Editorial

The genetic heterogeneity of hepatocellular carcinoma and the implications for personalised medicine

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Liver cancer is one from the most lethal cancers, ranking second only to lung cancer in terms of its global mortality (1). Hepatocellular carcinoma (HCC) comprises about 90% of primary liver cancer, with high prevalence in association with hepatitis B virus (HBV) infection or chronic inflammation and cirrhosis attributed to hepatitis C virus (HCV), alcohol excess or obesity related non-alcoholic fatty liver disease. Although there are potentially curative surgical treatments available for those presenting with earlier stage disease (2-4), recurrence is common post resection and liver transplantation is limited by organ availability. Furthermore, the majority of patients have intermediate or advanced stage disease at diagnosis (2-5). For these individuals, locoregional therapies are considered where liver function and performance status are good. For those who progress, or who have more advanced disease at presentation, sorafenib was accepted as the standard of care following landmark trials published in 2008 (6) and 2009 (7). While sorafenib treatment was associated with only a modest median survival benefit of 2.5 months, the hope was that it heralded the onset of a host of novel agents that would offer additional benefit. Unfortunately, despite major investment in phase II and phase III trials over the last decade, predominantly assessing therapies targeting kinases and angiogenic pathways (sunitinib, brivanib, linifanib, everolimus and erlotinib), none has—as yet—shown any survival benefit. The reasons for these failures in comparison to the success of sorafenib have been reviewed (8). Sorafenib is a multikinase inhibitor, rather than targeting one specific pathway, which may be one reason. It was better tolerated than some, with less severe and manageable toxicity. In addition has come the appreciation that 'personalised medicine', especially if using therapeutic agents that target a particular signalling pathway specifically, should prospectively take into account the expression or not of the target in question. Thus a number of enrichment trials are ongoing for patients with HCC. Enrichment employs the use of a biomarker for stratification and trials are currently exploring the predictive value of biopsy derived immunohistochemical expression of C-met and glypican-3, as well as serum AFP [to stratify treatment with ramucirumab (9)] and circulating ras mutation (to stratify for the MEK inhibitor refametinib). Another recognised key aspect when considering the previous treatment failures, is the need to understand the biology of HCC initiation and progression, so that the most appropriate therapeutic targets are selected for development and the treatments are delivered most effectively.

Both the need for stratification biomarkers as well as the need to understand HCC biology have fuelled further the longstanding debate over the role of biopsy in the management of patients with HCC—there being little need to expose patients to the risks of biopsy for diagnostic purposes in the majority given the high specificity of radiology and the absence of treatments based on stratification on offer (10), versus the criticism that we will not advance treatment for this disease in line with other cancer types if we continue to practice without the benefit of tissue studies (11). Much of the heated discussions have centred on ethical issues, with less attention thus far—

owing to limited evidence—given to the actual practical value of biopsy of HCC as a stratification tool. In fact, HCC is a heterogenous tumour on multiple levels—morphological, immunohistochemical as well as genetic (12-14). While major advances have been made in the genomic classification of HCC using resection specimens, with identification of candidate driver mutations (15-19), the evidence that information provided by a single biopsy from a single tumour will facilitate effective stratification of any therapy is unproven.

A paper by Xue *et al.* (20) recently published in *Gastroenterology*, has added another level of complexity to these debates, highlighting further the real challenge tumour heterogeneity poses for the practice of personalised medicine. Outside of the HCC field, whole genome and exome sequencing of multiple tumours from the same individual has contributed to a growing understanding of the genetic selection that contributes to 'cancer evolution'. These kinds of studies have enabled researchers to identify mutations or genetic abnormalities that are ubiquitously present in every cell of every tumour in an individual person, versus those that are present only in subsets of cells in the primary tumour and/or its satellite lesions and metastases. These data are presented as phylogenetic trees, where the 'trunk' of the tree represents the ubiquitous mutations—the longer trunks showing greater degrees of homogeneity within a tumour—and the 'stems' or final 'branches' represent changes restricted to subsets of tumour(s). These phylogenetic trees help to visualise the evolution of a cancer, from its primary lesion to the development of multiple lesions. They can also portray the genetic heterogeneity of an individual person cancer, enabling researchers to appreciate just how much of a cancer with multiple lesions would be treated with a particular 'targeted' therapy. A distinct possibility is that unless a targeted anticancer therapy is directed at a driving mutation present in the 'trunk' of the tree, it would stand little chance, if given in isolation, of being curative or effective in the longer term. Targeting a 'branch' driver might be perceived as 'pruning' the cancer tree, rather than cutting it down.

The paper by Xue *et al.* (20) brings HCC phylogenetic trees to the heart of the liver cancer community for the first time. The team based at Tianjin Cancer Hospital have studied 43 HCC tumour lesions collected from 10 patients with HBV who underwent surgical resection between January 2013 and May 2014. Multiple lesions from the same individual included intrahepatic metastasis, satellite nodules and tumour thrombi. Matched control samples from the same individuals were from blood or non-cancerous liver. DNA from these tissues was analysed using exome and low depth whole genome sequencing, reporting mutations, copy number variations (CNVs) and HBV integrations. Mutations in previously described candidate HCC driver genes (*TP53*, *AXIN1*, *RB1* and *CTNNB1*) (15-17) were identified, as were mutations in a number of genes (*COL14A1*, *PLCB4* and *ACY1*) not commonly described. The team went on to assess intra-tumour heterogeneity (ITH) by calculating the percentage of ubiquitous mutations shared by all lesions in a single patient, showing that this was highly variable—ranging from 8–97%. Patients with HCC tumour size larger than 5 cm showed a greater degree of ITH.

Phylogenetic trees based on mutations, CNVs and HBV integrations were drawn for each patient. Some trees had short trunks, indicating cases which metastasized early. The patterns within the trees suggested that both intrahepatic metastases and tumour thrombi could appear as either early or late events. The team went on to annotate mutations likely to be HCC drivers based on prior literature. While these trees represent just 10 patients with HBV related multifocal HCC, and generalisations should be avoided, it was apparent that 'driver' mutations could be acquired as early or late events, often driving just one tumour branch rather than occurring in a common stem or trunk. The authors noted mutations in genes like *LAMA5* and *COL4A4* were more often detected in the branches of the trees, possibly supporting dysfunction in extracellular matrix pathways as playing a role in HCC metastatic spread. Perhaps the mutations and CNVs we should take most note of, however, are those that were commonly shared in trunks or stems, rather than those seen in individual branches. These included mutation or HBV integration in some of the usual suspects—*TP53*, *CTNBB1*, *AXIN1*, *RB1*, *MLL4*, as well as a number of recurrent CNVs that were suggested to be key in tumour evolution.

Faced with such diversity between multiple lesions, the possibility of personalised medicine for HCC appears ever more challenging. The survival benefit of sorafenib being largely a result of it being a 'multikinase' inhibitor targeting both HCC proliferative pathways as well as the extra cellular environment seems likely, with the chance of agents targeting one pathway very specifically—unless used in combination—perhaps more doubtful. The genetic changes are not typically what the therapeutic agents target, but given different pathways are likely to drive individual

lesions, choosing personalised combinations of agents may not be possible without a more global picture of individual ITH. Multiple biopsies of lesions above a particular size might be advocated in the research setting, aiming to characterise a panel of changes if not to perform whole genome sequencing, but at what risk?

While these challenges appear daunting, perhaps the prospects for our future patients are not as dismal as they at first appear. This paper by Xue *et al.* is exciting in that it provides important insights into HCC evolution and potentially points the way to more effective treatment approaches for our patients-even if as yet we have neither the tools nor the resource to deliver this. But as our understanding evolves, so do the technologies we employ. While liver tissues to define an individual ITH may never be available unless the entire tumour is surgically removed, short fragments of 'cell free' DNA are present in our circulation at significant levels (1–2,000 copies of the healthy genome per mL) all the time. Furthermore, a number of solid tumour types, including HCC, release their DNA into the circulation as 'circulating tumour' DNA (ctDNA) (21). We might expect that the quantity of ctDNA will relate to tumour burden and that this may yield prognostic information. The potential to characterise ctDNA mutations to aid diagnosis or to use in a predictive fashion is an emerging possibility, as is tracking known mutations to determine prognosis in response to therapy (22,23). Exome wide characterisation of ctDNA is also possible (24), as is the detection of CNV and epigenetic change (25). The 'liquid biopsy' concept, characterising more global tumour changes in blood at the levels of ctDNA, circulating tumour cells and microRNAs, is in its infancy, but perhaps has the potential to provide the necessary insight that will inform effective personalised medicine in the future.

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Footnote

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