













Antitumour activity of neratinib in patients with *HER2*-mutant advanced biliary tract cancers

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HER2 mutations are infrequent genomic events in biliary tract cancers (BTCs). Neratinib, an irreversible, pan-HER, oral tyrosine kinase inhibitor, interferes with constitutive receptor kinase activation and has activity in *HER2*-mutant tumours. SUMMIT is an open-label, single-arm, multi-cohort, phase 2, ‘basket’ trial of neratinib in patients with solid tumours harbouring oncogenic *HER2* somatic mutations (ClinicalTrials.gov: NCT01953926). The primary objective of the BTC cohort, which is now complete, is first objective response rate (ORR) to neratinib 240 mg orally daily. Secondary objectives include confirmed ORR, clinical benefit rate, progression-free survival, duration of response, overall survival, safety and tolerability. Genomic analyses were exploratory. Among 25 treatment-refractory patients (11 cholangiocarcinoma, 10 gallbladder, 4 ampullary cancers), the ORR is 16% (95% CI 4.5–36.1%). The most common *HER2* mutations are S310F (n = 11; 48%) and V777L (n = 4; 17%). Outcomes appear worse for ampullary tumours or those with co-occurring oncogenic *TP53* and *CDKN2A* alterations. Loss of amplified *HER2* S310F and acquisition of multiple previously undetected oncogenic co-mutations are identified at progression in one responder. Diarrhoea is the most common adverse event, with any-grade diarrhoea in 14 patients (56%). Although neratinib demonstrates antitumour activity in patients with refractory BTC harbouring *HER2* mutations, the primary endpoint was not met and combinations may be explored.

Biliary tract cancers (BTCs) represent an uncommon group of neoplasia that comprise tumours of the intrahepatic and extrahepatic biliary tree, gallbladder, and ampulla of Vater^{1,2}. For over a decade, gemcitabine plus cisplatin has been an established first-line systemic treatment for patients with locally advanced/metastatic disease, although recent data

indicate that the addition of anti-programmed cell death ligand-1 (PD-L1) therapy further improves outcomes^{3,4}. The combination of gemcitabine and cisplatin plus durvalumab leads to an objective response rate (ORR) of 26.7%, progression-free survival (PFS) of 7.2 months, and overall survival (OS) of 12.8 months. In the second-line setting in

genomically unselected populations, fluoropyrimidine doublets exhibit anticancer activity and modestly improve patient outcomes versus monotherapy or placebo, with response rates of 5% and 15% for FOLFOX and liposomal irinotecan plus fluorouracil and leucovorin, respectively^{5,6}. Implementation of precision medicine is an increasingly relevant strategy in the second-line setting, given the high proportion of druggable alterations identified in tumours of the biliary tree^{7–10}.

HER2 is a receptor tyrosine kinase encoded by the *HER2 (ERBB2)* gene¹¹. HER2 protein overexpression, gene amplification, and less commonly, somatic *HER2* mutations (ie, kinase domain missense and insertion mutations, extracellular domain missense mutations, and transmembrane domain mutations), drive uncontrolled cellular signalling, promoting tumour growth and survival¹¹. Pharmacological inhibition of HER2 signalling has antitumour activity in preclinical models and is a validated therapeutic strategy in HER2-positive breast, gastric, and lung cancers¹¹.

HER2 alterations have been identified in a subset of BTCs and somatic *HER2* mutations have been reported at frequencies of up to 10% in this setting^{7,9,12}. *HER2* alterations (amplifications or mutations) were associated with poor overall survival (OS) in patients with metastatic disease in a retrospective dataset⁹ and HER2 overexpression was associated with increased risk of disease recurrence in patients with resected BTC¹³. Case reports, case series, and single-arm prospective studies suggest that targeting HER2 has therapeutic potential in patients with *HER2*-altered BTC; however, published data are limited by retrospective designs, small sample sizes, and in some cases lack of comprehensive genomic annotation^{14–17}. Published studies have focused on targeting *HER2*-amplified or *HER2*-overexpressing tumours¹⁸ and few prospective studies have sought to target HER2 in BTC harbouring activating somatic *HER2* mutations^{14,15,17,19}.

Neratinib, an irreversible pan-HER oral tyrosine kinase inhibitor (TKI), interferes with constitutive receptor kinase activation, leading to cancer regression in preclinical models^{20–22}. In the clinic, neratinib extends OS of patients with early- and late-stage HER2-positive breast cancer and is approved by the United States Food and Drug Administration for use as monotherapy in adjuvant breast cancer following 1 year of trastuzumab, and in combination with capecitabine in third-line metastatic breast cancer^{23,24}. SUMMIT was an open-label, international phase 2 ‘basket’ trial investigating the activity and safety of neratinib across a broad spectrum of cancers in patients whose tumours harbour activating somatic *HER2* mutations²⁵. In the initial study report, the antitumour activity of neratinib appeared to be dependent on both histology and mutation. One of the first seven patients enrolled in the *HER2*-mutant BTC cohort in SUMMIT achieved a partial response (PR), meeting Simon two-stage criteria for cohort expansion²⁵. Here, we report the final results of the expanded *HER2*-mutant BTC cohort in SUMMIT.

Results

Between April 3, 2014, and August 1, 2019, 25 patients with metastatic BTC harbouring *HER2* mutations were enrolled at hospitals in the USA, Australia, Denmark, France, Israel, and Spain, and treated on study (Fig. 1).

Patient characteristics are summarised in Table 1.

At data cut-off (January 22, 2021), all 25 patients (100%) had discontinued treatment; at the end of study, 19 (76%) had died of disease, four (16%) had withdrawn consent for additional follow-up, and 2 (8%) were ongoing survival follow-up. All patients received at least one dose of study drug. Median time on treatment was 6.7 (interquartile range [IQR] 4.0–16.4) months for the overall cohort; median follow-up duration was 9.0 (IQR 3.7–18.4) months.

Efficacy

Six of the 25 patients with BTC discontinued the study because of clinical deterioration or clinical progression and were not evaluable for

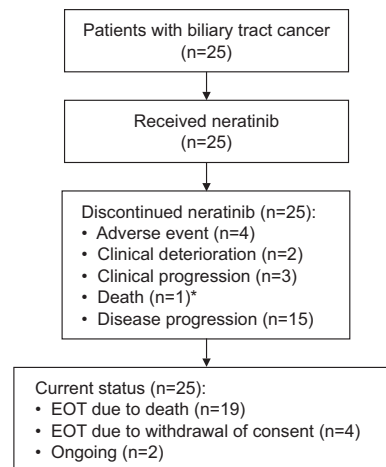


Fig. 1 | Study flow. *Death was due to progressive disease. EOT end of study.

Table 1 | Baseline demographics and clinical characteristics

	HER2-mutant biliary cohort (n = 25)
Age, years	65 (49–78)
Female sex	13 (52%)
ECOG performance status	
0	6 (24%)
1	17 (68%)
2	2 (8%)
Tumour site	
Cholangiocarcinoma	11 (44%)
Intrahepatic	6 (24%)
Extrahepatic	5 (20%)
Gallbladder	10 (40%)
Ampulla of Vater ^a	4 (16%)
Histology⁴⁶	
Adenocarcinoma	22 (88%)
Well differentiated	2 (8%)
Moderately differentiated	9 (36%)
Poorly differentiated	7 (28%)
Unknown	4 (16%)
Other	3 (12%)
M category at enrolment	
M0	1 (4%)
M1	24 (96%)
Patients with prior surgery	16 (64%)
Patients with prior radiation	5 (20%)
Prior systemic regimens	2 (0–7)
Prior systemic therapy	
Gemcitabine-based	24 (96%)
Platinum-based	23 (92%)
Fluoropyrimidine-based	18 (72%)
None	1 (4%)

Values are median (range) or n (%) unless otherwise indicated. ECOG Eastern Cooperative Oncology Group. Data cut-off: Jan 22, 2021.

^aOne of four ampullary cancers had intestinal morphology.

response. Among the remaining 19, four had a confirmed PR (Fig. 2a; Table 2), for a confirmed objective response rate (ORR) of 16% (95% confidence interval [CI] 4.5–36.1%). These confirmed PRs were observed in three patients with gallbladder carcinoma (3/10; 30%) and one with cholangiocarcinoma (1/11; 9%). Another patient with cholangiocarcinoma had an unconfirmed PR. None of four patients with

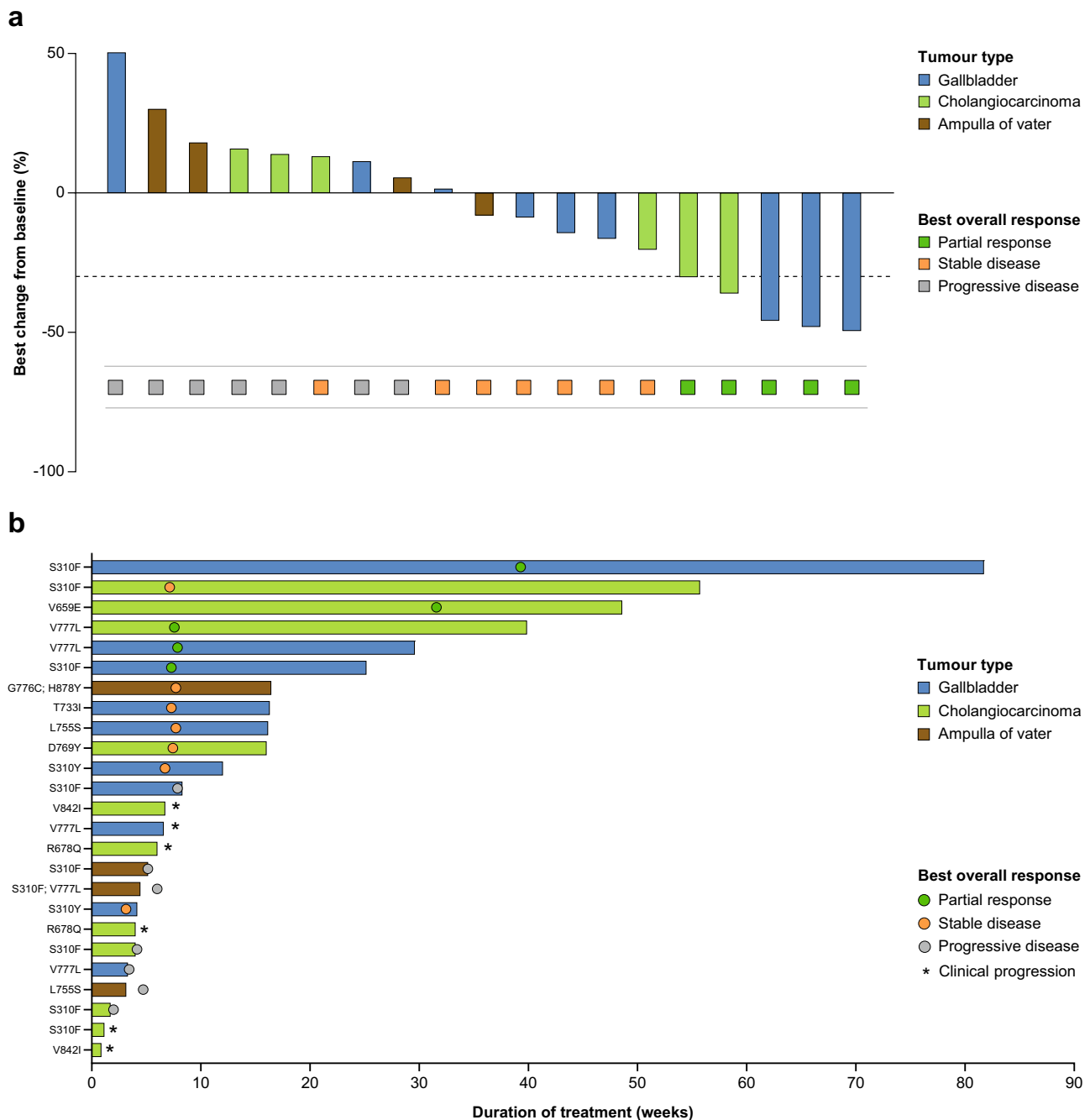


Fig. 2 | Activity of treatment (n = 25). **a** Waterfall plot for 19 patients with Response Evaluation Criteria in Solid Tumours (version 1.1)-evaluable disease; six patients who were not evaluable are not depicted. Dotted line indicates -30%

tumor shrinkage; **b** Time on treatment and response assessment for all 25 study patients. Data cut-off: January 22, 2021. CI confidence interval. Source data are provided as a Source Data file.

cancer of the ampulla of Vater had a response. The duration of response (DoR) for the four patients with PR were 3.0, 3.7 (censored), 3.8, and 4.7 months (Fig. 2b). The best overall response (BOR) rate was 20% (95% CI 6.8–40.7%); the clinical benefit rate (CBR) was 28.0% (95% CI 12.1–49.4%), and the disease control rate (DCR; not a prespecified analysis) was 24.0% (95% CI 9.3–45.1%).

Median progression-free survival (PFS) was 2.8 (95% CI 1.1–3.7) months (Fig. 3a; Table 2); median PFS for the gallbladder, cholangiocarcinoma, and ampulla subsets were 3.7 (95% CI 0.8–6.4), 1.4 (95% CI 0.5–9.1), and 1.1 (95% CI 1.1–3.8) months, respectively. Median OS was 5.4 (95% CI 3.7–11.7) months (Fig. 3b; Table 2); median OS for the gallbladder, cholangiocarcinoma, and ampulla subsets were 9.8

(95% CI 2.4–not estimable), 5.4 (95% CI 0.8–16.2), and 5.0 (95% CI 3.7–10.2) months, respectively.

Safety

All 25 patients in the BTC cohort had at least one adverse event (AE; Table 3); 16 (64%) had one or more serious AEs, two (8%) had serious treatment-related AEs (diarrhoea, dehydration, acute kidney injury; Supplementary Table 2), and five (20%) had treatment-emergent AEs and/or clinical progression leading to treatment discontinuation. Diarrhoea was the most common AE in the BTC cohort; 14 patients (56%) reported diarrhoea of any grade and six (24%) had a grade 3 diarrhoea event (Supplementary Table 3). There was no grade 4

diarrhoea. Two patients had a grade 5 AE: one died because of general deterioration and one because of sepsis.

Exploratory genomic analysis

Twenty-three patients (92%) had either sufficient pre-treatment plasma-derived cell-free DNA (cfDNA), tumour tissue, or both, for retrospective central next-generation sequencing (NGS; Supplementary Table 1). Fifteen patients (60%) had adequate archival or pre-treatment tissue for NGS analysis, an additional eight (32%) had pre-treatment cfDNA. In one of 23 patients, a *HER2* V842I reported by enrolment assay was not identified on central NGS, and a custom single-gene assay was inconclusive. In three of 23 patients, central testing not only confirmed the *HER2* mutation reported on enrolment assay but also identified additional *HER2* mutations (D277Y, V842I,

L253V). In the remaining patients, *HER2* mutations detected by enrolment assay and central NGS were concordant.

Based on retrospective central NGS (23 of 25 patients), *HER2* mutations were distributed as shown in Fig. 4a. The most common mutation was S310F, an extracellular domain hotspot mutation ($n = 11$; 48%), followed by kinase domain hotspot mutation V777L ($n = 4$, 17%). Objective responses occurred in patients with S310F ($n = 2$), V659E ($n = 1$; unconfirmed PR), and V777L ($n = 2$) mutations. The most common co-occurring alterations included *TP53* ($n = 13$; 57%), *CDKN2A* ($n = 5$; 22%), *ERBB3* ($n = 4$; 17%, 2 oncogenic and 2 variants of unknown significance), *SMAD4* ($n = 4$; 17%), and *SKT11* ($n = 4$; 17%) (Fig. 4b). Two patients (9%) had tumours harbouring co-occurring *HER2* copy-

Table 2 | Activity summary

Activity endpoint ^a	<i>HER2</i> -mutant biliary cohort ($n = 25$)
Objective response at first assessment (week 8)	2/18 (11.1%)
Objective response (confirmed)^b	
CR	0
PR	4 (16%)
ORR	16.0% (4.5–36.1%)
BOR	5 (20%)
DOR for each responder, months	3.0, 3.6 ^c , 3.7, 4.7
Median time on treatment (IQR), months	6.7 (4.0–16.4)
CBR ^d	28.0% (12.1–49.4%)
CR	0
PR	4 (16%)
SD ≥ 16 weeks	3 (12%)
Median PFS (95% CI), months ^e	2.8 (1.1–3.7)
Median OS (95% CI), months	5.4 (3.7–11.7)

Data are n (%) or % (95% CI) unless otherwise indicated. CBR clinical benefit rate, CI confidence interval, CR complete response, DoR duration of response, IQR interquartile range, ORR objective response rate, OS overall survival, PFS progression-free survival, PR partial response, SD stable disease. ^aResponse is based on investigator tumour assessments per Response Evaluation Criteria in Solid Tumours (version 1.1). ^bObjective response rate is defined as either a complete or partial response that is confirmed no less than 4 weeks after the criteria for response are initially met. ^cCensored. ^dClinical benefit rate is defined as confirmed complete or partial response or stable disease for at least 16 weeks (within ± 7 -day visit window). ^eKaplan–Meier analysis.

Table 3 | Incidence of treatment-emergent adverse events (occurring in $\geq 10\%$ of patients) ($n = 25$)

Adverse event, n (%)	All grades	Grade 1/2	Grade 3/4
Diarrhoea ^a	14 (56%)	8 (32%)	6 (24%) ^b
Vomiting	12 (48%)	11 (44%)	1 (4%)
Fatigue	10 (40%)	10 (40%)	0
Nausea	10 (40%)	10 (40%)	0
Abdominal pain	8 (32%)	6 (24%)	2 (8%)
Decreased appetite	7 (28%)	7 (28%)	0
Constipation	6 (24%)	6 (24%)	0
Aspartate aminotransferase increased	4 (16%)	3 (12%)	1 (4%)
Dehydration	4 (16%)	2 (8%)	2 (8%)
Dizziness	4 (16%)	4 (16%)	0
Dry mouth	4 (16%)	4 (16%)	0
Pyrexia	4 (16%)	4 (16%)	0
Abdominal distension	3 (12%)	3 (12%)	0
Anaemia	3 (12%)	2 (8%)	1 (4%)
Ascites	3 (12%)	2 (8%)	1 (4%)
Asthenia	3 (12%)	2 (8%)	1 (4%)
Blood alkaline phosphatase increased	3 (12%)	1 (4%)	2 (8%)
Blood bilirubin increased	3 (12%)	1 (4%)	2 (8%)
Hypokalaemia	3 (12%)	3 (12%)	0
Rash	3 (12%)	3 (12%)	0
Weight decreased	3 (12%)	3 (12%)	0

^aNone of the diarrhoea events resulted in dose discontinuation; one patient was hospitalised, and four patients reduced study drug due to diarrhoea events. ^bNo grade 4 diarrhoea events were reported.

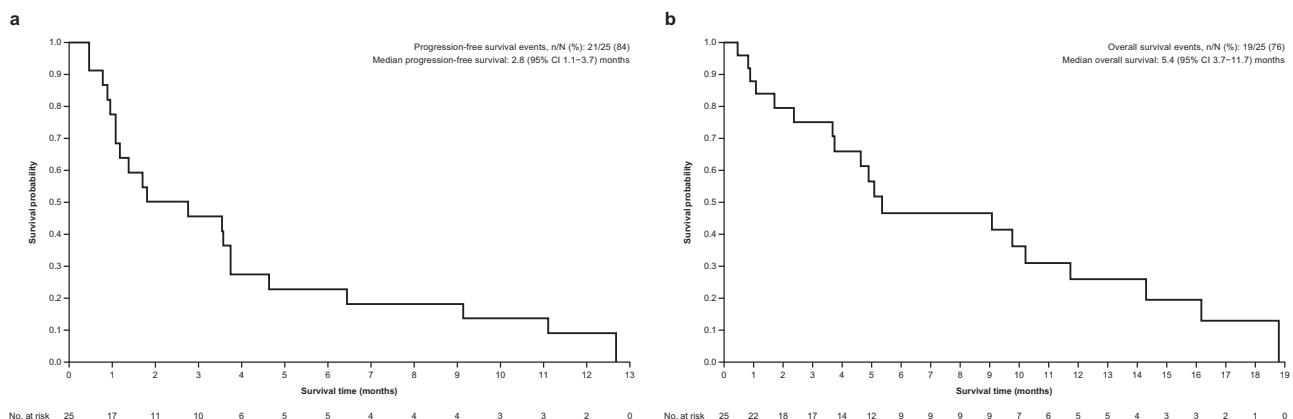


Fig. 3 | Estimated Survival For *HER2*-mutant Biliary Tract Cancer Patients Treated with Neratinib. Kaplan–Meier curves for **a** progression-free survival and **b** overall survival. Source data are provided as a Source Data file.

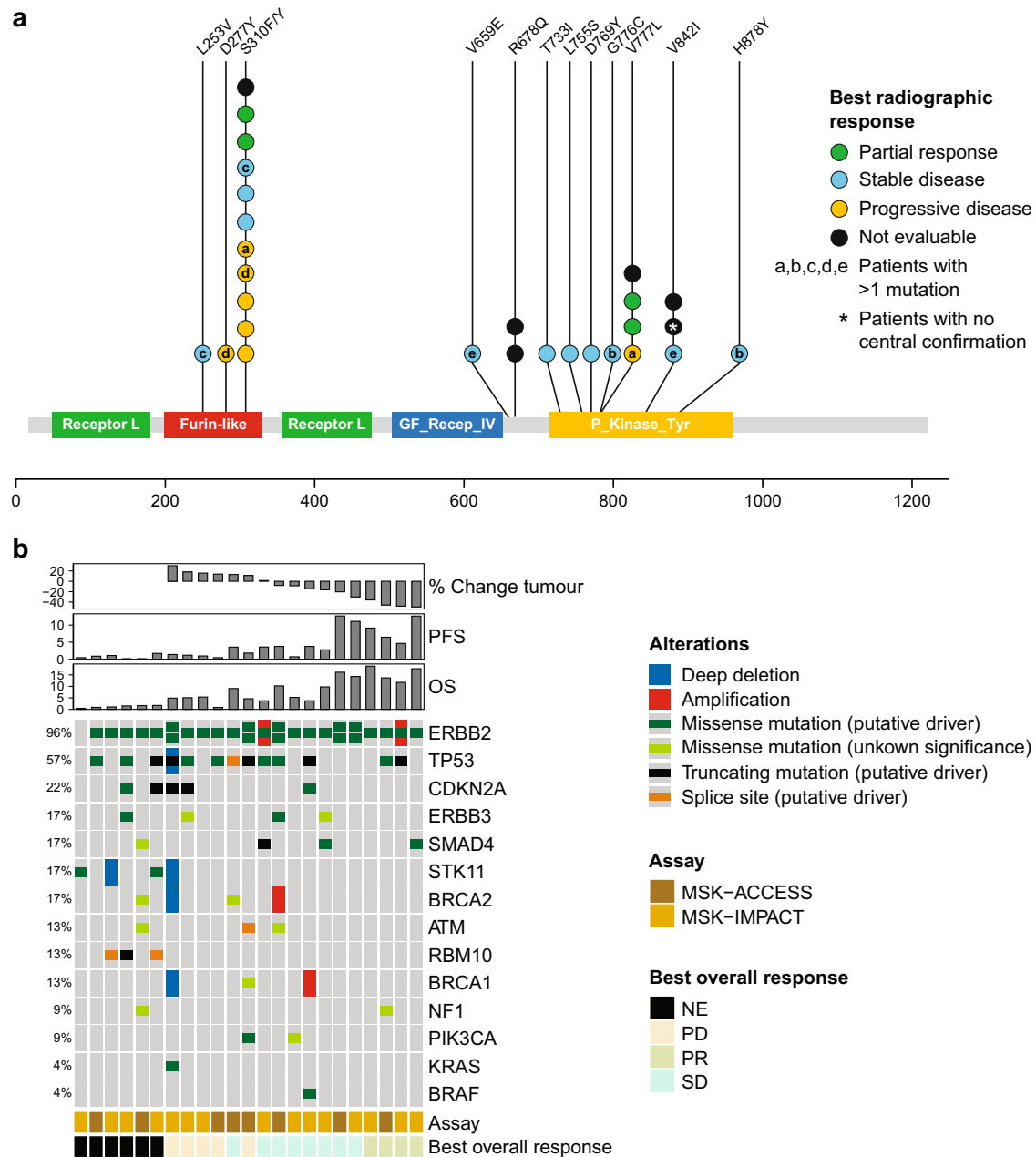


Fig. 4 | Genomic determinates of response to neratinib (n = 23). **a** Lollipop diagram of the *HER2* gene annotated with centrally confirmed mutations and tumour responses. Letters within circles indicate matching samples, i.e., patients with more than one mutation; **b** OncoPrint of co-occurring genomic alterations annotated

with objective response, central next-generation sequencing confirmation assay best tumour shrinkage, PFS, and OS. NE not estimable, OS overall survival, PD progressive disease, PFS progression-free survival, PR partial response, SD stable disease. Source data are provided as a Source Data file.

number amplifications, one mutant allele and one wild-type allele. Two patients (9%) had tumours harbouring co-occurring *PIK3CA* mutations (one oncogenic E545K alteration and an E81K variant of unknown significance). Oncogenic MAPK pathway co-alterations included *KRAS* Q61H (n = 1; 4%) and *BRAF* D594N (n = 1; 4%). No *IDH1*, *EGFR*, or *FGFR2* alterations were observed.

As retrospective analysis suggested worse outcomes for those patients whose tumours harboured *TP53* and *CDK2NA* mutations, we descriptively explored potential association of these co-occurring mutations on outcome²⁶. Among 10 patients with tumours wild type for both *TP53* and *CDK2NA*, two (20%) achieved a PR; the median OS for this group was 14.3 (95% CI 0.46–18.8) months. Two of eight patients with co-occurring *TP53* mutations without *CDK2NA* mutations (25%) achieved a PR; median OS was 9.1 (95% CI 0.82–11.7) months. There

were no PRs among five patients with co-occurring *TP53* and *CDK2NA* mutations; median OS for this group was 4.9 (95% CI 1.7–5.1) months. All *TP53* and *CDK2NA* mutations identified were either oncogenic or likely oncogenic according to OncoKB.

To explore hypothesis-generating changes in genomic profile coincident with progression on neratinib, paired tumour biopsies and serially collected cfDNA were interrogated by next-generation sequencing (NGS) in the four responders. One had paired pre-treatment and at-progression biopsies, and sequentially collected cfDNA during treatment. In the remaining three patients, before-treatment, on-treatment, and at-progression cfDNA samples were available for analysis. No genomic alterations were detected in cfDNA collected from two patients. One patient had adenocarcinoma of the gallbladder harbouring a *HER2* S310F mutation whose

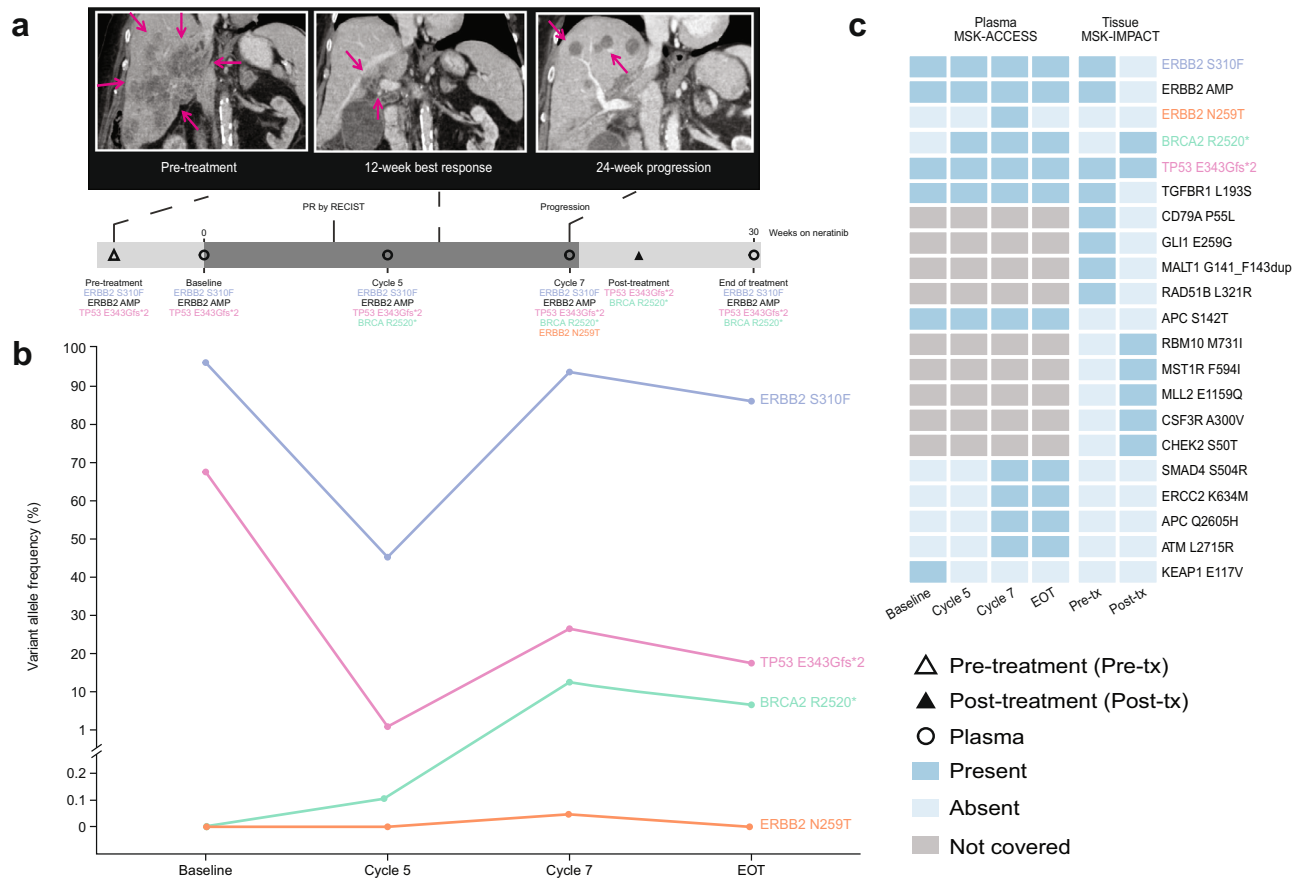


Fig. 5 | Polyclonal resistance to neratinib. A patient with adenocarcinoma of the gallbladder harbouring a *HER2* amplified/S310F mutation who had progression of disease on gemcitabine plus cisplatin, FOLFOX, and FOLFIRI achieved a confirmed PR on treatment with neratinib. **a** Cross-sectional and treatment course imaging showing tumour response and progression (purple arrow); **b** serial cfDNA and **c** paired paired-tissue next-generation sequencing. cfDNA cell-

free DNA, *EOT* end of treatment, *FOLFIRI* 5-fluorouracil/folinic acid + irinotecan, *FOLFOX* 5-fluorouracil/folinic acid + oxaliplatin, *MSK-ACCESS* Memorial Sloan Kettering-Analysis of Circulating cfDNA to Evaluate Somatic Status, *MSK-IMPACT* Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets, *PR* partial response, *tx* treatment. Source data are provided as a Source Data file.

disease had progressed on gemcitabine + cisplatin, 5-fluorouracil/folinic acid + oxaliplatin, and 5-fluorouracil/folinic acid + irinotecan (Fig. 5). NGS sequencing of a pre-treatment liver metastasis revealed amplification of the mutant *HER2* S310F allele, a truncating *TP53* mutation, and 19 additional molecular aberrations (six variants of unknown significance and 14 copy-number amplifications). Treatment with neratinib resulted in a PR (best response –48% reduction in target lesions). Biopsy and NGS of a progressing liver lesion revealed loss of *HER2* amplification with reduction in variant allele fraction (VAF) of the original *HER2* S310F mutation (VAF 90.2% pre-treatment vs <0.4% at disease progression). In addition, five mutations and seven copy-number alterations not seen in the pre-treatment sample were detected following disease progression, including a truncating *BRCA2* mutation (R2520*). NGS of peripheral blood before treatment detected *HER2* S310F and *TP53* E343Gfs*2 but not *BRCA2* R2520*. On-treatment plasma sampling revealed reduction in allele frequencies of *HER2* S310F and *TP53* mutations, consistent with response to neratinib treatment; both increased at the time of progression. Of note, the *BRCA2* truncating mutation and an additional *HER2* N259T mutation were detected in the progression plasma sample.

Discussion

Patients with advanced BTC have limited treatment options and poor overall prognosis, so it is critical to develop new treatments for this disease. This cohort of SUMMIT identified and selected patients with

advanced BTC harbouring infrequent somatic, activating *HER2* mutations and describe their genome-guided treatment with a selective pan-HER TKI²⁷. In this BTC cohort, neratinib was safe and exhibited modest antitumour activity, with a confirmed ORR of 16% (95% CI 4.5–36.1%) and CBR of 28.0% (95% CI 12.1–49.4%). The observed antitumour activity as assessed by ORR in the present study is similar to the observed anticancer activity of the currently available second-line cytotoxic chemotherapies (ORR 5–15%) and is in line with the modest ORR of 22% trastuzumab and pertuzumab in *HER2* positive BTC^{4,5}. These data give credence to the oncogenic role of somatic *HER2* mutations in BTC, provide further support for targeting *HER2* missense mutations as a therapeutic approach, and provide a much-needed benchmark for the operating characteristics of *HER2*-targeted therapy in the rare instance of somatic *HER2*-activating mutations⁹. That said, the lower proportion of patients with tumour shrinkage, short DoR, and the failure of the cohort to meet the prespecified rules that were required for the results to be considered positive argue for both a deeper understanding of *ERBB2* oncogene addiction in this rare cancer and the need to focus on strategies such as combination therapy approaches with potential to enhance antitumour activity and improve outcomes.

Objective responses were only seen in patients with cholangiocarcinoma and gallbladder cancers; minimal to no anticancer activity was observed in ampulla of Vater cancers. Interestingly, three of the four ampullary cancers harboured dual *HER2* mutations based on

central sequencing, one of which also had an activating *KRAS* co-mutation. The fourth was not available for central sequencing. In patients with breast cancer, either pre-existent dual *HER2* mutation or *HER2/HER3* co-mutation has been reported to be associated with lack of clinical benefit to neratinib as either a single agent or combined with anti-oestrogen therapy²⁸. In addition, one of four patients exhibited intestinal differentiation. Acknowledging the caveats of drawing conclusions given the small sample size, these findings suggest that responses to *HER2*-targeted therapy may differ based on anatomic site, histology, and/or underlying genomic profile, and future studies should seek to address discrete anatomic pathologies as separate cohorts or stratify accordingly²⁹. Such findings have also been observed in *HER2*-positive (amplification or overexpression) BTC tumours treated with monoclonal antibodies; in the MyPathway study, patients with gallbladder cancers tended to have better outcomes than other sub-histologies¹⁸.

Although the genomics for our study were descriptive in nature and objective responses were infrequent, outcomes appeared worse for patients with co-occurring inactivating alterations in cell-cycle regulators *TP53* and *CDKN2A*. A recent, large retrospective study reported that *TP53* and *CDKN2A* alterations have prognostic implications in cholangiocarcinoma, with worse outcomes after adjusting for stage and other known prognostic factors²⁶. Furthermore, other reports of *HER2*-targeted TKIs and *HER2*-directed antibodies in *HER2*-positive cancers suggest that *TP53* alterations and other cell-cycle regulators are important genomic modifiers of response and outcome^{25,30,31}. Further prospective studies in larger cohorts of patients with BTC harbouring *HER2* mutations will be needed to confirm the predictive and prognostic role of co-occurring *TP53* mutations with or without *CDKN2A* mutations.

Although tumour shrinkage was clearly documented in a subset of patients, responses to neratinib were relatively short-lived, indicating acquisition of resistance (DoR 3.0–4.7 months). We attempted to nominate potential resistance mechanisms though NGS on paired tumour biopsies and on serial cfDNA collection. One of four responders lost *HER2* alterations during progressive sampling, with the emergence of several mutations unique to this resistant clone. At the time of disease progression, analysis of a second metastatic lesion indicated loss of the initial *HER2* copy-number amplification and a marked decrease in VAF of the *HER2* S310F mutation, with emergence of an inactivating *BRCA2* mutation and several other alterations not observed on the pre-treatment sample. In addition, other alterations were observed in cfDNA, including a low-level *HER2* N259T variant of unknown significance, which were not observed in pre-treatment or post-progression tumours. Acquisition of additional *HER2* mutations, whether oncogenic or not, has been reported upon progression with neratinib-containing therapy in *HER2*-mutant breast cancer^{28,32}. In a second patient, serial cfDNA analysis did not reveal emergence of an alternative genomic driver, either due to assay detection limitations or via a more complex functional resistance mechanism not measurable by NSG or cfDNA. Given the limited sample size, it is challenging to speculate on generalised mechanisms of acquired resistance in BTC based on these results; preclinical modelling could potentially help inform such pathways.

Neratinib treatment was generally well tolerated in this BTC cohort. The AE profile was comparable with previous reports: diarrhoea and vomiting were the most common all-grade events, and diarrhoea and abdominal pain were the most common grade 3/4 events. No new safety signals were observed. The pattern of AEs observed in the BTC cohort was generally similar to the overall SUMMIT population, in which diarrhoea, nausea, and vomiting were the most common all-grade AEs, affecting 74%, 43%, and 41% of patients, respectively²⁵.

The study has several notable strengths including: ability to identify rare genomic variants in an uncommon patient population; correlative design that allowed for hypothesis-generating observations

regarding prognostic implications of co-occurring mutations, as well as those related to acquired resistance to neratinib; and implementation of the potential utility of cfDNA in identifying patients with BTC for molecular therapeutics, as the genomic driver of interest was detected in a high proportion of pre-treatment samples and was often concordant with tumour tissue.

Limitations of this study include the small sample size, inability to confirm centrally the oncogenic driver in three of 25 patients, and lack of available pre-treatment tissue for central confirmation in approximately 40% of patients, which hampered correlative analysis. These observations suggest pre-treatment tissue acquisition may be required in future molecularly targeted studies in BTC. In retrospect, as response appeared to differ based on anatomic site, exclusion of ampullary cancers or at least defining anatomic cohorts of sufficient sample size would have led to better estimation of antitumour activity. It is also important to acknowledge, as observed in other studies in patients with BTC^{5,6,18,33–36}, that a subset of patients progressed rapidly on treatment; eight patients died as a result of their disease within 8 weeks of study initiation. This highlights the complexity of drug development in BTC.

In summary, analysis of the genomically driven, multi-histology SUMMIT trial suggests that selected *HER2*-mutant BTCs are sensitive to inhibition by the pan-HER TKI neratinib. Neratinib was well tolerated and showed antitumour activity in patients with metastatic gallbladder cancer or cholangiocarcinoma harbouring *HER2* mutations. Addition of a second targeted agent prolonged and deepened responses to neratinib in the breast and lung cohorts of SUMMIT^{28,37} and a parallel approach could have similar utility for patients with *HER2*-mutant BTC. Likewise, prospective modelling (i.e., I-PREDICT) has illustrated that co-targeting oncogenic drivers with more than one precision medicine might further enhance response³⁸. Further studies are needed to evaluate the role of additional agents in enhancing response to neratinib in patients with *HER2*-mutant metastatic BTC.

Methods

Study design and participants

This research complies with all relevant ethical regulations regarding the use of human study participants. The study was conducted in accordance with International Conference on Harmonisation Good Clinical Practice guidelines, Declaration of Helsinki, and local regulations. Approval was obtained from institutional review boards at each of the participating institutions (Supplementary Table 4). The SUMMIT protocol, which is available on request to the lead author, is not yet published online as several elements of the SUMMIT trial are ongoing. The protocol will be published on clinicaltrials.gov after completion of the study. Written informed consent was obtained for all patients before performing study-related procedures. Patients were not compensated for taking part in the study.

SUMMIT (NCT01953926) is an open-label, single-arm, multi-cohort, multi-tumour, phase 2, 'basket' trial conducted at 58 centres internationally²⁵. Patients were recruited between April 3, 2014, and August 1, 2019 by investigators at each site based on *HER2*-mutation status; 16 sites contributed at least one patient to the BTC cohort.

Inclusion and exclusion criteria are as follows:

Inclusion criteria

1. Men and women who are ≥ 18 years old at signing of informed consent.
2. Histologically confirmed cancers in patients with activating *ERBB* mutations and who are refractory to standard therapy or for which standard or curative therapy does not exist or is not considered sufficient or appropriate by the Investigator.
3. At the time of screening, a previously documented mutation: *HER2* mutation in breast, bladder/urinary tract, biliary tract,

colorectal, endometrial, gastroesophageal, lung, ovarian, and any other cancers.

4. At least one measurable lesion, preferably as defined by Response Evaluation Criteria in Solid Tumours (version 1.1)³⁹.
5. Left ventricular ejection fraction $\geq 50\%$ measured by multiple-gated acquisition scan or echocardiogram.
6. Eastern Cooperative Oncology Group performance status of 0–2.
7. Female patients with cancers known to secrete β -human chorionic gonadotropin (β -hCG), ie, germinomas, are eligible if the pattern of serum β -hCG is suggestive of the malignancy and the pelvic ultrasound is negative for pregnancy.
8. Men must agree and commit to use a barrier method of contraception while on treatment and for 3 months after the last dose of the investigational product. Women of child-bearing potential must agree and commit to the use of a highly effective double-barrier method of contraception (e.g., a combination of male condom with an intravaginal device such as the cervical cap, diaphragm, or vaginal sponge with spermicide) or a non-hormonal method, from the signing of the informed consent until:
 - i. 28 days after the last dose of neratinib monotherapy, or
 - ii. 6 months after the last dose of paclitaxel, or
 - iii. 1 year after the last dose of fulvestrant.
9. Provide written informed consent to participate in the study and follow the study procedures.
9. Demonstrates a QTc interval >450 ms for men or >470 ms for women or known history of congenital QT prolongation or torsade de pointes.
10. Inadequate bone marrow, renal, or hepatic function as defined on screening laboratory assessments.
11. Uncontrolled concurrent malignancy (early-stage or chronic disease is allowed if not requiring active therapy or intervention and is under control).
12. Active infection or unexplained fever >38.5 °C (101.3 °F).
13. Women who are pregnant, are planning on becoming pregnant, or are breast-feeding.
14. Significant chronic gastrointestinal disorder with diarrhoea as a major symptom (e.g., Crohn's disease, malabsorption, or grade ≥ 2 NCI CTCAE [version 4.0] diarrhoea of any aetiology at baseline).
15. Clinically active infection with a hepatitis virus.
16. Evidence of significant medical illness, abnormal laboratory finding, or psychiatric illness/social situations that could, in the Investigator's judgement, make the patient inappropriate for this study.
17. Known hypersensitivity to any component of the investigational product, required combination therapy, or loperamide.
18. Unable or unwilling to swallow tablets.
19. Patients bearing certain somatic *HER* mutations, such as those that are subclonal in nature, or resulting in the expression of truncated proteins including alterations that result in a premature stop codon or a change in reading frame (i.e., frameshift mutations) may not be considered for eligibility.
20. Patients with known activating *KRAS* mutations.

Exclusion criteria

1. Prior treatment with any *HER2*-directed tyrosine kinase inhibitor (e.g., lapatinib, afatinib, dacomitinib, neratinib) with the exception of patients with non-small cell lung cancer (NSCLC) who may have received afatinib and remain eligible.
2. Not recovered to at least grade 1 or baseline (National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE] version 4.0) from all clinically significant adverse events related to prior therapies (excluding alopecia).
3. Received chemotherapy or biologic therapy ≤ 2 weeks or five half-lives of the agent used, whichever is shorter, prior to the start of neratinib.
4. Received radiation therapy ≤ 14 days prior to initiation of investigational product, except primary brain tumour patients.
5. Patients who are receiving any other anticancer agents with the exception of patients on (1) a stable dose of bisphosphonates or denosumab or (2) sex hormone therapy in the case of breast, prostate, or gynaecological cancers.
6. Received prior therapy resulting in a cumulative epirubicin dose >900 mg/m² or cumulative doxorubicin dose >450 mg/m². If another anthracycline or more than one anthracycline has been used, the cumulative dose must not exceed the equivalent of 450 mg/m² doxorubicin.
7. Symptomatic or unstable brain metastases. (Note: Asymptomatic patients with metastatic brain disease who have been on a stable dose of corticosteroids for treatment of brain metastases for at least 14 days are eligible to participate in the study.) Patients with primary central nervous system tumours are eligible.
8. Active uncontrolled cardiac disease, including cardiomyopathy, congestive heart failure (New York Heart Association functional classification of ≥ 2), unstable angina (symptomatic angina pectoris within the past 180 days that required the initiation of or increase in anti-anginal medication or other intervention), myocardial infarction within 12 months of enrolment, or ventricular arrhythmia (except for benign premature ventricular contractions). For patients with NSCLC, the following are additionally excluded: conduction abnormality requiring a pacemaker; supraventricular and/or nodal arrhythmias not controlled

- with medication; valvular disease with documented compromise in cardiac function; symptomatic pericarditis; any history of myocardial infarction documented by elevated cardiac enzymes or persistent regional wall abnormalities on assessment of left ventricular function; any history of documented congestive heart failure and/or cardiomyopathy.

Procedures

Patients received neratinib 240 mg orally daily. One cycle was 28 days or 4 weeks of treatment. Patients received mandatory loperamide prophylaxis. Patients were treated until disease progression, unacceptable toxicity, or consent withdrawal.

Tumour response was assessed locally using RECIST (version 1.1) every 8 weeks by computed tomography or magnetic resonance imaging. AEs were recorded using Common Terminology Criteria for AEs (version 4.0) from consent until day 28 after study treatment discontinuation.

All clinical data were collected under an IRB-approved protocol and store in a encrypted and secure database that underwent regular review and cross referencing in the local electronic medical record. Upon the completion of the protocol, this database was locked. Genomic data were generated for each patient on study and stored in cBioPortal. All data were generated using cBioPortal for Cancer Genomics: MutationMapper (cBioportal front end version 3.7.23)^{40,41} (Fig. 3) and ComplexHeatMap (version 2.8.0) package with ggplot2 (3.3.5) and R (4.1.3) (Figs. 4 and 5). All figures were refined using Adobe Illustrator 2021 (25.2.3).

Exploratory genomic analysis

Archival or pre-treatment formalin-fixed, paraffin-embedded (FFPE) tumour tissue was required for study entry. Plasma was collected before treatment, on treatment (every other cycle), and at treatment discontinuation. Tumour DNA was extracted from FFPE tissue or plasma, and sequenced using Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)⁴² or MSK-ACCESS⁴³. Custom targeted *HER2* single-gene sequencing was performed in select cases using plasma samples. Somatic alterations were annotated with OncoKB (version date December 24, 2021)⁴⁴.

Outcomes and endpoints

The primary objective of the BTC cohort was to determine the response rate (ORR). Other objectives included determination of: CBR (confirmed CR, PR, or stable disease [SD] for ≥ 16 weeks within ± 7 -day visit window); PFS (interval from treatment start to first date on which recurrence, progression, or any-cause death was documented); OS (defined as the interval from start of treatment to death for those who died; for those who did not die, censored at the last known alive time); DoR (time from date measurement criteria were met for CR or PR until first date of documented disease progression); safety; and tolerability. Disease control rate (DCR), which was not a prespecified endpoint, was defined as confirmed CR, PR, or SD for ≥ 24 weeks. Exploratory correlative objectives included retrospective central confirmation of locally reported *HER2* mutation via NGS on archival or fresh tumour tissue or in cfDNA extracted from plasma, and description of patient outcomes based on pre-treatment genomics, and genomic clonal evolution with treatment via NGS on serial cfDNA.

Statistical analysis

A Simon two-stage optimal design was used to determine whether neratinib monotherapy had sufficient activity to warrant further development. Early study termination was permitted if data at the first stage indicated treatment was ineffective. Using Simon's optimal two-stage design (significance level 10%, power 80%), a true ORR at 8 weeks of $\leq 10\%$ was considered unacceptable (null hypothesis) and a true ORR at 8 weeks of minimally 30% (alternative hypothesis) merited further study. In the first stage, enrolment continued until seven patients had completed two neratinib cycles and appropriate activity assessment was completed. If one or more responses, defined as a PR or CR at the first post-baseline assessment, were observed, the cohort was expanded to include 11 additional patients for the second stage. Additional enrolment beyond the first 18 patients was allowed to ensure that at least 18 patients were evaluable for a radiographic response. This led to over-enrolment of the study. If four or more responses were seen in stage 2, the cohort could be expanded to a maximum of 30 patients. As the biliary tract cohort did not meet the criteria for continued enrolment, this cohort was closed to recruitment. The overall SUMMIT study, of which this is a component, is ongoing.

All endpoints were descriptive. No sex- or gender-based analyses were performed as there are no biological data to support a difference in outcome based on sex or gender. Analyses for correlates, which were prespecified, were descriptive. Baseline characteristics, activity, and safety were summarised in the safety analysis set (all patients receiving at least one neratinib dose). The Clopper-Pearson method was used to calculate ORR and CBR 95% CIs. Kaplan–Meier methodology was used to determine PFS estimates with 95% CIs. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) or the survival package (version 3.1–12) from R (version 4.0.2)⁴⁵. This study is registered with ClinicalTrials.gov, NCT01953926 and European Union Drug Regulating Authorities Clinical Trials Database, EudraCT 2013-002872-42.

Data availability

Puma Biotechnology is committed to sharing clinical trial data and information to help physicians and patients make informed treatment decisions, and to help researchers advance scientific knowledge. The authors declare that the data supporting the findings of this study are available within the article and source data for the figures are provided with this paper. The SUMMIT protocol is not yet published online as several elements of the SUMMIT trial are ongoing but it will be published on ClinicalTrials.gov within 1 year of completion of the study. Raw patient data are under restricted access for privacy reasons. Puma makes patient-level, de-identified data sets, and associated documents available as set forth in Puma's data sharing policy (https://pumabiotechnology.com/data_sharing_policy.html). Requests for

study protocol, other study documentation and clinical trial data may be submitted to clinicaltrials@pumabiotechnology.com for consideration. Once approved, a data sharing agreement will be provided for timely access to these data for the time required to perform the analysis. Genomic data have been deposited in the cBioPortal repository (available at https://www.cbioportal.org/study/summary?id=biliary_tract_summit_2022). Source data are provided with this paper.

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Author contributions

J.J.H., S.A.P.-P., D.DiP, M.F.B., L.D.E., F.M.-B., D.B.S., and G.K.A.-A. were responsible for the conception and design of the work. J.J.H., S.A.P.-P., R.H.S., J.J.M., J.Z., D.DiP., C.S., S.D.S., M.F.B., L.D.E., F.M.-B., K.J., D.B.S., and G.K.A.-A. were responsible for data analysis. J.J.H., S.A.P.-P., R.H.S., J.M.C., G.I.S., D.I.Q., I.B., V.M., M.B., S.L., I.S., H.P., J.M.F., M.A., S.M.S., CdelaF, C.F., J.Z., DDiP, C.S., S.D.S., M.F.B., L.D.E., F.M.-B., K.J., D.B.S., and G.K.A.-A. were responsible for data acquisition. J.J.H., S.A.P.-P., R.H.S., J.M.C., D.I.Q., I.B., V.M., M.B., S.L., I.S., H.P., J.M.F., M.A., S.M.S., CdelaF, C.F., J.Z., D.DiP., M.F.B., L.D.E., F.M.-B., K.J., D.B.S., and G.K.A.-A. were responsible for data interpretation. J.J.H., R.H.S., J.Z., D.DiP., M.F.B., L.D.E., D.B.S., and G.K.A.-A. were responsible for drafting the work. All authors critically revised the work, approved the final draft of the manuscript, and agree to be accountable for all aspects of the work. J.J.H., R.H.S., J.J.M., J.Z., D.DiP., M.F.B., L.D.E., D.B.S., and G.K.A.-A. verify the underlying data.

Competing interests

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Additional information

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