Precision oncology in liver cancer

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Abstract: With the widespread adoption of molecular profiling in clinical oncology practice, many physicians are faced with making therapeutic decisions based upon isolated genomic alterations. For example, epidermal growth factor receptor tyrosine kinase inhibitors (TKIs) are effective in EGFR-mutant non-small cell lung cancers (NSCLC) while anti-EGFR monoclonal antibodies are ineffective in Ras-mutant colorectal cancers. The matching of mutations with drugs aimed at their respective gene products represents the current state of "precision" oncology. Despite the great expectations of this approach, only a fraction of cancers responds to 'targeted' interventions, and many early responders will ultimately develop resistance to these agents. The underwhelming success of mutation-driven therapies across all cancer types is not due to an inability to detect genetic changes in tumors; rather a deficit in functional insight into the genomic alterations that give rise to each cancer. The Achilles heel of precision oncology thus remains the lack of a robust functional understanding of an individual cancer genome that then allows prediction of the best therapy and resultant outcome for that patient. Current practice focuses on one 'actionable' mutation at a time, while solid cancers typically possess many mutations that involve different cellular sub-populations within a tumor. No method or platform currently exists to guide the interpretation of these complex data, nor to accurately predict response to treatment. This problem is particularly germane to primary liver cancers (PLC), for which only a handful of targeted therapies have been introduced. Here, we will review strategies aimed at overcoming some of these challenges in precision oncology, using liver cancer as an example.

Keywords: Hepatocellular carcinoma (HCC); intrahepatic cholangiocarcinoma (ICC); fibrolamellar cancer; organotypic culture; immunotherapy; molecular profile; kinase inhibitors

Introduction

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two most common forms of primary liver cancer (PLC); together, they are the third most common cause of cancer-related death worldwide. According to the Global Burden of Disease Study, in 2015 there were over 850,000 incident cases of liver cancer globally, and over 800,000 deaths (1). While the age-standardized mortality attributable to liver cancer has

significantly decreased over the past several decades in many high-prevalence regions including Asia, Africa and South America, Western countries such as the United States and Canada have experienced increases. Chronic liver disease predisposes patients to develop HCC; almost 85% of cases result from arguably preventable causes such as alcohol and viral hepatitis. We thus expect that with improvements in health education, practice, policy and prevention, the global burden of liver cancer driven by these risk factors may continue to reduce. For example, anti-viral treatments

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have become highly effective and widely available. Direct antiviral agents (DAA) against HCC have made it possible for the majority of chronically-infected patients to clear the virus, with subsequent stabilization or improvement in liver health (2,3). Unfortunately, some studies have reported that the eradication of HCV by DAA may promote new or recurrent HCC (4-7). While this finding has not been borne out in larger studies, the debate continues regarding the early impact of DAA on HCC (8-11). Nonetheless, it is prudent to continue to monitor and surveil HCV-treated livers for the development of PLC until longer term studies can provide better evidence to establish guidelines for more accurate and efficient screening of the HCV population.

In contrast to the decreasing incidence of HCV-induced HCCs, tumors driven by non-alcoholic fatty liver disease (NAFLD), which is associated with the metabolic syndrome, are becoming more prevalent. As the obesity epidemic continues to climb worldwide, a much larger population will be at risk for developing liver cancer (12,13), thus new strategies are urgently needed to address this formidable epidemic (14).

Unlike most other solid tumors, PLCs respond poorly to conventional chemotherapy such that no agents are currently FDA-approved for the treatment of advanced HCC; rather several tyrosine kinase inhibitors (TKIs) have demonstrated efficacy in patients with preserved liver function (15). Both sorafenib and regorafenib are non-specific kinase inhibitors that are currently used in HCC, and their clinical benefits extend survival by a mere 2–3 months. The efficacy of these drugs suggests that aberrant kinase activities are relevant in HCC, but yet their poor response rates highlight a significant deficiency in our understanding of the role of specific kinases and other potential therapeutic targets in HCC.

Tumor heterogeneity & limitations of molecular profiling

Widespread application of next-generation sequencing (NGS) has led to detailed molecular characterization of HCC and ICC (16-21). These studies confirm that HCC and ICC are genetically distinct, with recurrent mutations in KRAS, IDH1/2, FGFR, EGFR-ERBB in ICC, as compared to Wnt-βcatenin, TERT, JAK-STAT, and PI3K-AKT-mTORC1 mutations in HCC. Despite these frequent mutations, each tumor contains many more somatic mutations (up to hundreds) that may contribute to its pathogenesis. Therefore, each cancer bears a unique

genetic signature, which continues to evolve as the cancer progresses and gives rise to heterogeneity within that tumor. Such complexity poses a major roadblock given our current approach to precision oncology, which focuses on one target at a time.

'Druggable' targets in ICC include IDH1/2, FGFR2/4, EGFR/ERBB2 and PIK3CA, but studies of molecularly targeted agents in these patients had mixed results. A preliminary 'basket' trial evaluating compounds targeting FGFR, IDH1, MET and MEK in biliary tract cancers reported that only 4 out of 13 patients responded to treatment (22). In a sub-analysis of ICCs included in the MOSCATO-01 trial, 23 out of 34 patients undergoing molecular profiling were found to have a potentially 'actionable' mutation; among them, 18 received targeted therapies, with an overall response rate of 33% (23). Other clinical trials reported similarly inconclusive benefits based on molecular findings (24), supporting the notion that we are far from effective precision oncology for patients with ICC.

A large number of kinase inhibitors have been used in patients with advanced HCC; only a few agents have demonstrated efficacy: sorafenib, levatinib, and regorafenib. Interestingly all of these agents are relatively non-specific in terms of their kinase targets, and many of these targets are involved in angiogenesis (i.e., VEGFR1, VEGFR2, VEGFR3, PDGFR, RET). Therefore, anti-angiogenesis may represent an important means of HCC stabilization, but these agents may not be directly inducing cytotoxicity of the cancer cells. Currently, sorafenib remains first-line systemic treatment for advanced HCC, but only has an objective response rate of 2–3%. In a phase III non-inferiority trial, lenvatinib lead to a clinically meaningful improvement in outcome compared with sorafenib with a higher objective response rate of 24% (25); it is expected to be approved as an alternative first-line therapy in 2018. On the other hand, regorafenib, which is structurally similar to sorafenib, has been approved as a second-line option in patients who tolerated but progressed on sorafenib. In this subset of patients, regorafenib achieved an objective response in 11%, leading to a median overall survival of 10.6 months (26).

In contrast to the promiscuous TKIs discussed above, agents with more restricted substrate specificity, such as rapamycin analogs or FGFR and c-MET inhibitors, have not been shown to impact survival as first-line therapy for patients with advanced HCC. This lack of an effect is particularly intriguing given the frequency with which

these pathways are affected in HCC based on molecular studies. Further, there are no specific predictors of response to any of the FDA-approved TKIs. The modest success of kinase inhibitors in HCC highlights the critical knowledge gap in our understanding of the molecular pathogenesis of HCC, and our inability to translate the knowledge that we do have to the bedside. Some specific challenges that impede the success of targeted therapies include (I) tumor heterogeneity, (II) a lack of predictive biomarkers, (III) the inability to distinguish 'driver' from 'passenger' mutations, (IV) involvement of multiple signaling pathways in carcinogenesis, and (V) the development of drug resistance. In this review, we discuss a number of approaches that address these hurdles, with the goal of improving our delivery of precision oncology to patients with PLC.

HCC is usually the end-product of chronic liver injury of various etiologies (viral, metabolic, alcohol) and comes in many different flavors of disease, each with a unique molecular profile and cancer biology (21). These genetic changes have been categorized into 'baskets' involving ~10 pathways with only a handful of these being currently 'actionable'. In addition, extensive cross-talk exists between these pathways, such that upregulation of one pathway can compensate for drug-induced inhibition of another pathway to maintain cellular functions. It can be argued that our current state of knowledge does not account for the complex biology that takes place within each tumor. New methods are needed to understand the sum total of the underlying molecular alterations found in individual cancer. Precision medicine holds great promise as we strive to treat disease based on genetic variation, but to do so we must bridge the gaps between genetic information, biology, and clinical medicine.

General approaches to precision oncology

Since cancer is the result of genetic and epigenetic mishaps, it is reasonable to suggest that the first step in achieving success in precision oncology is to define the genomic alterations of individual tumors. In fact, obtaining this type of data is now common and widespread in clinical practice. From the provider's perspective, however, molecular driven therapies have often failed to achieve the desired clinical outcome. We now know that targeting the same mutation in different tumors does not lead to consistent response. This phenomenon was demonstrated in a study using vemurafenib to treat a variety of non-melanoma cancers that shared the same mutation (BRAF V600E), and the

response rates varied greatly between tumor types, arguing that this BRAF mutation has varying functional significance in different cancers (27). Understanding the relationship between specific oncogenic mutations, tumor histology, and patient history and outcome is of paramount importance in predicting and designing tailored therapies with the aim of inducing clinically-meaningful responses.

Successes of 'targeted' therapy for solid cancers to date include imatinib for gastrointestinal stromal tumors, trastuzumab for HER2-positive breast and gastric cancers, and EGFR inhibitors for subset of NSCLCs, all of which have been validated in large phase III randomized studies. Outside of the context of clinical trials, however, precision oncology has yet to produce the anticipated widespread improvements in patient outcome. To date, few randomized trials have compared molecularly-informed treatments with standard selection by providers. The SHIVA trial was designed to match a panel of well-known cancer mutations with 10 FDA-approved drugs (28). Eligible patients (n=293) with 'actionable' mutations were randomly assigned to molecularly-targeted drugs or treatment at their physician's choice. At a median follow-up of 11.3 months, the median progression-free survival was 2.3 months for the experimental (molecular-targeted) group and 2.0 months for the control group (P=0.41). While this trail has many limitations, it highlights the need for additional well-designed, prospective studies that consider both histology and genomic profile. Examples of 'basket' trials that are ongoing include the NCI-sponsored MATCH (NCT02465060) and MPACT (NCT01827384) studies, the ASCO-sponsored TAPUR (NCT02693535) registration study, and a number of European and Asian studies. These trials should provide much needed insight into the relationship between tumor response and molecular profile. In PLCs, however, inhibition of the 'actionable' pathways that are commonly mutated (PI3K-AKT-mTOR, FGF, c-MET) have already been tested in clinical studies; none have demonstrated significant clinical benefit as first-line therapy (29). Moving beyond 'basket' trials, new strategies are needed to improve the outcome of patients with advanced HCC. Here, we discuss three potential approaches to address the barriers noted above.

Targeting a specific HCC subtype

Though no two cancers are exactly alike, experts agree that common driver mutations exist that are shared among

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tumors, allowing molecular subclassification. It follows that if one can fully delineate molecular pathogenesis of each subclass of HCC, we could design therapies for individual cancer type. One example of a distinct, yet homogenous subgroup of HCC is fibrolamellar HCC (FLC). FLC is unusual in that it occurs in children and young adults without a history of underlying liver disease. Genomic analyses have identified a common mutation in these rare cancers that involves an in-frame deletion of ~400 kb on chromosome 19, resulting in a novel fusion protein, DNAJ-PKAc (30). The mutant gene product replaces the first exon of the catalytic subunit of protein kinase A (PKAc) with the N-terminus of a heat shock protein, DNAJ (a.k.a. HSP40). No other recurrent genomic changes have been found in FLCs, and the overall mutation burden in these cancers is low, thus the modified PKAc appears to be the dominant oncogenic driver of these cancers (31-33). In fact, recent studies have shown that the expression of DNAJ-PKAc is necessary and sufficient to induce liver tumorigenesis in mice, but the mechanism behind this transformation is unknown (34,35).

Patients with FLC typically present late with bulky disease; up to 70% have lymph node involvement at the time of diagnosis, leading to a poor overall 5-year survival rate of ~35% in these otherwise healthy young people (36,37). The disease has a distinct histology defined by large polygonal cells with eosinophilic cytoplasm and prominent nucleoli that grow in clusters surrounded by lamella of fibrous stroma, hence the name fibrolamellar (38). Unlike 'classic' forms of HCC, FLC often metastasizes to lymph nodes, and can be associated with non-hepatic encephalopathy in its advanced stages. Further, they do not respond to conventional chemotherapies or targeted therapies such as sorafenib. Surgical resection is the only effective therapy for FLC, but is not curative once disease has spread outside the liver.

Intuitively, one would assume that a mutation involving a kinase that causes cancer would involve constitutive activation of its kinase activity to drive cellular transformation, as is the case for ABL, EGFR, BRAF, and SRC-mutated cancers. However, biochemical studies of DNAJ-PKAc show that the fusion kinase has similar basal kinase activity as the wild-type protein and remains under the regulation of its R subunits in a cAMP-dependent manner (39,40). In FLCs, the fusion kinase is overexpressed compared to wild-type PKAc due to higher basal promoter activity of *DNAJB1*, which leads to increased kinase activity in response to cAMP. In mice, over-expression of *Dnajb1-*

Prkaca in the liver, but not a kinase-dead mutant, causes tumor formation (35). Little is presently known about the events downstream of DNAJ-PKAc, and there is no evidence that inhibition of global PKAc activity is safe or of therapeutic value. Due to the ubiquitous function of PKA, non-selective inhibition would likely lead to severe cellular and organ dysfunction, hence an alternative approach will be necessary for these patients. In preliminary studies using a novel cell model of FLC, we identified an important function of the heat-shock protein (HSP) scaffold in regulating growth-factor associated AKAP-PKAc signaling and chemoresistance (manuscript in preparation). We are currently exploring the utility of blocking HSP function to expose specific vulnerabilities of FLC cells to kinase inhibition. These and other studies that address the molecular biology and pharmacology of FLC will provide critical insights to finding effective therapies for this unique PLC.

Functional validation in genomic medicine

The majority of PLCs do not fall into genetically homogenous subtypes like FLC; rather they share many overlapping mutations across multiple pathways. It is impractical to study each tumor in extensive detail in order to understand the biologic nuances of their complex genomic disarray. An alternative strategy is to use a functional assay to test and validate targets that are identified through molecular profiling of individual cancers. Determining the relative efficacy of different compounds or combinations is particularly important when genomic profiling highlights more than 1 potential drug target, or when multiple drugs are available for a given target. *Table 1* lists common methods used to query the biologic impact of genetic or pharmacologic manipulations in human cancers. While no one approach is perfect, each technique has advantages that can be exploited to investigate tumor biology and response to treatment. Recent advances allow propagation of human cancers either *in vitro* or in surrogate hosts. For example, 'conditional reprogramming', which entails co-culture of tumor cells with irradiated fibroblasts and a Rho kinase inhibitor greatly enhances the ability of primary human cells to be maintained *in vitro* (41). This technique has led to the development of many human cancer cell lines, as outlined in the Cancer Cell Line Encyclopedia. While these cell lines represent a valuable resource for large-scale screening, it is unclear whether these cells are representative of the diversity of cancer cells

Table 1 Comparison of human-derived cancer models

Method	Success rate	Renewable	Micro-environment	Immune component	Time to establish	Cost
1° Cell line (conditional re-programming)	High	Yes	No	No	Months	Medium
Organoid	High	Yes	No	No	Days–weeks	Medium
PDX mice	Low	Yes	Yes	No*	Months	High
Organotypic culture	High	No	Yes	Yes	Days–weeks	Low

*, Humanized models reconstitute specific components of the human immune system.

within one tumor following a period of *in vitro* selection. As such, resultant clones may not represent the heterogeneity that exists within the primary cancer (42).

3D-organoid cultures have recently attracted a great deal of attention given their ease of propagation and close resemblance of the physiological niche found *in vivo*. Refined protocols have improved the efficiency and speed of generating primary tumor spheroids, which can then be used for molecular analyses and drug screening. Broutier *et al.* describe a novel system of long-term *in vitro* propagation of PLCs that preserves their histologic architecture, gene expression, and genomic alterations (43). Using these organoids in drug screens, they identified ERK as a potential therapeutic target in HCC, but did not specify the specific patient subtype for which these agents could be effective (43). Pauli *et al.* reported the use of a similar approach to identify effective drugs for individual cancers (44); the predictive value of this method in clinical care remains to be proven in prospective studies.

A major drawback of 2D and 3D cultures is the absence of the native tumor microenvironment, which is known to play a critical role in tumor development and response to therapy. This deficit is particularly relevant in ICCs, in which a desmoplastic stromal reaction is often seen adjacent to tumor cells. In fact, the phenotype of cancer-associated fibroblasts has been associated with more advanced stage and poorer 5-year survival in these patients (45). Patient-derived xenografts (PDX) in immuno-deficient mice were created in an attempt to overcome this shortcoming of purely *in vitro* systems. Recent evidence suggests that the murine stroma adopts a metabolic phenotype that resembles the human cancer microenvironment (46), highlighting the importance of tumor-stromal interaction. While engraftment efficiency varies among tumor types, once established, PDX models recapitulate many features of primary human cancers including histology, gene expression, and drug response. A major limitation of the use of PDX models in clinical precision oncology is the long lag period necessary for engraftment and passaging, rendering this approach impractical for individuals seeking tailored cancer treatment. Secondary concerns about PDX models include selection bias in favor of more advanced tumors with high-grade features (47,48), variable engraftment efficiencies (49), and the high costs of PDX development, maintenance, and testing. In our experience, both HCCs and ICCs engraft subcutaneously in NSG mice, albeit at lower efficiency than colorectal cancers (unpublished data). At present, PLC PDX models are not readily available from commercial sources such as JAX or the Living Tumor Laboratory, therefore access is limited to a few investigators.

Organotypic slice cultures were originally described decades ago in neurophysiology studies (50,51), but have recently gained popularity in the study of human solid tumors. Tumor slice cultures (TSC) have multiple advantages, including methodologic simplicity and preservation of the tumor architecture. It has been used to assess relative drug sensitivity (52). Additional benefits of a standardized TSC platform are a high success rate, minimal lag period, and low cost. In our experience of >150 cases, viable TSCs have been generated from ~90% of tumors, and can be maintained *in vitro* for weeks, allowing for extensive experimental manipulation. *Figure 1* demonstrates PLC TSCs that show variable responses to drug treatments. Since there is no *in vitro* selection, TSCs maintain tumor heterogeneity including the immune environment that is intrinsic to the human disease (53). Despite the advantages listed above, TSCs are limited by their finite lifespan without the ability for renewal.

In summary, these 'functional' assays of human cancers fill an important gap in our ability to provide biologically relevant molecular data to guide therapy. We still struggle to make these assays readily available to patients, however, as most remain in the realm of research investigation, and none have been subjected to the rigor of a prospective
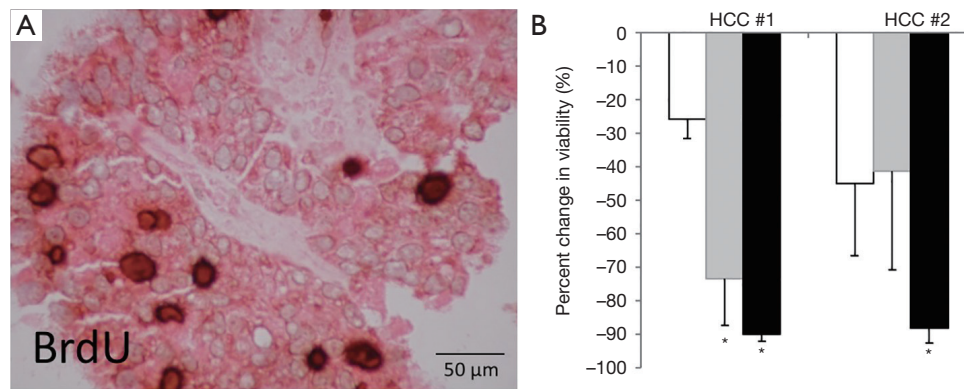
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Figure 1 Organotypic slice culture of human HCC. (A) HCC slice culture (day 12) showing intact tumor architecture with BrdU-labelled proliferating tumor cells; (B) example of two independent HCCs with differential response to sorafenib (gray). DMSO, white bars; staurosporine (10 µM), black bars. *, P<0.05 compared to DMSO. HCC, hepatocellular carcinoma; DMSO, dimethyl sulfoxide.

clinical trial. Given these limitations, significant refinements in technique, data analyses and integration will be needed to allow us to realize the full potential of precision oncology.

Immunotherapies for HCC

Predictions by scientists over half a century ago suggested that a patient's own immune system could selectively target and eliminate cancer cells from the body (54). This form of precision oncology has finally gained wide application with the use of immune checkpoint inhibitors in treating cancers such as melanoma, urothelial carcinoma, and NSCLC. Recent FDA-approval of the anti-programmed cell death protein 1 (PD1) monoclonal antibody, pembrolizumab, marks the first time a drug has been recommended based on a biomarker predictive of mutational burden irrespective of tumor histology (55). In addition to PLCs with microsatellite unstable phenotype, interest in immunotherapy for HCC has been spurred by cases of spontaneous regression in the setting of systemic inflammatory responses (56). Immune checkpoint inhibitors target molecules such as cytotoxic T-lymphocyte protein 4 (CTLA-4), PD-1, or programmed cell death ligand 1 (PD-L1). The end effect of antibody-mediated inhibition of these immune inhibitory molecules is an overall activation or reactivation of the antitumor effects of endogenous cytotoxic T cells. The liver poses unique challenges to effective immunotherapy, however, as it is generally considered be relatively immune tolerant given its exposure to a high number of antigens from the gut. For example, several liver-specific non-parenchymal cells such

as liver sinusoidal endothelial cells and Kupffer cells (KCs) release IL-10, which leads to T cell tolerance rather than immunity (57). Therefore, insight into the liver immune landscape may unveil novel therapeutic strategies, and help identify patients that may benefit the most from immune-modulation.

An additional factor to consider in the immune milieu in HCC is that the chronic inflammation from viral hepatitis or alcohol that predisposes to these cancers leads to an overall environment of self-tolerance within tumor and surrounding liver. In analyses of the immune tumor microenvironment in HCC, an immunosuppressive phenotype has been demonstrated by high expression of IL-10 and low expression of inflammatory molecules such as granzyme B (58), as well as an increased clonal population of infiltrating regulatory and exhausted CD8 T cells (59). Several studies have shown higher levels of Th2-like cytokines, such as IL-4, IL-8, IL-10, and IL-15, in HCCs that are more aggressive or metastatic (60), and elevated serum IL-10 levels have been associated with poorer prognosis after resection (61). By contrast, longer patient survival correlates with higher densities of infiltrating CD3^{+} and CD8^{+} cells in tumors (62), and those in which tumor infiltrating lymphocytes express a higher level of pro-inflammatory genes, such as TNF, IL-6, and CCL2 (63). While CD8^{+} T cells recognizing tumor-associated antigens are found in the peripheral blood of patients with HCC, their number and ability to produce IFNγ has been shown to be reduced within the tumor itself, which also contains regulatory T cells (64). The presence of greater numbers of regulatory T cells also correlates with higher TNM stage

and poorer survival (65,66). In addition to high levels of immunosuppressive cytokines and cell types, several studies have demonstrated the presence of immune checkpoint inhibitors and their effects on clinical outcome in HCC. CD8+ T cells expressing PD-1 are present within HCCs, and the number of PD-1 positive CD8+ cells correlates with disease progression and post-operative; analogous findings have been shown for high PD-L1 expressing tumors (67).

Several strategies aimed at overcoming immune tolerance in the liver to enhance the immune response to HCC have been evaluated in clinical trials. Alpha-fetoprotein (AFP) was initially investigated as a potential antigen for vaccine therapy, but early results were unimpressive and the few partial responses were short (68). A vaccine against peptide components of glypican-3 (GPC3), a proteoglycan that is frequently overexpressed on the cell membrane in HCC, was found to be safe in a phase I trial and increased CD8+ T cell infiltration in tumors in most patients. Overall survival was longer in patients who developed increased GPC3 specific T cells, but only 1 of 33 patients exhibited an objective tumor response (69). A vaccine against the telomerase-derived peptide GV1001 did lead to any anti-tumor effects (70). Several small prospective studies have evaluated dendritic cell (rather than peptide) based vaccines; while these vaccines appear to be safe and can generate immune responses, no objective tumor responses have been observed in most studies (67).

Adoptive cellular therapy (ACT) is an alternative immunotherapeutic approach involving the removal of T cells from a patient and manipulating them *in vitro* prior to returning them to the patient. Cytokine induced killer (CIK) therapy involves isolating peripheral blood mononuclear cells (PBMCs) from a patient and activating the autologous lymphocytes with IL-2; this technique has been studied in Asia with several prospective and retrospective studies. Two randomized controlled trials showed improvement in recurrence-free survival, but no difference in overall survival when adjuvant CIK was following HCC resection (71,72). A third randomized controlled trial did demonstrate an overall survival benefit of CIK when given as adjuvant treatment in conjunction with radiofrequency ablation or transarterial chemoembolization as local control for patients who were not surgical candidates (73). Other immunomodulatory strategies are further from clinical application but are potentially promising. Chimeric antigen receptor (CAR) T cells are engineered to express specific T cell receptors that recognize pre-defined tumor antigens, and when given back to the patient should elicit an effective anti-tumor response. CAR T cells against GPC3 have shown promise in their ability to reduce HCC tumor volume in mouse models (74), but have not yet been used in human PLCs.

On September 22, 2017 the FDA granted accelerated approval to nivolumab, an anti-PD-1 monoclonal antibody, for HCC patients who failed first line sorafenib based on the CheckMate 040 trial (75). In this phase I/II non-comparative, multicenter, open-label study of 202 HCC patients with Childs A cirrhosis, nivolumab had a manageable safety profile, 20% of patients had an objective response, and 3 patients achieved a complete response. Ongoing phase III trials are needed to confirm these findings. Tremelimumab, a blocking antibody against CTLA-4, which is highly expressed on regulatory T cells, was studied in a clinical trial including 17 patients with hepatitis C related HCCs that were not amenable to other therapy. Similar to the results seen with nivolumab, 17% of patients had an objective remission and 76% had disease control (76), providing further support for the role of immune checkpoint inhibition in HCC treatment. When tremelimumab was evaluated in combination with radiofrequency ablation or chemo-ablation, 26% of patients achieved partial responses outside of the ablation zone with the addition of CTLA-4 blockade; in those patients the number of activated CD4+ and CD8+ cells in PBMCs also increased (77). The next steps in refining our immunotherapeutic strategy for HCC include evaluation of combination therapy, such as the ongoing trial of anti-CTLA-4 and anti-PD-1 therapy, and the identification and utilization of biomarkers to predict positive responses to specific treatments. Once again, the availability of models such as the TSC may aid in the discovery of clinically effective protocols.

Summary

Parallel advances in genome science, molecularly targeted- and immuno-therapies have revolutionized our approach to treating traditionally chemoresistant cancers such as HCC. This new era of molecular medicine brings hope and precision to personalized care; however, the current state of information can be overwhelming. Complex genomic data cannot be directly translated to the bedside without analyzing them in the appropriate biologic framework and providing evidence to support their functional relevance. The strategies presented here aim to bridge the existing

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gaps between molecular profiles, immune landscape, and meaningful therapies for patients.

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Footnote

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