Liquid Biopsy for HCC Management: How Close Are We?

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Value of biomarkers in prognosis and prediction of response in HCC

The National Cancer Institute defines a biomarker as a biological molecule-from blood, tissue or other fluids - able to identify a normal or abnormal process, disease or able to define response of humans to a given therapy[1]. In HCC, there are few established biomarkers. Among those, plasma levels of alfa-fetoproteín (AFP) is universally recognized as a prognostic biomarker for survival (cut-off of AFP > 200 ng/ml or >400 ng/ml) according to EASL guidelines[2]. High plasma AFP levels are associated with poor outcome at early stages of the disease and at advanced stages of the disease. In fact, in the later stages patients with AFP > 200-400 ng/ml have shown poor prognosis in the setting of phase III trials[3 6], with median survival of 5 months[6]. In parallel, genomic studies have defined molecular signatures from adjacent tissue,[7] or HCC tissue[8 9] able to predict outcome along known clinical variables.

In solid tumors, several biomarkers have been acknowledged to identify responders to molecular therapies, and more than 35 indications are currently approved in oncology for drugs based on biomarker selection[10]. Such is the case for instance for ALK fusions in NSCLC responding to crizotinib. Unfortunately, in HCC they do not have drivers as biomarkers of response because either they are not actionable (TERT, CTNNB1, TP53) or the studies have not yet provided high- level evidence (FGF19, IGF2, VEGFA, TSC1/TSC2) [11 12].

Five drugs are currently accepted in terms of providing survival advantage in advanced HCC based on phase III data: sorafenib and lenvatinib in front line and regorafenib, cabozantinib and ramucirumab in second line[11]. While no biomarkers predictors of response to sorafenib have been identified [13], recent studies have shown liquid biopsy biomarkers associated to better survival in patients treated with regorafenib[14]. Similarly, plasma AFP levels >400 ng/ml define responders to ramucirumab in patients previously exposed to sorafenib[6]. This study is certainly the first biomarker/driven positive phase III in HCC therapeutics. While checkpoint inhibitors have shown positive signals of efficacy (objective response 14-18%), no biomarker is known to predict such responses in HCC [15]. This would be very relevant in light of the recent negative RCT comparing permbrolizumab vs placebo in second-line HCC.

Liquid biopsy in cancer

Liquid biopsy enables non-invasive analysis of tumor molecular alterations through isolation of tumor components that are released to the bloodstream or other body fluids, including circulating tumor DNA (ctDNA) or RNA, circulating tumor cells (CTC), and exosomes[16 18]. Almost all biomarkers approved by regulatory agencies defining prognosis or prediction of response are based using surgical or biopsy tissue samples10. Nonetheless, these samples might be subject of sample bias (in case of heterogeneity particularly in the context of acquired resistance) and can not be obtained repeatedly. In solid tumors, genomic profiles of ctDNA have been shown to recapitulate those of corresponding tumors in 80-90% of cases, particularly for key driver genes[19]. Nowadays, several studies have shown the utility of liquid biopsy in three main areas a) monitor response to treatments, b) assess emergence of drug resistance[16 18]. Next generation sequencing and drop-let digital PCR allow to identify and quantify alterations present at al-

lele frequencies of 0.01%[20] opening the path to use these techniques for surveillance strategies[21]. Two different liquid biopsy companion diagnostic tests for EGFR mutations in plasma ctDNA have been approved by regulatory agencies to guide precision oncology therapies in NSCLC[22]. Finally, c) CTC and ctDNA been proposed to quantify minimal residual disease[18]. For instance, post-operative detection of ctDNA correlated with recurrence irrespective of adjuvant chemotherapy and remained an independent predictor of recurrence-free survival after adjustment for established risk factors.

Liquid biopsy in HCC

The EASL guidelines identified as an unmet need in HCC research the development of new tools for early detection or prediction of response or resistance to systemic therapeutics by liquid biopsy[2]. Besides allowing the quantitation of ctDNA, NGS enables identifying specific genomic alterations, which expands its applications to predictive biomarkers, monitoring of tumor clonal composition and identifying mechanisms of treatment resistance to systemic therapies. This is particularly interesting considering the difficulties to access tumor tissue in HCC patients, and the inherited constraints of sequential tissue biopsies. In the early HCC stages, no such study with liquid biopsy has been conducted in HCC, but a recent study using several blood testing such as ctDNA and tumor protein markers, in several cancer types (including 39 HCC cases), showed that this approach is promising in early detection[21].

The current role of liquid biopsy in the management of HCC is still far beyond other solid tumors. Mutation detection by ultra-deep targeted ctDNA sequencing in early stage HCC along with correlation of corresponding multiregional tissue samples is feasible by using a panel of 58 frequently mutated and/or actionable genes in HCC, where a 70% correlation with tissue mutations was identified[23]. In the clinical setting few studies have used liquid biopsy to define molecular aberration. The most prominent study assessed liquid biopsy by using BEAMing technique to define KRAS status in HCC, which was positive in around 4% of cases 24. Additional validation of these seminal studies in the setting of therapeutic management is needed, considering that targeted sequencing in advanced HCC has identified 25% actionable events[11] [12]. One of such cases might be biomarkers predicting response or primary resistance to checkpoint inhibitors in HCC, which so far are unknown[15]. The identification of the immune excluded HCC class (cold tumors) was previously characterized by CTNNB1 mutations[25] and these tumors are those expected to be resistant to immunotherapy[26]. Since CTNNB1 mutations can be identified in blood this could represent a read-out for resistant cases. Effective systemic agents in HCC are increasing (i.e., sorafenib, lenvatinib, regorafenib, cabozantinib, ramucirumab), and it will be paramount to maximize treatment response and implement precision oncology.

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