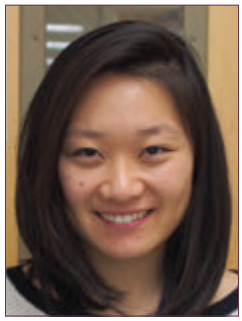
Blue Faery: The Adrienne Wilson Liver Cancer Association is proud to announce the annual Blue Faery Award (BFA) for Excellence in Liver Cancer Research. Primary liver cancer, also known as hepatocellular carcinoma (HCC), is the third leading cause of cancer deaths worldwide. Blue Faery created the award to recognise medical professionals who develop innovative research in the fight against HCC, which currently has no cure. This year the BFA the award winner is, Dr Xin W Wang, Senior Investigator, Chief, Liver Carcinogenesis Section, Deputy Chief Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, USA.

For further information on the Blue Faery Award visit: www.bluefaery.org



C Billie Bian,
Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

Xin W Wang,
Senior Investigator
Chief, Liver Carcinogenesis Section, Deputy Chief Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

Acknowledgements:
The authors disclose no conflicts. This work was supported by grants from the Intramural Research Program of the Center for Cancer Research, the National Cancer Institute.

Correspondence address:
E: xw3u@nih.gov

Targeting heterogeneity in hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the second most lethal cancer in the world among men. Given the difficulty of early detection, most patients are diagnosed at stages too late for potentially curative treatments. As a result, HCC, a clinically and biologically heterogeneous disease, is highly resistant to treatment, making it one of the most difficult malignancies to manage, the 5-year survival rate being <16% in the United States. Why has there been so little progress in finding therapeutics against this disease, despite the plethora of research occurring within fields of HCC and all other cancers? Why have so many recent clinical trials of small molecules failed [1]? Perhaps some drugs have been quite successful in a specific subpopulation of patients, but the results are overshadowed by the general (apparent) ineffectiveness. Perhaps there should be a shift in research dogma of how HCC is examined and diagnosed. Rather than assuming all HCC cases to be a single disease, we need to consider HCC as a group of related but different cancers, each with its own unique tumour biology and treatment protocol correlating to a particular phenotypic, genetic or aetiologic moiety.

The diverse aetiology of HCC, including viral infections from Hepatitis B (HBV) and C (HCV), environmental carcinogens such as aflatoxin B1, excessive alcohol consumption and obesity is among the factors contributing to a high incidence of HCC. The endemic nature of HBV and HCV in some parts of Asia has exacerbated the disease burden. These disparate origins of the disease, compounded by multiple environmental factors that both aggravate and mask disease progression, are seen in the biological and clinical heterogeneity of tumours, making early HCC detection and curative treatment both very ineffective.

Tumour resection and liver transplantation remain the only forms of targeted therapy for HCC with a curative potential, though both procedures are often restricted to patients in early stages of the disease, and postoperative recurrence is common [2]. Other locoregional therapies, such as radiofrequency ablation (RFA) and transarterial chemoembolisation (TACE), are also commonly used, but tumour relapse is usually inevitable. In systemic therapy, little progress has been made in discovering effective therapeutic targets against HCC, especially compared to the plethora of options for other carcinomas. The inherent high expression of multidrug resistance proteins (MDRP) in hepatocytes, a facet aimed at filtering and expelling toxic foreign molecules from the liver, makes HCC innately drug resistant. The only approved drug on the market, sorafenib, prolongs average survival by 2.8 months [3]. The approved use of sorafenib, a multikinase inhibitor targeting the vascular endothelial growth factor receptor (VEGFR) and Raf, is based on the success of the initial Sorafenib HCC Assessment Randomised Protocol (SHARP) trial [3]. While this landmark study offers promising hope to HCC patients, the treatment is limited to Child-Pugh class A, indicating functional liver and minimal cirrhosis. Success of sorafenib in patients of greater disease severity remains uncertain. Nevertheless, the success of SHARP — both in drug effectiveness and trial design — led to a substantial increase in phase two and three trials for small molecules similar to sorafenib. These trials imitated the completely blinded model of SHARP, randomising all eligible patients and disregarding aetiologic background, environmental factors, patient phenotype and even known tumour mutations. Nearly all subsequent trials were unsuccessful [1].

In contrast, some evidence indicates that patient subpopulations with similar tumour biology and genetic background may respond favorably to specific treatments. For example, while initial results of a phase II study with tivantinib as second-line

treatment for advanced HCC were negative, subsequent analyses indicated effectiveness in a subgroup of MET-high patients [4]. Tivantinib is an inhibitor of MET, a high affinity tyrosine kinase receptor for human growth factor (HGF); secretion by stromal cells aids tumourigenesis. Tivantinib efficacy in MET-high patients has subsequently been confirmed in an ongoing phase III METIV-HCC trial, perhaps the first successful trial using a biologically-selected cohort [5]. HCC patients with low miR-26 levels have a shorter survival time, but a better response to interferon therapy [6], indicating miR-26 as a useful biomarker to enrich patients for this treatment [7]. A recent study of a Chinese cohort treated with TACE showed statistically significant cumulative correlation between a number of known single nucleotide polymorphisms (SNPs) in the gene expressing isocitrate dehydrogenase (IDH) and decreased overall survival. IDH, an enzyme of the citric acid cycle, greatly impacts tumour metabolism when differentially expressed [8]. These encouraging results emphasise the importance that we become cognisant of the heterogenic background of the patient population and their respective tumours. Studies should be designed in a manner so that such heterogenic differences can be analysed and used to improve diagnosis and treatment.

As well as inter-tumour heterogeneity, much evidence already exists regarding intra-tumour genetic heterogeneity, the most pivotal involving repeated observation of mutually exclusive nucleotide level heterogeneity in different regions of the same tumour, regardless of tumour type [9]. This revelation counters the current practice of single point biopsies, suggesting an incomplete and biased examination of the tumour genome. This discovery also seemingly counters the accepted clonal theory of cancer biology, modeled on the assumption that all cancer cells originate from a single ancestor, with each generation adaptively acquiring additional mutations. This intra-tumour heterogeneity poses additional challenges in accurately defining druggable targets in HCC. A potential solution to these challenges is to implement mandatory guidelines to bank high quality tumour specimens in clinical trials, allowing better understanding of their individual tumour biology and more precise selection of effective treatment. New methods are needed to assess more accurately molecular changes in formadehyde-fixed paraffin-embedded (FFPE) tumours that are easily stored and transported, compared to flash-frozen tumour specimens. But, increased efforts are also needed to comprehensively gather flash-frozen samples, which fully preserve nucleotide integrity. Another important approach is to study the genome of circulating tumour cells (CTC) and circulating tumour DNA (ctDNA), suspected of being shedded from primary tumours. CTCs, the assumed culprit of metastasis, can now be easily enriched and isolated. Because CTCs are associated with metastasis and tumour relapse following curative treatments, it is imperative to understand the biology of CTCs. Recent technological developments will allow for genetic examination of tumours without the need for invasive tissue sampling. More importantly, such tumours can be examined at the single-cell level, which has not been developed to its full potential in cancer biology, especially in a paraclinical setting.

Clinically, interrogating CTCs and ctDNA can be used to monitor tumour progression and phenotype; any genetic changes in the primary or metastatic tumour – including mutations that may predict resistance or susceptibility to certain chemotherapeutics – would be known, information that might be vital for the attending physician. Yet despite these promising avenues, CTCs and ctDNA may themselves present inherent bias, as the collected information is completely dependent on what is detectable (and is capable of being detected). The potential inherent bias also induced by the hospitality — or lack thereof — of the peripheral blood must also be taken into account. It is possible that any detected ctDNA may in fact be biased towards those cells that are easily engulfed by the host immune system. It is also possible that the genetic mutations in singularly detected CTCs may actually be mutations that are unfavorable to metastasis. Yet even with these persistent questions, these possibilities need to be probed in light of the inevitable microscopic and macroscopic heterogeneity of tumours.

HCC seems to be a small field of lower profile, yet the disease is ironically among the most fatal of diagnoses, accompanied by one of the lowest survival rates. Progress in the field is limited in part to the lack of available biopsy samples to allow active monitoring of disease progression. Small sample sizes, especially when coming from diverse origins, have often led to pooling of samples, which can potentially mask distinct aetiologically-based tumour characteristics. Priority needs to be placed on recognising aetiologic, phenotypic and genetic differences amongst HCC cases, and the need for targeted/individualised (customised) treatment. The rapid pace of clinical trial development and growth that lacks a biomarker-enriched patient selection strategy can negatively impact the overall goal of finding curative drugs against HCC; promising drugs that can only target a subpopulation of HCC patients can potentially be halted indefinitely due to poor results seen in analysis of larger heterogenetic HCC populations. Recent technological developments will help direct attention towards macroscopic, and especially microscopic, heterogeneity of tumours — primary, metastatic and circulatory.

Medicine is evolving towards a future of precision and customised medicine. Perhaps that is the key to curing cancer – not just HCC – in the future, i.e. recognising that all cancers are heterogenic and requiring unique treatment. This recognition, combined with the invaluable information available through retrospective analysis of clinical trials and the rapidly growing health technology industry, might lead to exponential increase in the field of HCC research in the future.

REFERENCES

1. Llovet JM, Hernandez-Gea V. *Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design.* Clin Cancer Res 2014;20:2072-9.
2. Zhou XD, Tang ZY, Yang BH, Lin ZY, Ma ZC, Ye SL, Wu ZQ, et al. *Experience of 1000 patients who underwent hepatectomy for small hepatocellular carcinoma.* Cancer 2001;91:1479-86.
3. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, et al. *Sorafenib in advanced hepatocellular carcinoma.* N.Engl.J.Med. 2008;359:378-90.
4. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van VH, et al. *Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study.* Lancet Oncol. 2013;14:55-63.
5. Rimassa L, Porta C, Borbath I, Daniele B, Finn RS, Raoul JL, Schwartz LH, et al. *Tivantinib in MET-high hepatocellular carcinoma patients and the ongoing Phase III clinical trial.* Hepat Oncol. 2014;1:181-8.
6. Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, et al. *MicroRNA expression, survival, and response to interferon in liver cancer.* N.Engl.J.Med. 2009;361:1437-47.
7. Ji J, Yu L, Yu Z, Forgues M, Uenishi T, Kubo S, Wakasa K, et al. *Development of a miR-26 companion diagnostic test for adjuvant interferon-alpha therapy in hepatocellular carcino ma.* Int.J.Biol.Sci. 2013;9:303-12.
8. Zhang H, Guo X, Dai J, Wu Y, Ge N, Yang Y, Ji J, et al. *Genetic variations in IDH gene as prognosis predictors in TACE-treated hepatocellular carcinoma patients.* Med Oncol 2014;31:278.
9. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, et al. *Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.* N.Engl.J.Med. 2012;366:883-92.