Genotyping of circulating tumor DNA in cholangiocarcinoma reveals diagnostic and prognostic information

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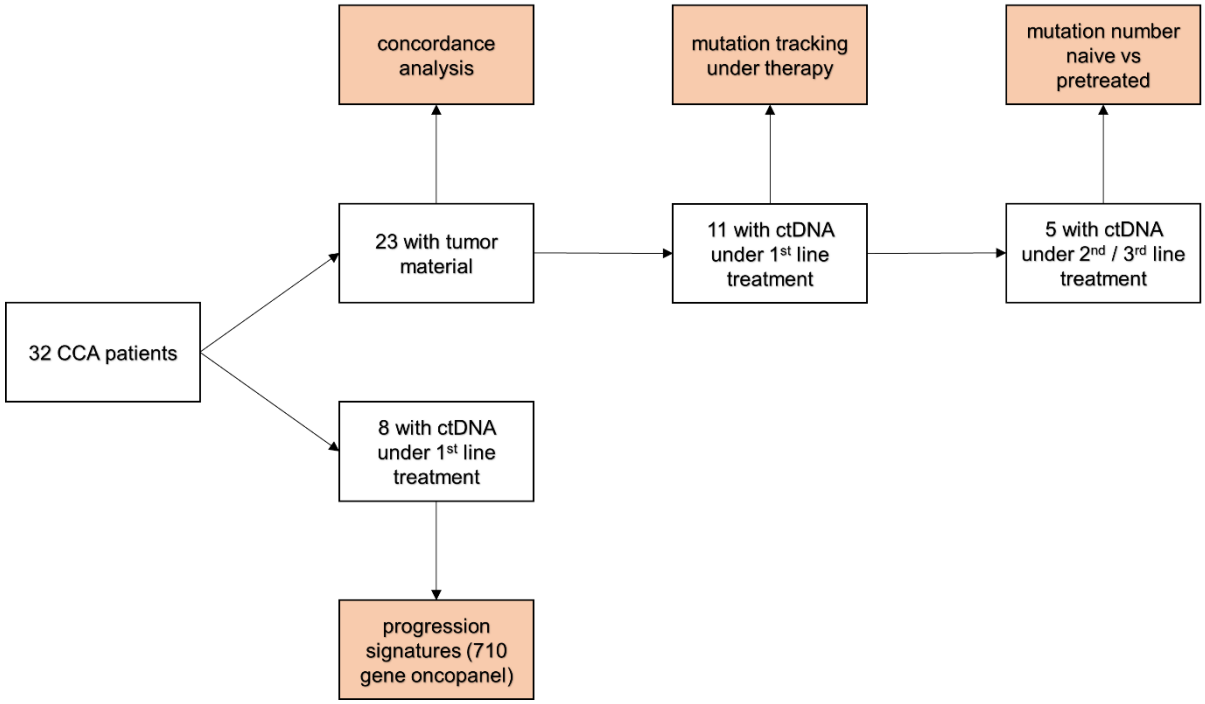
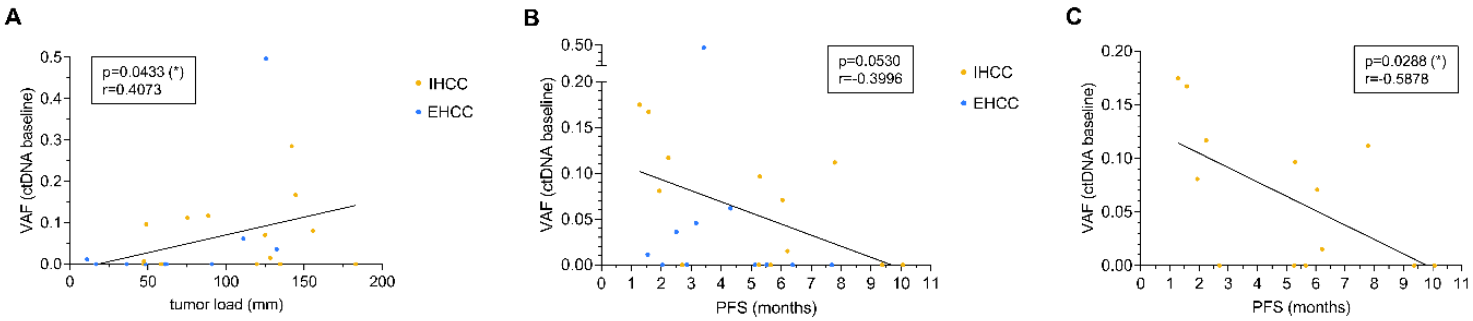
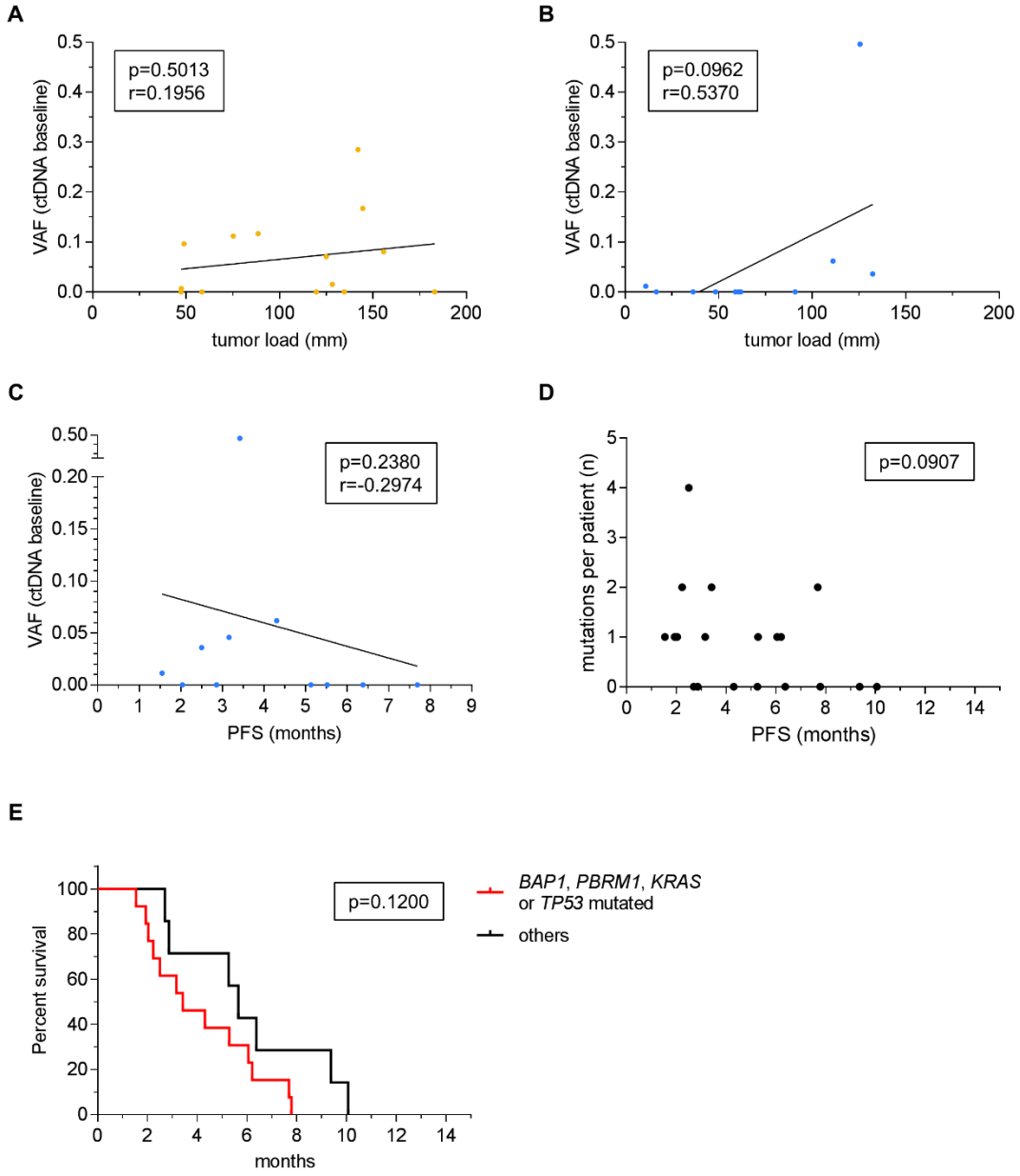
Supplementary Materials



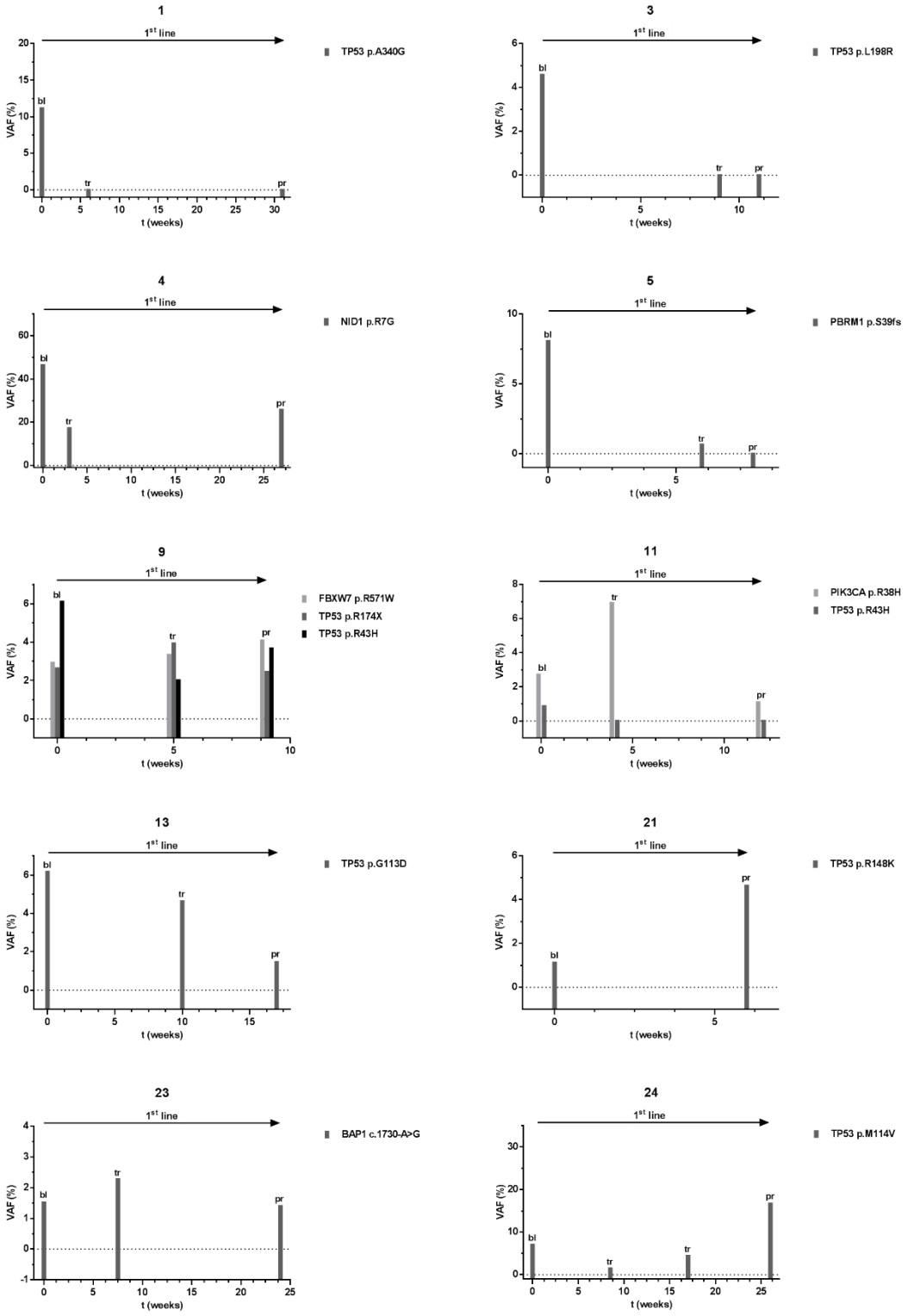
Supplementary Figure 1. Patient groups and applied methods.



Supplementary Figure 2. Correlation of ctDNA VAF baseline values and clinical parameters. **A.** VAF at baseline correlates with tumor load (RECIST 1.1) at baseline (P=0.0433, r=0.4037, Spearman). **B.** Lower VAF at baseline shows a trend towards prolonged progression-free survival (PFS) (P=0.0530, r=-0.3996, Spearman). **C.** VAF in the group of IHCC patients correlates with PFS (P=0.0288, r=-0.5878, Spearman). IHCC - intrahepatic cholangiocarcinoma, EHCC - extrahepatic cholangiocarcinoma.



Supplementary Figure 3. Correlation of ctDNA VAF baseline values with **A.** tumor load (RECIST 1.1) in the group of IHCC patients (*P*=0.5013, r=0.1956, Spearman), **B.** tumor load (RECIST 1.1) in the group of EHCC patients (*P*=0.0962, r=0.5370 Spearman), **C.** progression-free survival (PFS) in the group of EHCC patients (*P*=0.2380, r=-0.2974, Spearman). **D.** Correlation of the number of mutations per patient with PFS (*P*=0.0907, r=-0.3988, Spearman). **E.** Kaplan-Meier plot of PFS of patients with mutations in *BAP1*, *PBRM1*, *KRAS* or *TP53* vs. patients without mutations in these genes (*P*=0.1200, Mantel-Cox). IHCC - intrahepatic cholangiocarcinoma, EHCC - extrahepatic cholangiocarcinoma.



Supplementary Figure 4. Variant allele frequencies of detected mutations of all patients receiving first line chemotherapy from which cfDNA during the course of treatment was available (one patient was wild-type for the applied gene panel). bl - baseline, tr - treatment, pr - progression.

Supplementary Table 1. Clinical patient characteristics.

ID	sex	age	tumor localization	stage	metastases	chemotherapeutic regimen	
						therapy line	chemotherapy
1*	F	59	Intrahepatic	UICC IV	ADR, HEP, LYM, PER, OTH	1st, 2nd	Gem/Cis, FOLFIRI
2	F	78	Extrahepatic	UICC III	-	-	-
3	M	70	Extrahepatic	UICC IV	ADR, PLE	1st	Gem/Cis
4*	M	74	Extrahepatic	UICC IV	HEP, LYM, PUL	1st, 2nd, 3rd	Gem/Cis, FOLFIRI, Doce
5	M	70	Intrahepatic	UICC III	-	1st	Gem
6	F	55	Intrahepatic	UICC IV	ADR, LYM	1st	Gem/Cis
7	M	75	Intrahepatic	UICC III	-	1st	Gem/Cis
8	F	80	Intrahepatic	UICC IV	HEP, OSS, PUL	1st	Gem
9	M	84	Intrahepatic	UICC IV	LYM	1st	Gem
10	F	70	Extrahepatic	UICC IV	PER	1st	Gem/Ox
11	M	62	Extrahepatic	UICC IV	LYM	1st	Gem/Cis
12	M	75	Extrahepatic	UICC IV	PER	1st	Gem/Carbo
13	M	62	Extrahepatic	UICC IV	LYM	1st	Gem/Ox
14	M	74	Extrahepatic	UICC IV	PER	1st	Gem
15	F	54	Intrahepatic	UICC IV	LYM	1st	Gem/Cis
16	F	42	Intrahepatic	UICC IV	HEP, LYM	1st	Gem/Cis
17	M	39	Extrahepatic	UICC IV	LYM, OSS	1st	Gem/Cis
18*	F	59	Intrahepatic	UICC IV	HEP	2nd	FOLFIRI
19	F	49	Intrahepatic	UICC IV	LYM	1st	Gem/Ox
20	M	86	Extrahepatic	UICC IV	LYM, PUL	1st	Gem
21	M	73	Extrahepatic	UICC IV	PER, PUL, HEP, LYM	1st	Gem/Cis
22	M	72	Intrahepatic	UICC IV	LYM	1st	Gem/Ox
23	M	65	Intrahepatic	UICC IV	HEP, OSS, PUL	1st	Gem/Cis
24*	M	59	Intrahepatic	UICC IV	PUL	1st, 2nd	Gem/Cis, FOLFIRI

* = analyzed prior advanced therapy line(s); F = female; M = male; UICC = Union Internationale Contre le Cancer; ADR = adrenal; HEP = hepatic; LYM = lymphoid; OSS = osseous; PER = peritoneal; PUL = pulmonary; OTH = other; Gem = Gemcitabine; Cis = Cisplatin; Doce = Docetaxel; Ox = Oxaliplatin; Carbo = Carboplatin; FOLFIRI = folinic acid/5-fluorouracil/irinotecan

Supplementary Table 2. Molecular characteristics of patients

Supplementary Table 3. Baseline laboratory findings of patients in the study

Baseline laboratory findings	Median Value EHCC (min-max)	Median Value IHCC (min-max)	Unit
Hemoglobin	12.8 (11.6-15.8)	12.2 (9.7-16.1)	g/dl
Platelets	282 (161-511)	240 (173-335)	Giga/l
Leucocytes	7 (6-9.7)	6.7 (4-21.6)	Giga/l
Bilirubin	17 (2-54)	7 (5-171)	µmol/l
Aspartate Aminotransferase (AST)	53 (19-266)	45 (14-329)	U/l
Alanine Aminotransferase (ALT)	56 (12-378)	29 (17-292)	U/l
Gamma-Glutamyl Transferase (GGT)	371 (18-1525)	122 (22-1103)	U/l
Alkaline Phosphatase (AP)	226 (72-682)	133 (39-268)	U/l
Lactate Dehydrogenase (LDH)	238 (170-501)	213 (149-1114)	U/l
CA19-9	114 (5.4-1415)	103 (1.2-10000)	IU/ml
Carcinoembryonic antigen (CEA)	4 (1.2-451)	2.7 (1.3-31)	µg/l
Alpha-fetoprotein (AFP)	6.5 (1-105)	5 (2-41)	µg/l

Supplementary Table 4. Patient risk factors for cholangiocarcinoma in the study cohort

Characteristics	N (%)
Positive risk factors	**16/24 (66.7)**
Choledocholithiasis / Cholecystolithiasis	3 (12.5)
Cholecystectomy	2 (8.3)
Hepatitis C	1 (4.2)
Smoker	5 (20.8)
Chronic alcohol abuse	1 (4.2)
Type 2 diabetes	4 (16.7)
Negative risk factors	**8/24 (33.3)**

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