitabine-based + RT	4 (40.0)	NR	ß-actin	≥ 0.984[f]	NR	OS	19.2 (5.5 -34.9)	5
ERCC1	(Hwang et al., 2011)	Korea	93	2002 - 2008	all subtypes	advanced	platinum-based + RT	46 (49.5)	nucleus	endothelial cells	H-score ≥ 1.5	monoclonal mouse, clone 8F1	OS/PFS	7.3 (0.8 - 47.5)	6
	(Mian et al., 2016)	Canada	25	1992 - 2012	EHCC and GBC	resectable	gemcitabine	13 (52.0)	nucleus	NR	H-score > 0.5	monoclonal 8F1	OS/DFS	NR	5
	(Won et al., 2010)	Korea	66	1994 - 2003	GBC and EHCC	resectable	5-FU-based + RT	NR	NR	NR	> 49% of positive cells	NR	OS/DFS	NR	3
	(da Costa Miranda et al., 2014)	Brazil	44	2009 - 2011	all subtypes	advanced	gemcitabine-based	23 (52.0)	nucleus	stromal cells	H-score ≥ 0.5	NR	OS	NR	3
XRCC1	(Mian et al., 2016)	Canada	25	1992 - 2012	EHCC and GBC	resectable	gemcitabine	17 (68.0)	nucleus	NR	H-score > 1.3	monoclonal clone 32-2-5	OS/DFS	NR	5

(*continued on next page*)

Table 1 (*continued*)

Biomarker	Ref.	Country	N.	Inclusion period	BTC subtype	Disease stage[a]	Chemotherapy	High expression n (%)	Staining pattern	Reference cells	Cut-off for positivity (staining intensity score)	Origin of antibody	Survival analysis	Median follow-up, mo. (range)	mNOS scale
dCK	(Woo et al., 2017)	Korea	66	2010 - 2014	all subtypes	resectable	gemcitabine	21 (31.8)	cytoplasm	lymphocytes	NR	polyclonal rabbit	DFS	38.07 (3.68 - 68.25)	6
	(Murata et al., 2013)	Japan	28	2006 - 2011	EHCC and GBC	advanced	gemcitabine	15 (53.6)	cytoplasm/ nucleus	lymphocytes	≥ weak staining in > 50% of cells	polyclonal rabbit	OS/PFS	NR	5
TP	(Kim et al., 2013a)	Korea	99	1999 - 2009	EHCC and GBC[g]	resectable	5-FU or 5-FU-based	70 (70.7)	cytoplasm/ nucleus	NR	NR	sc-47702	OS	80.4 (41.3 - 149.9)	6
	(Won et al., 2010)	Korea	66	1994 - 2003	GBC and EHCC	resectable	5-FU-based + RT	NR	NR	NR	> 49% of positive cells	NR	OS/DFS	NR	3
TS	(Kim et al., 2013a)	Korea	99	1999 - 2009	EHCC and GBC[g]	resectable	5-FU or 5-FU-based	25 (25.3)	cytoplasm/ nucleus	NR	NR	clone TS106	OS	80.4 (41.3 - 149.9)	6
ALDH1A3	(Chen et al., 2016)	Taiwan	31	–	IHCC	advanced	gemcitabine-based	8(25.8)	cytoplasm	NR	H-score ≥ 100	clone N2C2	OS/PFS	NR	3
AQP-5	(Sekine et al., 2012)	Japan	10	1997 - 2010	all subtypes	resectable	gemcitabine	5 (50)	membrane	NR	sum of staining ≥ 3[h]	anti-AQP-5 H-200	OS/DFS	NR	4
CDA	(Woo et al., 2017)	Korea	66	2010 -2014	all subtypes	resectable	gemcitabine	38(57.6)	cytoplasm	NR	NR	polyclonal rabbit	DFS	38.07 (3.68 - 68.25)	6
c-Met	(Park et al., 2016)	Korea	86	2000 - 2006	EHCC	resectable	5-FU or capecitabine + RT	66 (76.7)	membrane/ cytoplasm	NR	≥ weak staining (1+ or 2+) in ≥ 30% of cells	NR	OS/DFS	NR	4
CD8/CD163[i]	(Miura et al., 2017)	Japan	45	2005 - 2011	EHCC	resectable	S-1[j]	12 (26.7)	NR	NR	median number of CD8 of ≥ 5.6 and CD163 < 71.3	monoclonal mouse C8/144B and MRQ-26	OS	38 (2 - 111)	5
CD24	(Kim et al., 2013b)	Korea	84	2000 - 2006	EHCC	resectable	5-FU + RT	36 (42.9)	NR	NR	any staining percentage (≥ 1+)	clone SN3b	OS/DFS	NR	5
DJ-1	(Zong et al., 2013)	China	72	1998 - 2007	IHCC	resectable	gemcitabine-based	39 (54.2)	cytoplasm/ nucleus	normal liver tissue	≥ moderate staining in > 25% of cells	NR	OS	NR	6
DPD	(Kim et al., 2013a)	Korea	99	1999 - 2009	EHCC and GBC[g]	resectable	5-FU or 5-FU-based	74 (74.7)	cytoplasm/ nucleus	NR	NR	sc-50521	OS	80.4 (41.3 - 149.9)	6
EGFR	(Park et al., 2016)	Korea	90	2000 - 2006	EHCC	resectable	5-FU or capecitabine + RT	64 (71.1)	NR	NR	≥ weak staining (1+ or 2+) in ≥ 30% of cells	NR	OS/DFS	NR	4
HER-1 and 2	(Ogo et al., 2006)	Japan	55	1983 - 2003	EHCC and GBC	resectable	uracil and tegafur-based	29 (52.7), 37 (67.3)[k]	membrane	lung cancer and breast cancer tissue[k]	HercepTest score 2+ or 3+ in > 80% of cells	monoclonal mouse, polyclonal rabbit[k]	OS	NR	8
HSP60	(Zong et al., 2013)	China	72	1998 - 2007	IHCC	resectable	gemcitabine-based	38 (52.8)	membrane/ cytoplasm	normal liver tissue	≥ moderate staining in > 25% of cells	NR	OS	NR	6
ID-2, 3 and 4	(Harder et al., 2013)	Germany	64	1996 - 2007	all subtypes	advanced	gemcitabine-based or 5-FU	13(20.3), 28(43.8), 33(51.6)[l]	cytoplasm/ nucleus	vascular smooth muscle cells	≥ moderate staining (2+ or 3+) in > 50% of cells	polyclonal rabbit sc-489, sc-490 and sc-13047[l]	OS	16.5	5
p53	(Puhalla et al., 2004)	Austria	21	1991 - 2002	GBC	resectable and advanced	gemcitabine or 5-FU-based	4 (19.0)	nucleus	NR	> 75% cells with positive staining (3+)	NR	OS	64.3 (0.3 - 83.6)	5

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Table 1 (*continued*)

Biomarker	Ref.	Country	N.	Inclusion period	BTC subtype	Disease stage[a]	Chemotherapy	High expression n (%)	Staining pattern	Reference cells	Cut-off for positivity (staining intensity score)	Origin of antibody	Survival analysis	Median follow-up, mo. (range)	mNOS scale
PD-L1	(Lim et al., 2015)	Korea	83	2000 - 2006	EHCC	resectable	5-FU or capecitabine + RT	56 (67.5)	membrane	NR	H-score > 5	monoclonal rabbit, clone E1L3 N	OS/DFS	27	5
PD-1/ CD8 TILs	(Lim et al., 2015)	Korea	83	2000 - 2006	EHCC	resectable	5-FU or capecitabine + RT	66 (79.5)	NR	NR	ratio ≥ 0.40	rabbit antibody, clone EPR4877 and clone SP16	OS/DFS	27	5
PGRN	(Kim et al., 2016)	Korea	80	2004 - 2014	all subtypes	advanced	gemcitabine- or 5-FU-based	35 (44)	cytoplasm/ stromal	NR	≥ moderate staining (2+ or 3+)	monoclonal mouse PG359-7	OS/PFS	17.7 (4.9 - 35.1)	6
RAM[m]	(Chiang et al., 2016)	Taiwan	42	2010 - 2012[n]	IHCC	advanced	gemcitabine-based	8 (19.0)	cytoplasm	NR	strong staining (3+) for any marker in > 10% of cells	anti-ROS1, anti-ALK clone ZAL4 and anti-c-MET clone C12	OS/PFS	10.3 (0.9 - 45.4)	9
TBX4	(Zong et al., 2013)	China	72	1998 - 2007	IHCC	resectable	gemcitabine-based	38 (52.8)	nucleus/ cytoplasm	normal liver tissue	≥ moderate staining in > 25% of cells	NR	OS	NR	6
VEGF	(Park et al., 2016)	Korea	87	2000 - 2006	EHCC	resectable	5-FU or capecitabine + RT	57 (65.5)	NR	NR	≥ weak staining (1+ or 2+) in ≥ 30% of cells	NR	OS/DFS	NR	4

[j]All patients received S-1 chemotherapy. One patient received uracil- and tegafur before S-1 and one patient received S-1 plus gemcitabine after S-1.

[a] Advance disease stage refers to patients with locally advanced, recurrent, and/or metastatic BTC.

[b] This information was provided by the author.

[c] Patients from the study of Kobayashi et al. (2012) were included in this study.

[d] The range of follow-up was calculated from the available data.

[e] This data was extracted from the mentioned reference study.

[f] The staining intensities were assessed by quantitative double-fluorescence IHC and the mean value of staining intensity of all cases was determined as the cut-off for positivity.

[g] Patients with ampullary carcinoma were also included in this study.

[h] This score was calculated as the sum of staining intensities (0, negative; 1, low; 2, moderate; 3, strong) and the percentage of positive tumour cells (0, < 10%; 1, ≥ 10 to < 50%; 2, ≥ 50%).

[i] Represents the ratio of high CD8 T-cells in cancer cell and low CD163 macrophages in tumour stroma.

[k] For Her-1 and 2 respectively.

[l] Data shown for ID-2, 3 and 4 respectively. Results of ID-1 were not shown in the original article.

[m] This study is a post-hoc analysis of patients participated in a phase II trial. Only the study-arm of patients who received chemotherapy alone are included in this review.

[n] This data was extracted from the original Phase II study (Chen et al., 2015).

Table 2
Association between biomarkers expression and survival in BTC patients treated with chemotherapy.

Biomarker	Ref.	N.	High expression, n (%)	Overall survival						Disease or progression-free survival					
				Median (mo.) or 5-year (%) of high vs. low staining	*P*-value	HR of univariate analysis (95% CI)	*P*-value	HR of multivariate analysis (95% CI)	*P*-value	Median (mo.) or 5-year (%) of high vs. low staining	*P*-value	HR of univariate analysis (95% CI)	*P*-value	HR of multivariate analysis (95% CI)	*P*-value
hENT1	(Brandi et al., 2016)	71	26 (36.6)	NR	NR	NR	NR	NR	NR	39.0 vs. 16.0 mo.	0.047	0.49 (0.24 - 0.99)	0.046	0.50 (0.26 - 0.97)	0.040
	(Sasaki et al., 2014)	68	45 (66.2)	55.0% vs. 32.0%	0.036	*0.43 (0.18 - 0.99)*	0.036	0.55 (0.26 - 1.21)	0.135	45.0% vs. 21.0%	0.005	*0.41 (0.20 - 0.86)*	0.005	0.49 (0.24 - 0.98)	0.044
	(Woo et al., 2017)	66	31 (47.0)	NR	NR	NR	NR	NR	NR	*17.5* vs. *11.6 mo.*	0.264	0.71 (0.38 - 1.31)	0.267	0.62 (0.33 - 1.16)	0.131
	(Kobayashi et al., 2012)	51	36 (70.6)	59.0% vs. 0%	0.008	*0.27 (0.10 - 0.71)*	0.008	0.36 (0.15 - 0.89)	0.027	50.0% vs. 0%	0.012	NR	NR	NR	NR
	(Deng et al., 2014)	44	26 (59.1)	13.0 vs. 7.0 mo.	0.135	0.413 *(0.21 - 0.81)*	0.048	NR	0.143	5.0 vs. 4.0 mo.	0.002	0.330 *(0.17 - 0.65)*	0.005	NR	0.005
	(Santini et al., 2011)	31	21 (67.7)	14.0 vs 7.0 mo.	0.128	0.48 (0.187 - 1.236)	0.128	NR	NR	6.33 vs. 2.83 mo.	0.03	0.345 (0.125 - 0.950)	0.039	NR	NR
	(Murata et al., 2013)	28	17 (60.7)	11.4 vs. 5.7 mo.	0.006	NR	NR	0.220 (0.077 - 0.629)	0.005	7.7 vs. 2.5 mo.	0.007	NR	NR	NR	NR
	(Borbath et al., 2012)	26	17 (65.4)	11.0 vs 5.0 mo.	0.036	0.41 (0.17 - 0.98)	0.046	0.41 (0.17 - 0.98)	0.046	6.0 vs. 2.0 mo.	0.012	0.35 (0.14 - 0.87)	0.023	0.35 (0.14 - 0.87)	0.048
RRM1	(Sasaki et al., 2014) [a]	68	35 (51.5)	33.0% vs. 59.0%	0.035	*2.16 (1.04 - 4.46)*	0.035	2.33 (1.12 - 5.0)	0.024	25.0% vs. 47.0%	0.015	*2.08 (1.08 - 4.02)*	0.015	2.44 (1.25 - 4.76)	0.009
	(Woo et al., 2017)	66	42 (63.6)	NR	NR	NR	NR	NR	NR	*13.0* vs. *16.1 mo.*	0.505	1.24 (0.66 - 2.32)	0.505	0.96 (0.50 - 1.83)	0.895
	(Deng et al., 2014) [a]	44	22 (50.0)	*10.0* vs. *18.2 mo.*	0.165	1.033 *(0.53 - 2.00)*	0.945	NR	0.173	*4.4* vs. *5.0 mo.*	0.483	1.304 *(0.67 - 253)*	0.507	NR	0.718
	(Murata et al., 2013)	28	17 (60.7)	10.0 vs. 10.2 mo.	0.594	NR	NR	2.315 (0.773 - 6.928)	0.133	2.5 vs. 7.4 mo.	0.135	NR	NR	NR	NR
	(Nakamura et al., 2010)	10	4 (40.0)	*13* vs. *33.6 mo.*	0.001	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
ERCC1	(Hwang et al., 2011)	93	46 (49.5)	7.8 vs. 7.0 mo.	0.143	*0.73 (0.47 - 1.11)*	0.143	NR	NR	2.9 vs. 4.2 mo.	0.116	*1.39 (0.92 - 2.10)*	0.116	NR	NR
	(Mian et al., 2016)	25	13 (52.0)	39.7 vs. 22.9 mo.	0.011	*0.30 (0.11 - 0.79)*	0.011	NR	NR	19.8 vs. 11.3 mo.	0.013	*0.31 (0.12 - 0.81)*	0.013	NR	NR
	(Won et al., 2010)	66	NR	NR	NR	NR	0.794	NR	NR	NR	NR	NR	0.591	NR	NR
	(da Costa Miranda et al., 2014)	44	23 (52.0)	*11* vs *8.3 mo.*	0.51	0.96 (0.71 - 1.30) [b]	0.82	NR	NR	NR	NR	NR	NR	NR	NR
XRCC1	(Mian et al., 2016)	25	17 (68.0)	33.8 vs. 14.6 mo.	0.005	*0.19 (0.06 - 0.63)*	0.005	NR	NR	16.3 vs. 11 mo.	0.052	*0.36 (0.12 - 1.04)*	0.052	NR	NR
dCK	(Woo et al., 2017)	66	21 (31.8)	NR	NR	NR	NR	NR	NR	34.95 vs. 11.41 mo.	0.154	0.61 (0.31 - 1.22)	0.160	0.49 (0.24 - 0.98)	0.043
	(Murata et al., 2013)	28	15 (53.6)	11.4 vs. 9.2 mo.	0.373	NR	NR	0.778 (0.284 - 2.132)	0.625	3.7 vs. 4.6 mo.	0.808	NR	NR	NR	NR
TP	(Kim et al., 2013a)	99	70 (70.7)	26.9% vs. 65.2%	0.007	NR	NR	2.014 (1.035 - 3.921)	0.039	NR	NR	NR	NR	NR	NR
	(Won et al., 2010)	66	NR	NR	NR	NR	0.212	NR	NR	NR	NR	NR	0.322	NR	NR
TS	(Kim et al., 2013a)	99	25 (25.3)	46.8% vs. 35.1%	0.222	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
TBX4	(Zong et al., 2013)	72	38 (52.8)	NR	NR	1.016 (0.623 - 1.656)	0.951	NR	NR	NR	NR	NR	NR	NR	NR
HSP60	(Zong et al., 2013)	72	38 (52.8)	NR	NR	0.967 (0.593 - 1.575)	0.892	NR	NR	NR	NR	NR	NR	NR	NR

(*continued on next page*)

Table 2 (*continued*)

Biomarker	Ref.	N.	High expression, n (%)	Overall survival						Disease or progression-free survival					
				Median (mo.) or 5-year (%) of high vs. low staining	*P*-value	HR of univariate analysis (95% CI)	*P*-value	HR of multivariate analysis (95% CI)	*P*-value	Median (mo.) or 5-year (%) of high vs. low staining	*P*-value	HR of univariate analysis (95% CI)	*P*-value	HR of multivariate analysis (95% CI)	*P*-value
DJ-1	(Zong et al., 2013)	72	39 (54.2)	NR	NR	1.673 (1.021 - 2.743)	0.041	1.980 (1.161 - 3.375)	0.012	NR	NR	NR	NR	NR	NR
ALDH1A3	(Chen et al., 2016)	31	8 (25.8)	7.5 vs. 14.6 mo.	0.006	NR	NR	NR	NR	2.7 vs. 7.4 mo.	0.005	NR	NR	NR	NR
AQP-5	(Sekine et al., 2012)	10	5 (50.0)	*100%* vs. *18.1%*[c]	0.033	NR	NR	NR	NR	*100%* vs. *0%*[c]	0.002	NR	NR	NR	NR
CDA	(Woo et al., 2017)	66	38(57.6)	NR	NR	NR	NR	NR	NR	*8.8* vs. *18 mo.*	0.249	1.43 (0.78 - 2.64)	0.251	1.09 (0.57 - 2.06)	0.795
c-Met	(Park et al., 2016)	86	66 (76.7)	43.0% vs. 25.0%	0.032	NR	NR	0.695 (0.352 - 1.372)	0.2940	48.2% vs. 33.0%[d]	0.126	NR	NR	NR	NR
CD8/CD163	(Miura et al., 2017)	45	12 (26.7)	NA vs. 44.1 mo.	0.036	NR	NR	0.139 (0.026 - 0.739)	0.021	NR	NR	NR	NR	NR	NR
CD24	(Kim et al., 2013b)	84	36 (42.9)	37.2% vs. 50.3%	0.187	NR	NR	NR	NR	29.0% vs. 55.1%[d]	0.0100	NR	NR	NR	0.073[d]
DPD	(Kim et al., 2013a)	99	74 (74.7)	36.0% vs. 44.0%	0.192	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
EGFR	(Park et al., 2016)	90	64 (71.1)	48.2% vs. 35.4%	0.872	NR	NR	NR	NR	48.8% vs. 45.4%[d]	0.979	NR	NR	NR	NR
HER-1	(Ogo et al., 2006)	55	29 (52.7)	9.3 vs. 73.9 mo.	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
HER-2	(Ogo et al., 2006)	55	37 (67.3)	69.5 vs. 30.6 mo.	ns	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
ID-2[e]	(Harder et al., 2013)	64	13(20.3)	6.0 vs. 15.6 mo.[g]	0.001	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
ID-3[f]	(Harder et al., 2013)	64	28(43.8)	12.0 vs. 13.2 mo.[g]	0.037	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
ID-4[f]	(Harder et al., 2013)	56	33(58.9)	6.0 vs. 13.2 mo.[g]	0.025	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
p53	(Puhalla et al., 2004)	21	4 (19.0)	NR	0.078	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
PD-L1	(Lim et al., 2015)	83	56 (67.5)	44.2% vs. 25.9%	0.192	NR	NR	NR	NR	31.7% vs. 27.2%	0.300	NR	NR	NR	NR
PD-1+/ CD8+ TILs	(Lim et al., 2015)	83	66 (79.5)	31.8% vs. 58.4%	0.032	NR	0.032	2.47 (1.04 - 5.86)	0.041	25.5% vs. 50.4%	0.024	NR	0.024	2.41 (1.08 - 5.41)	0.033
PGRN	(Kim et al., 2016)	80	35 (44.0)	9.1 vs. 9.5 mo.	0.746	NR	NR	NR	NR	2.7 vs. 5.0 mo.	0.023	NR	NR	1.69 (1.02 - 2.81)	0.044
RAM	(Chiang et al., 2016)	42	8 (19.0)	5.2 vs. 9.6 mo.	NR	NR	NR	NR	NR	4.5 vs. 4.9 mo.	NR	NR	NR	NR	NR
VEGF	(Park et al., 2016)	87	57 (65.5)	33.9% vs. 44.6%	0.864	NR	NR	NR	NR	47.4% vs. 42.9%[d]	0.794	NR	NR	NR	NR

Data in italic were estimated from the available data as explained in the methods. NA, not available; NR, not reported; ns, not significant;

[a] HRs were converted to HRs for high versus low RRM1.

[b] This is a HR for ERCC1 as a continue variable. The HR for ERCC1 as categorical variable was not given (*P*-value = 0.51).

[c] This represents the 2-year survival.

[d] Percentages of 5-year distant metastasis-free survival.

[e] Data are presented for nuclear ID-2 expression.

[f] Data are presented for cytoplasmic ID-3 and 4 expression.

[g] Data were converted from years to months.

Parkinson protein-7; DPD, dihydropyrimidine; EGFR or HER-1, epidermal growth factor receptor; EHCC, extrahepatic cholangiocarcinoma; ERCC1, Excision Repair Cross-Complementation 1; FU, Fluorouracil; GBC, gallbladder cancer; H-score, was calculated as the product of the staining intensity and the percentage of positive cells; hENT1, human Equilibrative Nucleoside Transporter 1; HER-2, c-erbB-2 protein; HSP60, heat shock protein-60; ID 1–4, inhibitor of differentiation 1–4; IHCC, intrahepatic cholangiocarcinoma; mNOS scale: modified Newcastle-Ottawa quality assessment Scale; mo., months; N, number of patients; NR, not reported; OS, overall survival; PD-1/CD8 TILs, programmed cell death protein 1 and CD8 tumor-infiltrating lymphocytes ratio; PD-1 L, programmed cell death 1 ligand 1 protein; PFS, progression-free survival; PGRN, Progranulin; RAM, ROS1, anaplastic lymphoma kinase (ALK) or c-MET; Ref., reference; RRM1, Ribonucleotide Reductase M1; RT, radiotherapy; TBX4, T-box transcription factor-4; TP, Thymidine Phosphorylase; TS, Thymidylate Synthase; VEGF, vascular endothelial growth factor; XRCC1, X-ray repair cross-complementing group 1.

3.3.1. Immunohistochemical analysis of hENT1

High hENT1 expression was found in 183 of 334 (54.8%) patients and varied between 36.6% and 70.6%. The expression of hENT1 was performed by IHC using the rabbit polyclonal antibody in 6 studies and the non-commercial available mouse monoclonal antibody in the remaining 2 studies (Table 1). Most studies (6 of 8) examined both the staining intensities and percentage of positive cells for hENT1. All studies used lymphocytes as the internal reference cells, except 1 study Woo et al. (2017) that did not report which internal reference was used. Of the 8 studies that assessed hENT1, 5 studies used moderate or weak staining in more than 50% of cells as a cut-off for high versus low hENT1 expression. One study (Deng et al., 2014) used an H-score ≥ 6 as a cut-off value, calculated as the product of staining intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and percentage of positive cells (1, < 10% positive cells; 2, 11%–50% positive cells; 3, > 50% positive cells). Brandi et al. (2016) defined strong staining of only the cell membrane as the threshold for high hENT1. One study Woo et al. (2017) did not report the cut-off value.

3.3.2. Clinical outcome by hENT1 status

Of the 8 studies that evaluated the correlation between hENT1 expression and survival in BTC patients treated with gemcitabine or gemcitabine-based chemotherapy, 6 studies reported OS (Santini et al., 2011; Deng et al., 2014; Murata et al., 2013; Borbath et al., 2012; Kobayashi et al., 2012; Sasaki et al., 2014) and DFS or PFS (Santini et al., 2011; Woo et al., 2017; Deng et al., 2014; Borbath et al., 2012; Brandi et al., 2016; Sasaki et al., 2014) (Table 1 and 2). All 6 studies found a prolonged OS in patients with high levels of hENT1 compared to patients with low hENT1 expression, and this difference in OS was statistically significant in 5 of 6 studies. After adjustment for different factors in the multivariate analysis, 4 of 5 studies found a statistical significant association between high hENT1 expression and OS. All 6 studies found a significantly longer DFS/PFS in patients with high hENT1 treated with gemcitabine-based chemotherapy. Furthermore, 4 of 5 studies found high hENT1 expression to be associated with improved DFS/PFS in the multivariate analysis. Table 2 summarizes the correlation between hENT1 expression and survival in patients with BTC treated with chemotherapy.

3.3.3. Pooled analysis

A meta-analysis of univariate hazard ratios (HR) was conducted to estimate the pooled HR of OS and DFS/PFS in patients with high versus low hENT1 expression treated with gemcitabine-based chemotherapy. There was no publication bias for studies included in the meta-analysis as assessed by funnel plots (Figures are not shown). Four of the 8 studies were incorporated in the meta-analysis of OS for hENT1 expression. Four studies were excluded from the meta-analysis, because 2 studies did not report OS and 1 study did not report enough data to calculate HR for OS. The study of Kobayashi et al. (2012) and Sasaki et al. (2014) partially used the same patient cohort, therefore the smallest study (Kobayashi et al., 2012) was excluded from the meta-analysis. In the meta-analysis of DFS/PFS for high versus low hENT1 expression, 6 studies were incorporated. Two of 8 studies were excluded. One study did not report DFS/PFS and the other study consisted of an overlapping patients' cohort as mentioned above Kobayashi et al. (2012).

The pooled HR of OS and DFS/PFS for high versus low hENT1 expression was 0.43 (95% CI: 0.28 to 0.64, P-value < 0.0001) and 0.45 (95% CI: 0.33 to 0.61, P-value < 0.00001), respectively. Fig. 2 demonstrates the forest plots of HRs for OS and DFS/PFS by hENT1 status.

A subgroup analysis of hENT1 expression was performed to evaluate the prognostic value of hENT1 in different subgroups of BTC patients. High hENT1 expression was associated with improved OS and DFS/PFS in resected as well as in advanced BTC patients, in patients treated with gemcitabine monotherapy and gemcitabine-based chemotherapy, in studies from Europe as well in studies from East-Asia (Table 3). Importantly, hENT1 expression was associated with OS and DFS/PFS regardless the used hENT1-antibodies (Table 3).

3.4. RRM1

RRM1 is a crucial enzyme for synthesis of deoxyribonucleotides, which are involved in DNA synthesis (Jordheim et al., 2011). Gemcitabine, a nucleoside analogue, interacts with RRM1 resulting in inhibition of DNA synthesis in dividing cells (Jordheim et al., 2011). Five studies, all from East-Asia, have analysed the association between RRM1 expression and clinical outcome in 216 patients with BTC treated with gemcitabine-based chemotherapy. Three studies included patients with advanced BTC and 2 studies involved patients who had undergone resection with curative intent. Three of 5 studies were of high quality as assessed by mNOS.

3.4.1. Immunohistochemical analysis of RRM1

Low RRM1 expression was present in 96 of 216 (44.4%) patients and varied between 36.4% and 60.0%. RRM1 expression was performed by IHC in 4 studies and in 1 study (Nakamura et al., 2010) quantitative double-fluorescence IHC was used. Two studies used plasma and stromal cells as the internal reference. In the remaining 3 studies, 1 study (Nakamura et al., 2010) used ß-actin as the internal reference and 2 studies did not report which internal reference cells were used. Different cut-off values were used for high RRM1, including moderate or weak staining in more than 50% of cells Murata et al. (2013), Sasaki et al. (2014), H-score of 6 points or more (Deng et al., 2014), the mean value of staining intensity (0.984) of all cases as assessed by quantitative double-fluorescence IHC (Nakamura et al., 2010), and 1 study did not report the used cut-off value (Woo et al., 2017). Three antibodies were used to evaluated RRM1 expression: 2 studies used polyclonal rabbit antibodies, 1 study used a monoclonal rabbit antibody and 1 study used monoclonal mouse antibody for RRM1. One study did not report the used antibody for RRM1 expression (Nakamura et al., 2010).

3.4.2. Clinical outcome by RRM1 status

In the 5 studies evaluating the correlation between RRM1 expression and survival, 5 and 4 studies reported the OS and DFS/PFS, respectively. Two studies found significantly prolonged OS in patients with low RRM1 expression (Nakamura et al., 2010; Sasaki et al., 2014), while in the other 2 studies the difference in OS between low and high RRM1 was not statistically significant (Deng et al., 2014; Murata et al., 2013) (Table 2). Prolonged PFS of patients with low RRM1 expression was observed in 1 study (Sasaki et al., 2014), but this difference was not statistical significant in the remaining 3 studies (Table 2).

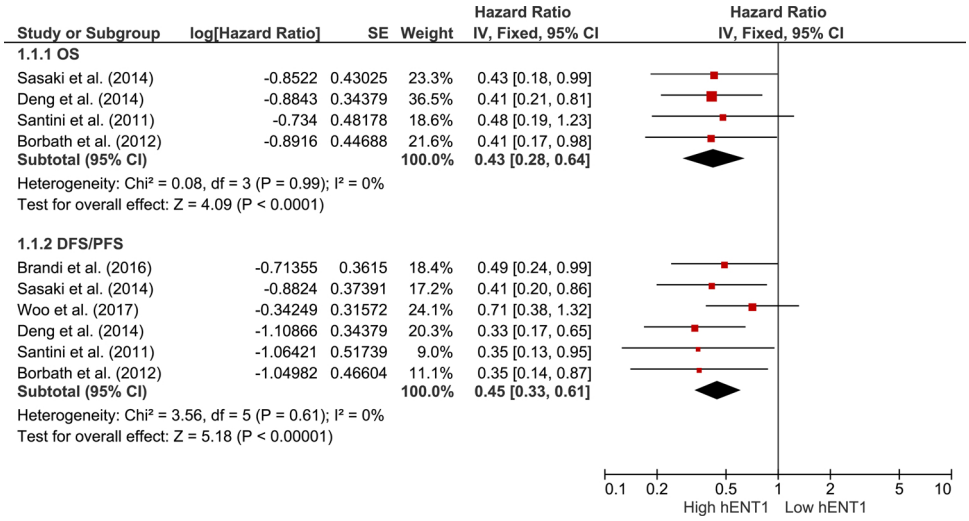

Fig. 2. Forest plot of hazard ratios for high versus low hENT1 expression in BTC treated with Gemcitabine-based chemotherapy.

3.5. Combined analysis of hENT1 and RRM1 expression

Sasaki et al. (2014) analysed the expression of both hENT1 and RRM1 in correlation with OS and DFS in BTC patients treated with gemcitabine-based chemotherapy after curative resection. The 5-year OS was 75% versus 25% in patients with high hENT1 combined with low RRM1, compared to the other 3 expression combinations of hENT1 and RRM1 (high hENT1 and high RRM1, Low hENT1 and high or low RRM1) Sasaki et al. (2014). In patients with high hENT1 and low RRM1 the 5-year DFS was 58% compared to 18% in patients with other 3 expression combinations of hENT1 and RRM1 Sasaki et al. (2014).

3.6. ERCC1

ERCC1 is an essential enzyme that recognizes and repairs DNA damage, especially crosslink damage (Besse et al., 2013). Cisplatin forms intrastrand adducts resulting in crosslinking of DNA strands which leads to apoptosis (Besse et al., 2013). The association between ERCC1 expression and clinical outcome was evaluated in 4 studies with a total of 228 patients (Table 2). ERCC1 expression was studied in 2 studies of patients treated with gemcitabine-based chemotherapy (da Costa Miranda et al., 2014; Mian et al., 2016), 1 study of patients who received platinum-based therapies (Hwang et al., 2011) and 1 study of patients treated with 5-FU-based chemotherapy (Won et al., 2010).

3.6.1. Immunohistochemical analysis of ERCC1

Low ERCC1 was observed in 48.0% to 50.5% of patients from 3 studies. One study did not report the number or percentage of patients with low or high ERCC1 expression (Won et al., 2010). Of the 4 studies that examined ERCC1 expression by IHC, 2 studies (Hwang et al., 2011; Mian et al., 2016) used monoclonal mouse (clone 8F1) antibodies and the remaining 2 studies (da Costa Miranda et al., 2014; Won et al., 2010) did not report the origin of the used antibodies.

The cut-offs for high versus low ERCC1 were, an H-score of 0.5 (Mian et al., 2016) or more than 0.5 (da Costa Miranda et al., 2014), an H-score of 1.5 or more (Hwang et al., 2011) and more than 49% of positive cells in the remaining study (Woo et al., 2017) (Table 1).

3.6.2. Clinical outcome by ERCC1 status

Mian et al. (2016) found a longer OS and DFS in resected patients receiving adjuvant gemcitabine after resection of gallbladder cancer or extrahepatic cholangiocarcinoma with high ERCC1 expression compared to low expression(Table 2). However, da Costa Miranda et al. (2014) did not observe a significant association between ERCC1 expression and OS in 44 advanced BTC patients treated with gemcitabine-based chemotherapy. Hwang et al. (2011) did not find a significant correlation between ERCC1 expression and OS or PFS in 93 patients with advanced BTC receiving platinum-based chemotherapy. A subgroup-analysis of 65 patients treated with cisplatin-based chemotherapy showed longer OS (9.1 vs. 7.3 months, *P*-value = 0.017) and PFS (4.6 vs. 1.9 months, *P*-value = 0.014) in patients with low ERCC1 expression Hwang et al. (2011). In patients treated with 5-Fluorouracil based chemotherapy, ERCC1 expression was not associated with OS or DFS (Won et al., 2010).

4. Discussion

Chemotherapy is the mainstay for systemic treatment of patients with BTC. The response to this treatment varies between patients and a significant number of patients do not benefit from chemotherapy. There are no clinical markers to predict who will benefit. Thus, it remains unclear which patients should be selected for this treatment. This systematic review evaluated the prognostic value of IHC biomarkers in patients with BTC treated with chemotherapy. High hENT1 expression in tumour tissue was associated with favourable clinical outcome in BTC patients treated with adjuvant or palliative gemcitabine-based chemotherapy. Because of limited number of studies which show inconsistent results, the prognostic value of RRM1 and ERCC1 remains unclear in BTC patients treated with chemotherapy.

The results of hENT1 in BTC patients are in line with findings in pancreatic cancer studies (Nordh and Andersson, 2014; Bird et al., 2017). Nordh et al. conducted a systematic review and evaluated hENT1 expression in resected as well as in advanced pancreatic cancer patients treated with gemcitabine (Nordh and Andersson, 2014). Nine of 10 studies found a statistical significant improved OS and 6 of 7 studies showed a prolonged DFS in patients with high hENT1 expression (Nordh and Andersson, 2014). Bird et al. performed a meta-analysis of resected pancreatic cancer patients treated with adjuvant gemcitabine-based chemotherapy and demonstrated a prolonged OS (HR 0.52) and DFS (HR 0.58) in patients with high hENT1 expression (Bird et al., 2017). These HRs for OS and DFS according to hENT1 status in pancreatic cancer are comparable with the pooled HRs in our meta-analysis for BTC patients (Fig. 2).

Previous studies in lung and pancreatic cancer patients have established the prognostic value of RRM1 for platinum and gemcitabine efficacy (Besse et al., 2013; Olaussen and Postel-Vinay, 2016; Gong et al., 2012; Han et al., 2018). Gong et al. performed a meta-analysis of 18 studies of advanced lung cancer patients treated with gemcitabine-

Table 3
Summary of hazard ratios for subgroup analyses of hENT1 expression in BTC.

Variables	Outcome	Studies	Patients	Model	HR (95% CI)	*P* value	References
All	OS	4	169	Fixed	0.43 (0.28 - 0.64)	< 0.001	(Santini et al., 2011; Deng et al., 2014; Borbath et al., 2012; Sasaki et al., 2014)
	DFS/PFS	6	306	Fixed	0.45 (0.33 - 0.61)	< 0.001	(Santini et al., 2011; Woo et al., 2017; Deng et al., 2014; Borbath et al., 2012; Brandi et al., 2016; Sasaki et al., 2014)
Disease stage*							
Resectable	OS	1	68	Fixed	0.43 (0.18 - 0.99)	0.036	(Sasaki et al., 2014)
	DFS	3	205	Fixed	0.54 (0.37 - 0.80)	0.002	(Woo et al., 2017; Brandi et al., 2016; Sasaki et al., 2014)
Advanced	OS	3	101	Fixed	0.43 (0.27 - 0.68)	< 0.001	(Santini et al., 2011; Deng et al., 2014; Borbath et al., 2012)
	PFS	3	101	Fixed	0.34 (0.21 - 0.55)	< 0.001	(Santini et al., 2011; Deng et al., 2014; Borbath et al., 2012)
Chemotherapy scheme*							
GEM	OS	1	26	Fixed	0.41 (0.17 - 0.98)	0.046	(Borbath et al., 2012)
	DFS/PFS	3	163	Fixed	0.54 (0.36 - 0.82)	0.004	(Woo et al., 2017; Borbath et al., 2012; Brandi et al., 2016)
GEM or GEM-based	OS	3	143	Fixed	0.43 (0.27 - 0.68)	< 0.001	(Santini et al., 2011; Deng et al., 2014; Sasaki et al., 2014)
	DFS/PFS	3	143	Fixed	0.36 (0.23 - 0.57)	< 0.001	(Santini et al., 2011; Deng et al., 2014; Sasaki et al., 2014)
Geographic region*							
East Asia	OS	2	112	Fixed	0.42 (0.25 - 0.71)	0.001	(Deng et al., 2014; Sasaki et al., 2014)
	DFS/PFS	3	178	Fixed	0.47 (0.32 - 0.70)	< 0.001	(Woo et al., 2017; Deng et al., 2014; Sasaki et al., 2014)
Europe	OS	2	57	Fixed	0.44 (0.23 - 0.84)	0.010	(Santini et al., 2011; Borbath et al., 2012)
	DFS/PFS	3	128	Fixed	0.41 (0.25 - 0.67)	< 0.001	(Santini et al., 2011; Borbath et al., 2012; Brandi et al., 2016)
Origin of antibodies*							
Polyclonal rabbit	OS	2	112	Fixed	0.42 (0.25 - 0.71)	0.001	(Deng et al., 2014; Sasaki et al., 2014)
	DFS/PFS	4	249	Fixed	0.48 (0.34 - 0.67)	< 0.001	(Woo et al., 2017; Deng et al., 2014; Brandi et al., 2016; Sasaki et al., 2014)
Monoclonal mouse	OS	2	57	Fixed	0.44 (0.23 - 0.84)	0.010	(Santini et al., 2011; Borbath et al., 2012)
	DFS/PFS	2	57	Fixed	0.35 (0.18 - 0.69)	0.002	(Santini et al., 2011; Borbath et al., 2012)

95% CI, 95% confidence interval; DFS, disease-free survival; GEM, Gemcitabine monotherapy; GEM-based, gemcitabine-based chemotherapy; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
The heterogeneity (I^2) was not statistical significant (*P*-values not shown in table) in all studies. I^2 test was 0% for all variables except for DFS/PFS of East-Asia studies (I^2 = 26%).
* The subgroup differences for OS and DFS/PFS were not statistical significant (*P*-values > 0.05) for all variables.

based chemotherapy and found prolonged OS and PFS in patients with low RRM1 expression compared to high RRM1 expression (Gong et al., 2012). High RRM1 was associated with poor OS (HR 1.70) and DFS (HR 1.84) in a meta-analysis of 9 studies of pancreatic cancer patients receiving gemcitabine (Han et al., 2018). In BTC patients, we could not confirm these results due to the limited number of RRM1 studies and contradictory outcomes

ERCC1 was evaluated in 4 BTC studies with small sample sizes, which do not allow us to draw clear conclusions about its prognostic value in BTC patients receiving chemotherapy. ERCC1 expression was evaluated in a meta-analysis of 23 studies of non-small cell lung cancer patients (Hubner et al., 2011). High ERCC1 was associated with poor OS and low objective response rates in patients receiving platinum-based chemotherapy, but not in chemotherapy-naïve patients (Hubner et al., 2011). Four prospective clinical trials evaluated the association between ERCC1 expression and clinical outcome in lung cancer patients (Mazzoni et al., 2013; Cobo et al., 2007; Lee et al., 2017; Heo et al., 2016). Of these 4 trials, ERCC1 was associated with a better objective response rate (Mazzoni et al., 2013; Cobo et al., 2007), but the remaining 2 trials did not show an association between ERCC1 expression and OS, PFS or response rate in patients treated with platinum chemotherapy (Lee et al., 2017; Heo et al., 2016). These inconsistent results could potentially be explained by a non-optimal ERCC1 IHC assay (Postel-Vinay and Soria, 2017).

Our aim was to identify potential prognostic IHC biomarkers for chemotherapy efficacy. The next step is to identify predictive biomarkers for chemotherapy in BTC because of their clinical importance. The only 2 available studies that evaluated the predictive value of hENT1 in an overlapping cohort of resected BTC patients, showed a significant association between hENT1 expression and OS and DFS in the adjuvant gemcitabine-based chemotherapy arm but not in de observation arm (Kobayashi et al., 2012; Sasaki et al., 2014). These results suggest that hENT1 may has also a predictive value in resected BTC patients treated with gemcitabine-based chemotherapy versus observation and could stratify resected BTC patients to those who will benefit from adjuvant gemcitabine-based chemotherapy in future clinical trials (Kobayashi et al., 2012).

In this article we focused on IHC biomarkers because this method is frequently used in clinical practice to assess biomarker expression and because of limited number of studies that assessed genetic biomarkers for chemotherapy efficacy in BTC. Of note, single nucleotide polymorphisms (SNPs) of hENT1 or RRM1 in tumour tissue of patients with BTC treated with gemcitabine-based chemotherapy were not correlated with clinical outcome (Woo et al., 2017; Yoon et al., 2015). There was also no correlation between genetic polymorphisms of ERCC1 and survival in patients with BTC treated with gemcitabine or cisplatin-based chemotherapy (Pongmaneratanakul et al., 2017; Pacetti et al., 2009). A possible explanation for the discrepancy in the prognostic value of hENT1 and RRM1 when assessed by IHC compared to real-time polymerase chain reaction (RT-PCR) could be that IHC measures the intensity, percentage and the localisation of these proteins that are responsible for uptake and metabolism of chemotherapy, while RT-PCR measures the overall expression of given SNPs of the hENT1 gen in the tumour and the surrounding normal tissue which may not always correlate with the actual protein expression (Besse et al., 2013). Prospective trials are needed to determine the prognostic and/or predictive value of genetic biomarkers in patients with BTC receiving chemotherapy.

Our systematic review has several limitations. Firstly, all included studies had a retrospective design or post-hoc analysis. Secondly, most studies included small number of patients and different BTC subtypes. Thirdly, studies of resected as well as advanced BTC patients treated with chemotherapy are included in this systematic review. Fourthly, different methodological techniques were used for the evaluation of biomarkers. In case of hENT1, 6 studies used the rabbit polyclonal antibody (SP120) and 2 studies used the mouse monoclonal antibody (10D7G2). There is currently no consensus which of these 2 antibodies or scoring system should be used to evaluate hENT1 expression

(Mazzoni et al., 2013). Two retrospective studies in pancreatic cancer evaluated hENT1 using both antibodies. One study found a significant correlation between high hENT1 and clinical outcome with murine-derived anti-hENT1 antibodies, but not with rabbit-derived antibodies (Mazzoni et al., 2013). The second study found that both antibodies can predict the response to gemcitabine (Kalloger et al., 2017). Finally, different cut-offs were used for high versus low hENT1 expression. This systematic review reveals that there is an urgent need to develop and validate a standard IHC protocol for hENT1 expression in BTC.

Despite these limitations, this is the first systematic review with meta-analysis on prognostic IHC biomarkers for chemotherapy outcome in BTC. Our meta-analysis did not reveal any heterogeneity between the results of the included hENT1 studies.

In conclusion, our study suggest that high hENT1 is associated with prolonged OS and DFS/PFS in patients with BTC treated with adjuvant and palliative gemcitabine-based chemotherapy. Investigation of hENT1 expression in large patient cohorts and prospective validation of this biomarker using a standardized IHC protocol is needed to confirm the clinical utility of this biomarker in clinical practice. Furthermore, future studies are required to investigate the potential correlation between RRM1 expression with clinical outcomes in BTC patients treated with gemcitabine-based chemotherapy.

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Conflict of interest statement

H. Klümpen has been a member of advisory board for IPSEN and his institution has received a research grant from BAYER outside of the submitted work. Other authors have nothing to be declared.

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