

Supplementary Table 3. Detailed clinical, laboratory, pathologic, molecular and follow-up characteristics of 26 patients with repeat ERCP and BiliSeq testing (successive testing is listed in chronologic order).

Set	Patient	Gender	Age (years)	History of PSC	Serum CA19-9 (Units/mL)	Location of Stricture	Malignant Bile Duct Pathology Brushing/Biopsy*	Specimen for BiliSeq Testing	Molecular Alterations	Follow-up Diagnosis	Method of Follow-up Diagnosis
Training	6	F	96	No	N/S	Distal	N/S / Negative (CG)	Biopsy (CG)	Negative	Distal Cholangiocarcinoma	Autopsy
	6	F	96	No	N/S	Distal	N/S / Positive (CG)	Biopsy (CG)	KRAS p.Q61L	Distal Cholangiocarcinoma	Autopsy
Training	14	M	65	No	120.7	Distal	N/S / Negative (CG)	Brushings	KRAS p.G12A	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	14	M	66	No	194.2	Distal	N/S / Negative (CG)	Biopsy (CG)	KRAS p.G12A	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	14	M	67	No	208.2	Distal	N/S / Negative (CG)	Biopsy (CG)	KRAS p.G12A	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	14	M	67	No	202.3	Distal	N/S / Negative (CG)	Biopsy (CG)	KRAS p.G12A	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	34	M	72	Yes	39	Intrahepatic	Negative / N/S	Brushings	GNAS p.R201C	Intrahepatic Cholangiocarcinoma	Clinicoradiographic Impression
Training	34	M	73	Yes	41	Intrahepatic	Negative / Negative (CG)	Brushings	GNAS p.R201C	Intrahepatic Cholangiocarcinoma	Clinicoradiographic Impression
	36	M	26	Yes	316.4	Distal	Negative / N/S	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
	36	M	29	Yes	N/S	Distal	N/S / Negative (CG)	Biopsy (CG)	Negative	Cholangiopathy	Clinicoradiographic Impression
	53	F	26	Yes	30	Distal	N/S / Negative (CG)	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
Training	53	F	27	Yes	31	Distal	Negative / N/S	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
	68	M	52	No	N/S	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
Validation	68	M	52	No	87.8	Distal	Negative / Negative (CG)	Both (CG)	Biopsy: ERBB2 p.V842I; Brushings: Negative	N/A	
	69	M	89	No	297	Distal	N/S / Negative (CG)	Biopsy (CG)	Negative	Distal Cholangiocarcinoma	Subsequent ERCP Biopsy
Validation	69	M	89	No	321	Distal	Positive / Positive (CG)	Both (CG)	Both Specimens: Negative	Distal Cholangiocarcinoma	Concurrent ERCP Biopsy
	79	F	79	No	164	Hilar	N/S / Negative (CG)	Biopsy (CG)	Negative	Perihilar Cholangiocarcinoma	Subsequent ERCP Biopsy
Validation	79	F	79	No	130	Hilar	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	Perihilar Cholangiocarcinoma	Subsequent ERCP Biopsy
	83	M	73	No	480	Hilar	Negative / Negative (CS)	Brushings	Negative	Perihilar Cholangiocarcinoma	Intraoperative Liver Biopsy
	83	M	74	No	1888	Hilar	N/S / Negative (CG)	Biopsy (CG)	Negative	Perihilar Cholangiocarcinoma	Intraoperative Liver Biopsy
	83	M	74	No	2003	Hilar	N/S / Negative (CG)	Biopsy (CG)	KRAS p.Q61H, TP53 p.Y236C	Perihilar Cholangiocarcinoma	Intraoperative Liver Biopsy
	96	M	81	No	760.8	Hilar	Negative / N/S	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
Validation	96	M	81	No	760.8	Hilar	Negative / N/S	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
	96	M	81	No	760	Hilar	Negative / N/S	Brushings	Negative	Cholangiopathy	Clinicoradiographic Impression
	121	F	73	No	41	Intrahepatic	Negative / N/S	Brushings	GNAS p.R201H	N/A	
	121	F	73	No	41	Intrahepatic	N/S / Negative (CG)	Biopsy (CG)	GNAS p.R201H	N/A	
Validation	139	M	73	No	1202	Distal	N/S / Negative (CG)	Biopsy (CG)	Negative	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	139	M	73	No	2239	Distal	Negative / Negative (CG)	Both (CG)	Biopsy: TP53 p.P278S; Brushings: Negative	Distal Cholangiocarcinoma	Clinicoradiographic Impression
Validation	142	M	81	No	25	Distal	Negative / N/S	Brushings	Negative	Pancreatic Ductal Adenocarcinoma	Pancreatic EUS-FNA
	142	M	81	No	16	Distal	Positive / Positive (CG)	Both (CG)	Both Specimens: Negative	Pancreatic Ductal Adenocarcinoma	Pancreatic EUS-FNA
Validation	151	F	72	No	4.2	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
	151	F	72	No	4.2	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
	151	F	72	No	4	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
Validation	153	F	55	No	90.4	Hilar	Negative / Negative (CS)	Both (CS)	Both Specimens: Negative	N/A	
	153	F	55	No	89.2	Hilar	Negative / Negative (CS)	Both (CS)	Both Specimens: Negative	N/A	
	153	F	55	No	93.5	Hilar	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
Validation	153	F	55	No	88.4	Hilar	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
	154	M	66	No	N/S	Distal	N/S / Negative (CG)	Biopsy (CG)	KRAS p.G12R	Ampullary adenocarcinoma	Whipple Resection
	154	M	66	No	N/S	Distal	N/S / Positive (CG)	Biopsy (CG)	KRAS p.G12R, CDKN2A copy number loss	Ampullary adenocarcinoma	Whipple Resection
Validation	155	M	66	No	53	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	Cholangiopathy	Clinicoradiographic Impression
	155	M	66	No	N/S	Distal	N/S / Negative (CG)	Biopsy (CG)	Negative	Cholangiopathy	Clinicoradiographic Impression
Validation	157	F	73	No	55	Intrahepatic	Negative / N/S	Brushings	ERBB2 amplification, TP53 p.C242fs*5	Intrahepatic Cholangiocarcinoma	Subsequent ERCP Biopsy
	157	F	73	No	422.4	Intrahepatic	Positive / Positive (CG)	Brushings	ERBB2 amplification, TP53 p.C242fs*5	Intrahepatic Cholangiocarcinoma	Concurrent ERCP Biopsy
Validation	174	M	83	No	6	Distal	Negative / Negative (CS)	Both (CS)	Both Specimens: Negative	N/A	
	174	M	84	No	9.1	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
Validation	175	M	57	No	43	Distal	Negative / Negative (CS)	Both (CS)	Both Specimens: Negative	Cholangiopathy	Clinicoradiographic Impression
	175	M	57	No	39.3	Distal	N/S / Negative (CS)	Biopsy (CS)	Negative	Cholangiopathy	Clinicoradiographic Impression
Validation	176	F	77	No	195.8	Hilar	Negative / Negative (CG)	Biopsy (CG)	FGFR2 p.S252W	Intrahepatic Cholangiocarcinoma	Clinicoradiographic Impression
	176	F	78	No	410.3	Hilar	Negative / Negative (CG)	Both (CG)	Both Specimens: FGFR2 p.S252W	Intrahepatic Cholangiocarcinoma	Clinicoradiographic Impression
Validation	177	M	68	Yes	6	Intrahepatic	Negative / Negative (CS)	Brushings	TP53 p.F338Lfs*6, CDKN2A p.R80*	High-grade dysplasia of the bile duct	Partial Hepatectomy
	177	M	68	Yes	5	Intrahepatic	Negative / Negative (CG)	Brushings	TP53 p.F338Lfs*6, CDKN2A p.R80*	High-grade dysplasia of the bile duct	Partial Hepatectomy
Validation	197	M	85	No	28.3	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: Negative	N/A	
	197	M	85	No	34.5	Distal	Negative / Negative (CG)	Brushings	Negative	N/A	
Validation	218	M	40	Yes	11.3	Intrahepatic	Negative / Negative (CS)	Both (CS)	Both Specimens: Negative	Intrahepatic Cholangiocarcinoma	Subsequent ERCP Biopsy
	218	M	40	Yes	11.3	Intrahepatic	Negative / Positive (CS)	Both (CS)	Biopsy: MET copy number gain; Brushings: Negative	Intrahepatic Cholangiocarcinoma	Concurrent ERCP Biopsy
Validation	237	F	70	Yes	137.2	Distal	Negative / Negative (CG)	Both (CG)	Biopsy: KRAS p.G12V, PIK3CA p.C901F; Brushings: Negative	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	237	F	70	Yes	137.2	Distal	Negative / N/S	Brushings	KRAS p.G12V, PIK3CA p.C901F	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	237	F	70	Yes	120	Distal	Negative / Negative (CG)	Both (CG)	Both Specimens: PIK3CA p.C901F	Distal Cholangiocarcinoma	Clinicoradiographic Impression
	237	F	70	Yes	88.8	Distal	Positive / Negative (CG)	Both (CG)	Brushings: PIK3CA p.C901F; Biopsy: Negative	Distal Cholangiocarcinoma	Clinicoradiographic Impression
Validation	243	F	91	No	64	Hilar	Negative / Negative (CS)	Brushings	PIK3CA p.H1047R	Intrahepatic Cholangiocarcinoma	Subsequent ERCP Biopsy
	243	F	91	No	64	Hilar	Positive / Positive (CS)	Both (CS)	Both Specimens: PIK3CA p.H1047R	Intrahepatic Cholangiocarcinoma	Concurrent ERCP Biopsy

Abbreviations: CG, cholangiographic-guided biopsy; CS, cholangioscopic-guided biopsy; N/A, not available; N/S, not submitted; PSC, primary sclerosing cholangitis

*Malignant biliary duct pathology of brushings/biopsies was defined as at least high-grade dysplasia or at least suspicious for malignancy.