

HER2 as a therapeutic target in gallbladder cancer—aye or nay?

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Gallbladder cancer (GBC) is an uncommon cancer. Prevalence varies across different geographical locations with Chile having the highest incidence at 9.7 per 100,000 age-standardised rate followed by Bolivia and the Republic of Korea (South Korea) (1). Most patients present in advanced stages of the disease which is associated with a poor prognosis. Even with palliative chemotherapy, the median overall survival in advanced disease is less than 12 months (2,3). As such, the search for new therapeutic targets with the hope of improving survival in patients with this disease is much needed.

The molecular biology behind the role of HER family of tyrosine kinases playing an important role in the pathogenesis of cancer is well established. Among the four HER family proteins, HER2 has the strongest catalytic kinase activity and is the preferred partner for dimerization with other HER family proteins (4). Even though the pathogenesis of GBC is not well-understood, the pathogenic ability of HER2 overexpression has been clearly demonstrated in animal models in which overexpression of HER2 in the basal layer of biliary tract epithelium led to the development of gallbladder adenocarcinoma in 100% of transgenic mice by 3 months of age (5).

In 2014, Roa *et al.* (6) published a study conducted with the aim to determine the frequency of HER2 overexpression in GBC and to identify a subgroup of patients who would benefit from targeted therapy. Specimens of 187 patients with GBC and 75 normal controls were tested for HER2 overexpression using immunohistochemical technique. HER2 positivity was defined according to the CAP/ASCO (College of American Pathologists/American Society of Clinical Oncology) criteria for breast cancer. The study

reported prevalence of HER2 overexpression in 12.8% of the GBC cases and there was a trend towards a worse 5-year overall survival in patients with HER2 overexpression although this was not statistically significant. More recently, Yan *et al.* (7) examined more than 37,000 tumour specimen across different histology for HER2 overexpression and found it to be present in 9.8% of 194 GBC specimens in the study. GBC was in fact the fourth highest in terms of frequencies of HER2 overexpression after bladder cancer, oesophageal and gastroesophageal junction cancers and breast cancer. Where do the findings of studies such as Roa *et al.*'s take us in our understanding of the relevance of HER2 in GBC and our pursuit of novel therapeutic targets for this disease?

Logically, in looking for a new therapeutic target for any disease, it would first involve determining the presence as well as prevalence of the molecular target in that specific disease. In this light, studies such as the one conducted by Roa *et al.* (6) support the relevance of studying HER2 as a target in GBC by informing us that a significant proportion of GBC demonstrates HER2 overexpression. Compared to earlier studies (8-16) on the prevalence of HER2 overexpression in GBC, the strength of Roa *et al.*'s study lies in its large sample size. However, examining his results together with these earlier studies, it is notable that the reported prevalence of HER2 overexpression ranged widely across different studies. This wide variability may be explained by the use of different criteria to define HER2 overexpression in each study and highlights the challenges we face in determining HER2 positivity in GBC.

Currently, there is no guideline on a standardised algorithm for testing and defining HER2 positivity

for GBC. HER2 overexpression may be tested for by immunohistochemical techniques or FISH for gene amplification. It is uncertain if it is valid to apply the same criteria used in defining HER2 positivity in breast or gastric cancer for GBC too. As we learnt from experience in studying HER2 in breast and gastric cancer, a clinically relevant definition of HER2 positivity may differ in different diseases at least in part due to intrinsic biological differences. It is hence plausible that GBC being a distinct disease entity also has its unique HER2 positivity definition still unbeknownst to us at the moment.

Following the study by Roa *et al.*, further insights into the relevance of HER2 in GBC have been gained using a combination of exome sequencing and ultra-deep sequencing of cancer-related genes on 57 GBC samples. Li *et al.* (17) reported that the ErbB signalling pathway is the most extensively mutated pathway occurring in 36.8% of the samples examined. A total of 37 non-silent somatic mutations of 15 genes in the ErbB pathway (*ERBB3*, *ERBB2*, *ERBB4*, *EGFR*, *KRAS*, *NRAS*, *HRAS*, *PIK3CA*, *BRAF*, *MAP2K4*, *MAPK10*, *SRC*, *MYC*, *NRG1* and *SOS2*) were detected. To determine the oncogenic effects of *ERBB3* and *ERBB2*, Li *et al.* overexpressed the mutants of *ERBB3* and *ERBB2* in GBC cell lines (GBC-SD, NOZ and OCUG-1) and found that overexpression of each *ERBB3* or *ERBB2* mutant resulted in a significant increase in proliferation in at least one cell line compared with mock transfection or expression of wild-type *ERBB3* or *ERBB2*. To further confirm these findings, *ERBB3* and *ERBB2* expression were tested in a non-malignant, *ERBB2* and *ERBB3* non-expressing cell line (HEK293T). It was noted that expression of *ERBB3* alone had no effect on cell proliferation and expression of wild-type or mutant *ERBB2* resulted in only a slight increase in proliferation of HEK293T cells, whereas co-expression of either *ERBB3* mutants with wild-type *ERBB2* or *ERBB2* mutants with wild-type *ERBB3* significantly enhanced proliferative effect. On the other hand, by using RNA interference to mediate loss of function, the study also found that silencing of *EGFR*, *ERBB2* and *ERBB3* inhibited growth of four lines of GBC cells. These data demonstrate the oncogenic potential and role of ErbB alterations in GBC pathogenesis and support the rationale for further exploration of HER2 as a therapeutic target in this disease.

HER2 is an attractive target also because we now have several drugs which could inhibit HER2 for anti-cancer effect with successes seen in the treatment of breast and gastric cancer. An anti-HER2 agent, lapatinib,

when combined with gemcitabine, had a synergistic anti-proliferative effect on a GBC cell line (TGBC1-TKB) *in vitro* (18). Kiguchi *et al.* (19), by studying a transgenic mouse model of GBC, gave us *in vivo* data of the chemopreventive and therapeutic efficacy of agents targeting the EGFR and HER2 pathway in this disease. Javle *et al.* (20) did a retrospective analysis of nine GBC patients treated with HER2 directed agents either as monotherapy or in combination with chemotherapy. Eight of these patients had either *HER2* gene amplification or overexpression, of which three had stable disease, four achieved partial responses and one complete response. One patient with HER2 mutation experienced mixed response after lapatinib treatment. Thus, there appears to be some activity with HER2 inhibition even as a single agent in the treatment of GBC.

However, despite these results, there has been disappointingly little progress made clinically in targeting HER2 for the treatment of GBC. There is no clinical trial conducted to evaluate the efficacy of anti-HER2 therapy specifically in GBC. Rather, GBC is often recruited under the umbrella of biliary tract cancer in clinical trials. Ramanathan *et al.* (21) conducted a phase II study of oral lapatinib dosed at 1,500 mg per day continuously in patients with advanced biliary tree and hepatocellular cancer previously treated with no more than one line of chemotherapy. Among the 17 patients with biliary tract cancer, including 5 GBC patients, response rate was 0%, progression free survival 1.8 months and median survival 5.2 months. Another trial attempted to evaluate the activity of trastuzumab as a single agent in HER2 positive advanced GBC and cholangiocarcinoma. This trial was closed prematurely after screening 53 patients with only four being enrolled onto study and three of these patients developed progressive disease while on treatment (<https://clinicaltrials.gov/show/NCT00478140>).

In face of limited and negative early clinical trial results thus far, one may wonder if HER2 is indeed a therapeutic target worth pursuing for GBC, but importantly we also need to explore and understand the possible reasons behind these failures. Firstly, the low prevalence rate of GBC in general makes it difficult to accrue patients onto clinical trial of adequately powered sample size. Furthermore, when the target population with HER2 overexpression may consist of only about 12% of all GBC (6), accrual onto trial for targeted therapy becomes even more challenging. The strategy used to overcome this by recruiting and studying patients under the umbrella of biliary tract cancers is not

ideal given the different disease biology between GBC and cholangiocarcinoma. Secondly, as mentioned earlier and similar to observations made in breast and gastric cancer trials, defining what is a clinically significant HER2 positive cut-off is crucial and may make a difference in anti-HER2 therapeutic outcomes but this cut-off in the setting of GBC is still unclear. Thirdly, single agent anti-HER2 therapy may have limited activity in GBC and further studies should examine its combination either with chemotherapy or other targeted agents.

In conclusion, the treatment of GBC is an area of unmet need and this tumour is often regarded as an orphan cancer. While there is rationale to further explore the use of anti-HER2 therapy in GBC, this will be difficult to do without collaborative efforts. The key challenges to making progress with HER2 therapy in GBC lie in designing better trials that require smaller numbers, selecting the right group of GBC patients and conducting these trials at multiple sites in countries with a higher prevalence of GBC.

Besides HER2, there have been a variety of other therapeutic targets which are currently also being intensively studied and investigated in a series of clinical trials (22). Genome wide analysis and scientific research to further our understanding of GBC pathogenesis together with a strong collaboration between scientists and clinicians from different centres to collectively evaluate drugs in clinical trials for this uncommon disease will likely help us to systematically establish more novel therapeutic targets for this disease in the future.

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