Receptor Tyrosine kinase co-amplification and benefit from HER2 inhibitors in Biliary Tract Cancers

Raffaella Casolino*[1], Francesco Amato*[1], Colin Rae[1], Srikanth Puttagunta[2], Chiara Braconi[1,2,3,#].

And collaborators:
Tamsin Nash[2,3], Martin MacLeod[2,3], Paula Sanchon-Sanchez[1,4], Patricia Roxburgh[1,2,3], Jeff Evans[1,2,3], Janet Graham[2,3], Fraser Duthie[2], Nicola Valeri[5], Pawel Herzyk[6,7] Julie Galbraith[6,7]

1. Institute of Cancer Science, University of Glasgow, UK
2. NHS Greater Glasgow and Clyde, Glasgow, UK
3. Beatson West of Scotland Cancer Centre, Glasgow, UK
4. Experimental Hepatology and Drug Targeting (HEVEPHARM), University of Salamanca, Institute for Biomedical Research of Salamanca (IBSAL), Center for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, ES
5. Division of Surgery and Cancer, Imperial College London, UK
6. Institute of Molecular Cell & Systems Biology, University of Glasgow, UK
7. Glasgow Polyomics, University of Glasgow, UK

* These authors have contributed equally

Correspondence to Dr Chiara Braconi, Institute of Cancer Sciences, University of Glasgow, Switchback Road, Glasgow, G61 1QH. Chiara.braconi@glasgow.ac.uk

Word count: 853

Number of figures: 1

Conflict of Interest
CB receives honoraria from Incyte. NV receives honoraria from Roche, Merck-Serono, Eli Lilly, Pfizer, Servier; and is a consultant for Benevolent AI. JE is consulting/advisory board member (payable to institution) for Ascelia, Astra Zeneca, Bayer, Bicycle Therapeutics, Bristol-Myers Squibb, Clovis, Eisai, Medivir, MSD, Nucana, Roche / Genentech; receives speaker's fees (payable to institution) from Ascelia, Astra Zeneca, Bayer, Bristol-Myers Squibb, Eisai, Medivir, MSD, Nucana, Roche / Genentech , and research funding (payable to institution) from Ascelia, Astra Zeneca, Bayer, Bicycle Therapeutics, Bristol-Myers Squibb, Eisai, Medivir, MSD, Nucana, Roche / Genentech, Adaptimmune, Astellas, Beigene, Boehringer, Ingelheim, Basilea, Celgene, Codiak, CytomX, GSK, Immunocore, iOnctura, Johnson & Johnson, Lilly, MiNa Therapeutics, Novartis, Pfizer, Sanofi, Sapience Therapeutics, Seagen, Sierra, Starpharma, UCB, Verastem ; he is Chair, Independent Data Monitoring Committee (honorarium payable to institution) Genmab; receives support to attend national & international congresses (personal) MSD, Bristol-Myers Squibb, Bayer, Roche, Nucana, Pierre Fabre.

Financial Support
This study has been supported by the Lord Kelvin Adam Smith readership (University Of Glasgow), the ISSF Excellence and Innovation Catalyst award (Wellcome Trust), and the COST-ACTION-18122 (Euro-Cholangio-Net).

Author's contribution
RC: collection of data and draft of the manuscript; FA: collection of data and draft of the manuscript; CR: collection of data; SP: collection of data; TN: collection of data; MM: collection of data; PSS: collection of data; PR: collection of data; JE: collection of data; JG: collection of data; FD: collection of data; NV: analysis of data and revision of manuscript; PH: collection of data; JG: collection of data: CB: ideation and design of project, analysis, revision of manuscript.

Data availability statement
Not applicable

Introduction

Targeting the human epidermal growth factor receptor *2 (HER2) has* emerged as a promising therapeutic strategy in advanced biliary cancers (BTC). Javle *et al* have recently reported a sub-analysis of the MyPathway trial on the efficacy of the dual HER2 inhibition with Pertuzumab plus Trastuzumab in advanced BTC with *ERBB2* amplification. After a median follow up of 8.1 months, 23% of patients achieved partial response, with a median duration of response of 10.8 months, median progression-free survival (PFS) of 4.0 months, and median overall survival of 10.9 months[1]. Whilst promising, these results indicate that resistance to anti-HER2 agents is still a significant challenge to overcome.

Several molecular mechanisms causing resistance to anti-HER2 blockade have been described in breast cancer [2] while in BTC they are still poorly understood as the investigation of this therapeutic strategy is still in its infancy [1, 3-6].

Here, we report the clinical course and the integrated molecular analysis of tissue and longitudinal liquid biopsies in a patient with co-amplified *ERBB2* and *EGFR* gallbladder cancer (GBC) treated with concurrent HER2 and EGFR inhibitors. Our data suggest that *EGFR* copy number gain might represent a mechanism of resistance to Trastuzumab in GBC and provide useful insights for the application of HER2 inhibitors in clinical practice.

Results

A 58-year-old female patient was diagnosed with a stage IV (CK7+, CK19+) GBC with multiple liver metastasis in February 2019 (Figure 1A). After progression to first line Cisplatin Gemcitabine (May 2019), she received a targeted inhibitor of thymidylate synthase within a phase I trial, which was withdrawn for hepatotoxicity. Third line Folfox chemotherapy was administered between September 2019 and March 2020, when due to stabilization of disease and onset of Covid19 pandemic she was switched to active surveillance. At progression (August 2020) the patient was re-challenged with Folfox, but treatment was halted two months later due to radiological progression. She was then considered for an off-licence targeted therapy based on the Next Generation Sequencing (NGS) results of her diagnostic liver biopsy [FoundationOne cDx 324 gene panel], which documented double amplification of *ERBB2* and *EGFR*, along with *SMAD* and *TP53* mutations.

In view of the double *ERBB2-EGFR* amplification, dual blockade with Trastuzumab (HER2-directed antibody; loading dose 8 mg/kg followed by 6mg/kg q21) and Lapatinib (reversible EGFR/HER2 kinase inhibitor; 1250 mg/day continuously) was initiated in November 2020. Monthly ctDNA and tumour markers were used to monitor response (Figure 1B&C).

Pre-treatment ctDNA analysis [Avenio ctDNA extended 77 gene assay] showed concordance with tissue data confirming the presence of *EGFR* and *ERBB2* amplifications (CNV scores 26.4 and 14.2 respectively) and p.Arg361His *SMAD4* and p.Tyr220Cys *TP53* mutations (VAF 48.12% and 53.46% respectively) (Figure 1D).
Liquid biopsy analysis after a month of treatment documented significant reduction in tumour markers and ctDNA load with normalisation of *SMAD4* and *TP53* mutations (VAF 0.32% and 0.36% respectively) and un-detectability of *ERBB2* and *EGFR* CNV (Figure 1C&E).

Lapatinib was discontinued 2.1 months later for G4 diarrhoea. As soon as EGFR inhibition was halted, tumour markers and ctDNA showed a steep increase. Trastuzumab was continued as single agent until March 2021, when was interrupted due to biochemical and radiological progression (PFS 4.1 months). ctDNA at progression showed *SMAD4* and *TP53* mutations (VAF 54% and 63% respectively) along with *ERBB2* and *EGFR* amplifications (CNV scores 26.62 and 14.6 respectively). Of note, de-novo mutations arose at 2.5 months (p.Glu545Lys *PIK3CA*) and 3.73 months (p.Val266Leu *PDGFRA* and p.Ile673Ile *EGFR*) (Figure 1E). The patient deceased in June 2021.

Discussion

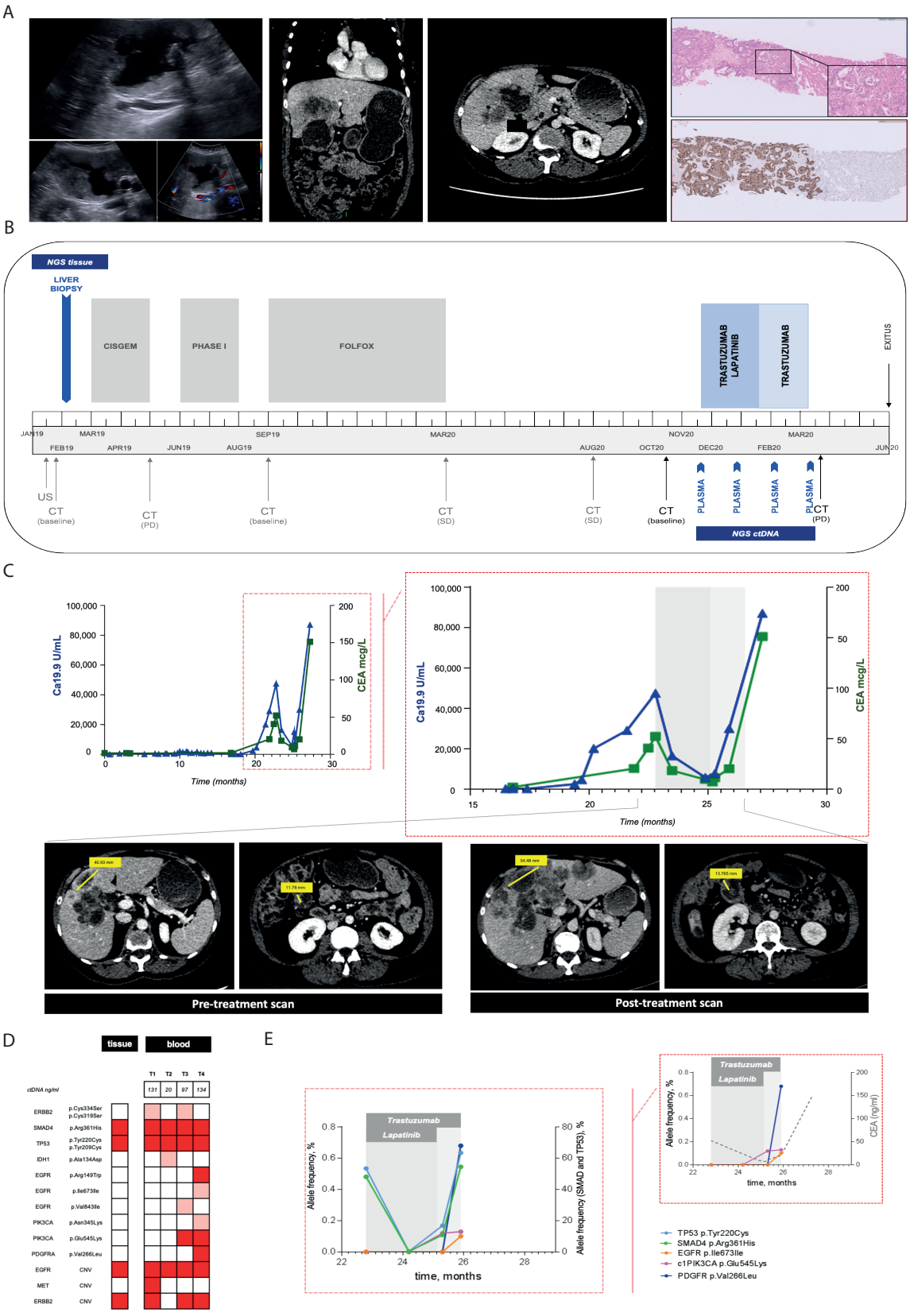
We report the clinical and molecular evolution of a patient whose GBC harbored a co-amplification of *ERBB2* and *EGFR:* dual ERBB2 and EGFR inhibition was initially associated with clinical, biochemical and ctDNA signs of response. As soon as EGFR inhibition was lifted due to ongoing toxicity from the tyrosine-kinase inhibitor, the patient showed disease progression. These observations suggest two, non-mutually exclusive hypotheses. First, *EGFR* copy number gain might have been the driver event in this patient progression, an assumption supported by observation that *EGFR*-amplified cancers have a significantly poorer prognosis than *ERBB2*-amplified cancers in a pan-cancer analysis (Suppl Figure 1). Second, similarly to other gastrointestinal cancers treated with *BRAF*, *KRAS* G12C and multi-TK inhibitor [7-9], *EGFR* signaling, especially in the context of an *EGFR*-amplification, might have represented a mechanism of bypass or rebound upon HER2 inhibition.
Although co-amplification of RTKs is relatively infrequent in BTC, our data suggest that testing for the presence of other RTK amplification might be a sound approach to identify mechanisms of resistance and optimize combinatorial therapeutic strategies such as the use of a pan-HER inhibitor like Afatinib in cases with *EGFR* and *ERBB2* co-amplifications. Under these assumptions, as HER2 inhibition becomes a widely used therapeutic approach in BTC, single testing with HER2 FISH appear to fall short in providing a comprehensive assessment of BTC molecular architecture prompting to consider broader genomic analyses for a more robust patient screening and selection. Finally, in our patient we observed emergence of *de novo* mutations in *PDGRA* and *PIK3CA* upon progression, which have been previously associated to resistance to Lapatinib[10] in colon cancer, underlining the paramount importance of ctDNA in identifying sequential adaptive therapies in this group of patients.

References

[1] Javle M, Borad MJ, Azad NS, Kurzrock R, Abou-Alfa GK, George B, et al. Pertuzumab and trastuzumab for HER2-positive, metastatic biliary tract cancer (MyPathway): a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol 2021;22:1290-1300.
[2] Rimawi MF, De Angelis C, Schiff R. Resistance to Anti-HER2 Therapies in Breast Cancer. Am Soc Clin Oncol Educ Book 2015:e157-164.
[3] Javle M, Churi C, Kang HC, Shroff R, Janku F, Surapaneni R, et al. HER2/neu-directed therapy for biliary tract cancer. Journal of Hematology and Oncology 2015.
[4] Sorscher S. Marked radiographic response of a HER-2-overexpressing biliary cancer to trastuzumab. Cancer Manag Res 2013;9:1-3.
[5] Nam AR, Kim JW, Cha Y, Ha H, Park JE, Bang JH, et al. Therapeutic implication of HER2 in advanced biliary tract cancer. Oncotarget 2016;7:58007-58021.
[6] Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018;554:189-194.
[7] Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100-103.
[8] Amodio V, Yaeger R, Arcella P, Cancelliere C, Lamba S, Lorenzato A, et al. EGFR Blockade Reverts Resistance to KRAS(G12C) Inhibition in Colorectal Cancer. Cancer Discov 2020;10:1129-1139.
[9] Jin H, Shi Y, Lv Y, Yuan S, Ramirez CFA, Lieftink C, et al. EGFR activation limits the response of liver cancer to lenvatinib. Nature 2021;595:730-734.
[10] Siravegna G, Lazzari L, Crisafulli G, Sartore-Bianchi A, Mussolin B, Cassingena A, et al. Radiologic and Genomic Evolution of Individual Metastases during HER2 Blockade in Colorectal Cancer. Cancer Cell 2018;34:148-162 e147.

Figure legend.

Figure1. A. Diagnostic abdomen US and CT scan images are shown, along with H&E and IHC (CK7) of the diagnostic liver biopsy. **B.** Schematic representation of the clinical course of patient RB001 enrolled within the translational study REG-Bil (ISRCTN15141439). **C**. Longitudinal assessment of tumour markers throughout the whole clinical course (left) and the treatment course with Trastuzumab and Lapatinib (right). On the bottom panel representative images of the radiological assessment performed before and after treatment with Trastuzumab. **D.** NGS profile in the diagnostic tissue (left) and the longitudinal blood samples taken throughout the targeted therapy (T1: Baseline C1; T2 1.3 month; T3 2.5 months, T4 3.73 months). **E.** Longitudinal course of the mutations assessed by NGS (left) with a focus on de novo mutations only emerged after Lapatinib (right).


A
B
NGS tissue
LIVER BIOPSY
CISGEM
PHASE I
FOLFOX
TRASTUZUMAB LAPATINIB
TRASTUZUMAB
EXITUS
JAN19
MAR19
SEP19
MAR20
NOV20
MAR20
FEB19
APR19
JUN19
AUG19
AUG20
OCT20
DEC20
FEB20
JUN20
US
CT (baseline)
CT (PD)
CT (baseline)
CT (SD)
CT (SD)
CT (baseline)
PLASMA
PLASMA
PLASMA
PLASMA
CT (PD)
NGS ctDNA
C
Ca19.9 U/mL
CEA mcg/L
Time (months)
Pre-treatment scan
Post-treatment scan
D
tissue
blood
ctDNA ng/ml
T1 131
T2 20
T3 97
T4 134
ERBB2 p.Cys334Ser p.Cys319Ser
SMAD4 p.Arg361His
TP53 p.Tyr220Cys p.Tyr209Cys
IDH1 p.Ala134Asp
EGFR p.Arg149Trp
EGFR p.Ile673Ile
EGFR p.Val843Ile
PIK3CA p.Asn345Lys
PIK3CA p.Glu545Lys
PDGFRA p.Val266Leu
EGFR CNV
MET CNV
ERBB2 CNV
E
Trastuzumab
Lapatinib
Allele frequency, %
Allele frequency (SMAD and TP53), %
time, months
CEA (ng/ml)
TP53 p.Tyr220Cys
SMAD4 p.Arg361His
EGFR p.Ile673Ile
c1PIK3CA p.Glu545Lys
PDGFR p.Val266Leu