Advancing Personalized Treatment for Hepatocellular Carcinoma: Integrating Targeted Therapies, Precision Medicine, and Bioengineering for Improved Outcomes

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Abstract

Background: Hepatocellular carcinoma (HCC) remains a global health challenge, ranking as the fourth leading cause of cancer-related death. Despite advancements in diagnostic and therapeutic strategies, HCC is often diagnosed at advanced stages where curative treatments are limited. Systemic therapies, including targeted therapies and immunotherapy, have become essential in managing advanced HCC. Methods: This review explores the latest progress in HCC treatment, focusing on the development and integration of targeted therapies and immunotherapy into personalized treatment strategies. We analyze the molecular mechanisms of HCC, highlighting key genetic drivers and signaling pathways, and discuss the role of bioengineering models in improving drug resistance and personalized treatment approaches. Results: Targeted therapies, including tyrosine kinase inhibitors (TKIs) like sorafenib, lenvatinib, regorafenib, and cabozantinib, have shown varying degrees of success in prolonging survival. Recent studies emphasize combination therapies, including immunotherapy with anti-angiogenic agents, as promising approaches for enhancing treatment efficacy. Bioengineered patient-derived liver cancer models have emerged as valuable tools for improving the precision of these therapies by reflecting HCC's molecular and phenotypic diversity. Conclusion: The future of HCC treatment lies in the integration of targeted therapies with precision medicine, guided by molecular profiling and advanced bioengineering models. These strategies promise to improve patient outcomes by addressing tumor heterogeneity and overcoming drug resistance, with bioengineering platforms and next-generation sequencing offering critical insights into personalized treatment regimens.

Keywords: Hepatocellular carcinoma (HCC), Targeted therapy, Molecular profiling, Anti-angiogenic therapy, Bioengineering technology, Precision medicine

Significance | Targeted therapies in hepatocellular carcinoma offer personalized treatment options, improving efficacy, reducing side effects, and overcoming drug resistance.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common and aggressive forms of liver cancer, representing the fourth leading cause of cancer-related deaths worldwide (Bray et al., 2018). With its incidence on the rise globally, HCC presents a significant public

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health challenge, particularly as it often remains asymptomatic in its early stages, making early detection and intervention difficult (Llovet et al., 2018). By the time most patients are diagnosed, the cancer has typically progressed to advanced stages, where curative treatments such as surgical resection, liver transplantation, or ablation are no longer viable options (Wong & Corley, 2008). As a result, systemic therapies, including targeted therapy and immunotherapy, have become integral components of treatment strategies for advanced HCC. These therapies aim to improve survival outcomes and quality of life for patients who are otherwise ineligible for curative treatments.

In recent years, the landscape of systemic treatment for HCC has evolved significantly. Targeted therapies and immunotherapies have shown considerable promise in slowing tumor progression and improving patient outcomes by focusing on the molecular mechanisms driving HCC development and immune evasion (Llovet et al., 2018). These approaches have marked a shift toward precision medicine, where treatments are tailored to the genetic and molecular profile of individual tumors, offering the potential for more effective and personalized care (McGranahan & Swanton, 2017). However, despite these advancements, significant challenges persist. HCC is an extremely heterogeneous disease, with substantial variation in genetic, molecular, and phenotypic characteristics both within individual tumors and across different patients (Fisher et al., 2013; Schulze et al., 2015). This heterogeneity complicates the development of universal treatment strategies, and the frequent emergence of drug resistance in response to targeted therapies further complicates treatment outcomes (Zucman-Rossi et al., 2015).

One of the major barriers to the development of effective therapies for HCC is the limitations of traditional tumor models. These models often fail to capture the full genetic and phenotypic diversity of the disease, leading to incomplete understanding of tumor biology and the mechanisms underlying treatment resistance (Cheng et al., 2009). Recent advances in bioengineering, however, have led to the creation of patient-derived liver cancer models that more accurately reflect the complexity of HCC tumors. These models offer the promise of better mimicking the tumor microenvironment and the genetic variations found in individual patients, making them ideal platforms for testing potential therapies and developing personalized treatment strategies (Cheng et al., 2009).

This review aims to explore the latest developments in the use of targeted therapies for liver cancer and the role of bioengineered models in advancing precision medicine for HCC. We will examine recent clinical trials, technological platforms, and the integration of personalized approaches in HCC treatment. By investigating these innovations, we hope to provide insight into how they can shape the future of liver cancer therapy, paving the way for more effective treatments and improved patient outcomes over the next decade. As the field progresses, personalized treatment strategies based on comprehensive tumor profiling and patient-derived models may become central to overcoming the challenges posed by HCC's complexity and heterogeneity.

Targeted therapies

Targeted therapy represents a promising approach for the precise treatment of hepatocellular carcinoma (HCC). With the completion of the Human Genome Project, the molecular landscape of HCC has become increasingly well-understood, providing insights into its genetic alterations (Wree et al., 2018). Numerous studies have identified key genes and signaling pathways frequently mutated in HCC, including those involved in the Wnt/β-catenin pathway, p53/cell cycle regulation, oxidative stress response, and epigenetic modification, among others (Boyault et al., 2007; Hoshida et al., 2009; Schulze et al., 2015). These molecular alterations have highlighted potential targets for therapeutic intervention, making molecularly targeted drugs a rapidly growing focus in liver cancer research.

However, despite the promise of targeted therapies, only a small fraction of HCC cases approximately 25% harbor identifiable, targetable genetic drivers (Schulze et al., 2015). This limited availability of targetable mutations presents a significant challenge in developing effective non-surgical treatments for HCC. Additionally, various cytokines and growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor-α (TGF-α), and insulin-like growth factor-II (IGF-II), have been extensively studied for their roles in HCC pathogenesis. Among these, anti-angiogenic therapies targeting VEGF have become a cornerstone of targeted treatment strategies for HCC (Weis and Cheresh, 2011).

The introduction of sorafenib marked a significant milestone in the treatment of advanced hepatocellular carcinoma (HCC). The landmark SHARP trial in 2007 was the first to demonstrate that sorafenib, a tyrosine kinase inhibitor (TKI), could extend overall survival (OS) in patients with advanced liver cancer by an average of three months compared to placebo, leading to its approval as a treatment option (Llovet et al., 2008, 2018). Subsequent studies validated sorafenib's impact on improving both OS and the objective response rate (ORR), establishing it as the only therapy with confirmed survival benefits for advanced HCC for nearly a decade (Cheng et al., 2009). Although sorafenib provided an important advancement, its benefits were still limited: the median survival for patients remained under one year (Bruix et al., 2012; Raoul et al., 2012; EASL Clinical Practice Guidelines and European Association for the Study of the Liver, 2018).

To improve these outcomes, research expanded to explore combination therapies involving sorafenib. Clinical trials revealed

that sorafenib, when combined with other treatment modalities such as transarterial chemoembolization (TACE) or external beam radiation therapy, extended progression-free survival and overall survival times more effectively than sorafenib alone (Qu et al., 2012; Meyer et al., 2017; Zhao et al., 2019). These findings indicated that combination therapy could enhance treatment efficacy, prompting further investigation into multi-modal approaches for advanced HCC.

In recent years, the field of targeted therapies for HCC has advanced considerably, introducing new first-line TKIs, including lenvatinib and sorafenib, and second-line options, such as regorafenib, cabozantinib and lapatinib, each with demonstrated efficacy in prolonging survival in patients with advanced liver cancer. These advancements in TKIs represent a crucial evolution in HCC treatment, expanding the range of therapeutic options beyond sorafenib monotherapy and underscoring the importance of ongoing research in targeted therapy (Table 1).

Lenvatinib

Lenvatinib has emerged as a promising alternative to sorafenib for the treatment of advanced hepatocellular carcinoma (HCC), particularly in addressing the challenge of drug resistance, which is a significant clinical limitation of prolonged sorafenib therapy. Sorafenib resistance in HCC is often attributed to upregulation of fibroblast growth factor (FGF), a pro-angiogenic factor that undermines the effectiveness of anti-VEGF treatments by fostering resistance in tumor cells (Park et al., 2015). Lenvatinib, however, offers a more comprehensive inhibitory profile as a multi-targeted tyrosine kinase inhibitor (TKI), selectively blocking VEGF receptors (VEGFR) 1-3 and FGF receptors 1-4, along with platelet-derived growth factor receptor-α (PDGFR-α), RET, and c-KIT. This broader targeting profile not only helps counteract FGF-related resistance but also enhances anti-tumor efficacy by disrupting multiple pathways crucial for tumor growth and vascularization (Zschäbitz, S., & Grüllich, C., 2018).

The efficacy of lenvatinib was demonstrated in the 2018 REFLECT trial, which established that lenvatinib was at least as effective as sorafenib in treating advanced HCC, particularly in terms of tumor inhibition (Kudo et al., 2018). In addition to showing non-inferiority to sorafenib, lenvatinib outperformed sorafenib on key secondary endpoints, significantly prolonging median progression-free survival and improving the objective response rate (ORR). This enhanced response was especially pronounced in patients with hepatitis B virus-related HCC, where lenvatinib demonstrated superior efficacy compared to sorafenib (Al-Salama et al., 2019).

As the second approved first-line treatment for advanced HCC, lenvatinib provides an important alternative for patients, expanding the therapeutic options available a decade after sorafenib's introduction. The success of lenvatinib in HCC treatment has not only offered patients new hope but also paved the way for further advancements in targeted drug development, setting a precedent for designing therapies that address multiple pathways and reduce resistance (Hiraoka et al., 2019).

Regorafenib

Regorafenib, along with cabozantinib, is a key second-line molecular targeted therapy for hepatocellular carcinoma (HCC) patients whose disease progresses following treatment with sorafenib. As the first drug approved specifically for second-line use in HCC, regorafenib marked the beginning of a new era in sequential and second-line therapy for advanced liver cancer (Finn et al., 2018). Regorafenib was developed by modifying the molecular structure of sorafenib to enhance its therapeutic efficacy. This structural optimization resulted in a multi-targeted tyrosine kinase inhibitor (TKI) with greater potency against VEGF receptors (VEGFR) and additional activity against TIE2, c-KIT, and RET kinases, enhancing its anti-tumor effect.

The efficacy of regorafenib in extending survival and slowing disease progression was confirmed in the pivotal RESORCE trial. In this study, regorafenib demonstrated significant clinical benefits for HCC patients who experienced tumor progression despite sorafenib treatment, prolonging overall survival (OS) by an average of 2.8 months compared to placebo. Additionally, regorafenib significantly improved progression-free survival (PFS) and time to progression (TTP), further establishing its role as an effective second-line therapy for advanced HCC (Bruix et al., 2017).

Further clinical studies have supported the efficacy of regorafenib as part of a sequential treatment regimen following sorafenib. This approach has shown positive outcomes even in complex cases, such as patients experiencing HCC recurrence after liver transplantation, where sequential therapy with sorafenib and regorafenib has proven beneficial (Iavarone et al., 2019; Yoo et al., 2019). By providing an effective second-line option, regorafenib has expanded treatment possibilities for HCC, enabling clinicians to offer a sequential therapy strategy that can help improve survival and disease control in patients who progress on first-line treatments.

Cabozantinib

Cabozantinib is a second-line tyrosine kinase inhibitor (TKI) used in the treatment of advanced hepatocellular carcinoma (HCC), specifically for patients who do not respond to or cannot tolerate sorafenib. It targets multiple kinases, including MET, AXL, and VEGFR1-3, which are associated with tumor growth, angiogenesis, and resistance to standard therapies. Cabozantinib's efficacy was confirmed in the Phase 3 CELESTIAL trial, which demonstrated that cabozantinib significantly extends overall survival (OS) by an average of 2.2 months compared to placebo. Additionally, the trial showed progression-free survival (PFS) and overall response rate (ORR) outcomes comparable to those observed with regorafenib in the RESORCE trial, further supporting cabozantinib's role as a viable option for sorafenib-resistant HCC (Abou-Alfa et al., 2018).

Notably, cabozantinib offers an advantage for patients who are unable to tolerate sorafenib, thereby expanding therapeutic options within the second-line setting (Kudo, 2018). However, its broader kinase inhibition can lead to higher toxicity levels than regorafenib, which necessitates careful monitoring and management of side effects during treatment. Additionally, cost-effectiveness analyses indicate that cabozantinib is associated with higher economic costs compared to regorafenib, which may impact its accessibility for some patients (Parikh et al., 2017; Soto-Perez-De-Celis et al., 2019). Selecting appropriate patients for cabozantinib therapy is therefore critical to maximize benefit and manage potential risks, making it an important addition to the tailored treatment strategies for advanced HCC.

Ramucirumab

Distinct from the second-line tyrosine kinase inhibitors (TKIs) such as regorafenib and cabozantinib, ramucirumab is a recombinant monoclonal antibody that specifically targets VEGFR2. By blocking the VEGFR2-ligand interaction and its downstream signaling pathways, ramucirumab exerts an anti-tumor effect by inhibiting angiogenesis, a key factor in HCC progression.

Initial findings from the REACH trial revealed that ramucirumab did not meet its primary endpoint for second-line therapy, as it showed only a modest increase in overall survival (OS) compared to placebo (9.2 months vs. 7.6 months) (Zhu et al., 2015). However, further analysis identified a specific subgroup of HCC patients with elevated alpha-fetoprotein (AFP ≥400 ng/mL) who experienced improved survival outcomes with ramucirumab treatment (Chau et al., 2017; Zhu et al., 2017; Gilabert and Raoul, 2018). This finding led to the REACH-2 trial, which specifically focused on this biomarker-selected group, and successfully confirmed ramucirumab's efficacy in patients with high AFP levels. As a result, ramucirumab became the first FDA-approved therapy for HCC based on a biomarker criterion (AFP ≥400 ng/mL), highlighting its potential in precision medicine for liver cancer (Zhu et al., 2019).

Despite this breakthrough, the precise biomarker-driven mechanisms underlying ramucirumab's efficacy in high-AFP patients remain unclear and warrant further investigation (Montal et al., 2019) This selective approval underscores the importance of biomarker-based treatments in HCC, paving the way for more individualized approaches in targeting advanced liver cancer.

Immune checkpoint inhibitors

Alongside molecularly targeted therapies, immunotherapy is emerging as a powerful approach for the systemic treatment of hepatocellular carcinoma (HCC). A key factor in HCC progression is immune evasion, where the tumor escapes detection and attack by the body's immune system. Immune checkpoint proteins, which are glycoproteins on the cell surface, play a major role in this process by transmitting inhibitory signals to T cells and natural killer (NK) cells. These proteins are commonly expressed on tumor cells as well as immune cells like macrophages and dendritic cells, helping the tumor evade the immune system by suppressing T cell activation and promoting immune tolerance (Zhu et al., 2019). Tumor cells exploit this mechanism by expressing immune checkpoint molecules, effectively avoiding immune surveillance and promoting their own survival.

Among immune checkpoint inhibitors (ICIs), anti-PD-1 and anti-PD-L1 monoclonal antibodies have shown the most promise in HCC, proving to be both clinically meaningful and extensively studied. As ongoing clinical trials yield results, the availability of first- and second-line immunotherapy options for HCC is steadily increasing. Currently approved first-line immunotherapies for HCC include atezolizumab, sintilimab, camrelizumab, and pembrolizumab. In addition, combinations of ICIs with anti-angiogenic targeted therapies are showing particularly encouraging results. Notable combinations include atezolizumab with bevacizumab (Edge et al., 2010), sintilimab with bevacizumab biosimilar IBI305, camrelizumab with apatinib, and pembrolizumab with lenvatinib (Ikeda et al., 2019). These dual approaches capitalize on the synergistic effects of immunotherapy and targeted therapies, improving outcomes in HCC patients who often have limited treatment options.

For second-line treatment, approved ICIs include camrelizumab, pembrolizumab, nivolumab, and ipilimumab. These agents may be used in monotherapy or in combination, either with anti-angiogenic targeted therapies or with other ICIs to further enhance efficacy (El-Khoueiry et al., 2017). Overall, combination therapies that integrate immunotherapy with other modalities have shown superior objective response rates (ORRs) and extended overall survival (OS) compared to monotherapy (Table 2). As evidence continues to build, combination immunotherapy strategies are set to play an increasingly central role in the treatment of advanced HCC, offering new hope for improved patient outcomes.

Challenges and opportunities of targeted therapy for HCC

Challenges

The progression of hepatocellular carcinoma (HCC) involves a complex network of pathways, making treatment challenging. Recent advancements in targeted therapies have offered renewed hope, with the development of new targeted drugs continuing apace. However, due to HCC's multifaceted nature, monotherapy often leads to severe, dose- or time-dependent adverse events (AEs), forcing treatment interruptions and limiting therapeutic impact. As a result, traditional single-agent treatments, such as tyrosine kinase inhibitors (TKIs) or immune checkpoint inhibitors (ICIs), have hit a ceiling, typically extending overall survival (OS) only to around 14–16 months. This suggests that a paradigm shift in targeted therapy development is needed, and in recent years, the combination of ICIs with anti-VEGF therapies has become a promising focus, significantly improving survival rates in advanced

HCC and establishing new therapeutic combinations (Topalian et al., 2015).

For first-line immunotherapy, combinations of ICIs with anti-VEGF antibodies, particularly atezolizumab with bevacizumab, have demonstrated superior clinical outcomes compared to sorafenib, without increasing the risk of AEs (Bruix et al., 2017). This approach has set a new standard for HCC treatment. Additionally, the phase II RESCUE study, which explored combining the VEGFR2-targeting TKI apatinib with camrelizumab, showed encouraging survival benefits and a favorable safety profile. Although an ongoing phase III trial (NCT03764293) is investigating this combination against sorafenib in advanced HCC, it has not yet reached its anticipated outcomes.

The LEAP-002 trial further examined combination therapy by pairing lenvatinib with pembrolizumab. Despite achieving a promising median OS of 21.2 months, the trial's co-primary endpoints of significantly extended OS and progression-free survival (PFS) were not met, highlighting the continued challenge of reaching statistically meaningful outcomes in this population (Kudo et al., 2018).

Opportunities

The continuous emergence of targeted drugs and combination therapies has significantly enhanced the outlook for hepatocellular carcinoma (HCC) treatment, providing new avenues for addressing this challenging disease. However, the complex tumor microenvironment (TME) of HCC poses substantial obstacles to treatment success. This microenvironment is composed of a variety of liver non-parenchymal cells, extracellular matrix proteins, and signaling molecules that contribute to tumor progression and therapeutic resistance. These components promote inflammation, angiogenesis, hypoxia, and fibrosis, all of which play key roles in tumor evolution and response to treatment. Moreover, HCC is highly heterogeneous at both the molecular and cellular levels, which complicates treatment strategies and contributes to the development of drug resistance. This heterogeneity, coupled with the tumor's ability to evolve over time, remains a primary barrier to effective targeted therapies, with drug resistance often leading to treatment failure. As a result, overcoming tumor heterogeneity and understanding the dynamic evolution of HCC are critical factors for advancing personalized treatment approaches.

The key to breakthrough progress in individualized treatment lies in identifying specific patient populations that will respond to targeted therapies and discovering clear drug-sensitivity markers. This would enable the customization of treatments based on the unique molecular profiles of each patient's tumor. The rapid advancement of next-generation sequencing (NGS) technology offers significant promise in achieving this goal. NGS allows for comprehensive genomic profiling of tumors, enabling the identification of molecular alterations that drive tumor growth and response to therapy (Collins and Varmus, 2015). By analyzing both tumor tissue and plasma samples, NGS can pinpoint biomarkers that guide treatment decisions, ushering in the era of precision medicine. However, translating NGS insights into clinical practice for HCC remains a challenge, and much work is needed to fully integrate these technologies into routine care.

Several studies have demonstrated the potential of NGS in refining targeted therapy for HCC. For example, in the RESORCE trial of regorafenib, a plasma microRNA panel and a gene mutation signature were found to predict response to treatment, providing valuable predictive markers for patient selection (Teufel et al., 2019). Similarly, the BIOSTORM study identified polygenic signatures associated with improved relapse-free survival (RFS) after sorafenib adjuvant therapy, suggesting that genetic profiling could guide post-hepatectomy treatment strategies (Pinyol et al., 2019). Furthermore, NGS has been instrumental in identifying mutations predictive of adverse outcomes in patients treated with sorafenib and immune checkpoint inhibitors (ICIs), using large genomic panels like the FDA-approved MSK-IMPACT platform (Harding et al., 2019). These studies underscore the potential of NGS to identify novel biomarkers and personalize treatment regimens for HCC patients.

While the success of molecularly targeted therapies in cancers such as lung and colorectal cancer has been driven by patient selection based on specific molecular characteristics, HCC presents unique challenges due to its significant molecular heterogeneity. However, improvements in NGS resolution are enabling the discovery of crucial tumor heterogeneity, which, with appropriately designed protocols, can lead to the identification of biomarkers predictive of responses to targeted

drug.

The integration of artificial intelligence (AI) and big data analytics is further enhancing the predictive capacity of these technologies. AI-driven deep learning models have shown great promise in predicting biomarkers for targeted therapy and managing the prognosis of HCC patients (McNamara, D.M. et al., 2019). By combining AI with NGS, it is expected that the accuracy and efficacy of precision medicine for HCC will be significantly improved.

In addition to identifying predictive biomarkers through NGS, establishing robust drug screening platforms is critical to overcoming the challenge of drug resistance in HCC. The rapid advancements in bioengineering have enabled the development of sophisticated preclinical models, such as patient-derived xenografts (PDX) and patient-derived organoids (PDO), which closely replicate the tumor microenvironment and progression of liver cancer. These models serve as powerful platforms for studying tumor evolution, exploring drug resistance mechanisms, and screening potential drug candidates. Furthermore, the development

of three-dimensional (3D) biology techniques has added another layer of complexity to these models, offering more realistic simulations of the in vivo tumor environment. As these bioengineering models continue to evolve, they hold great promise for advancing the understanding of HCC pathogenesis and improving the development of personalized treatments.

Bioengineering

Patient-derived xenografts

The patient-derived xenograft (PDX) model, originally developed over 50 years ago for colorectal cancer research (Rygaard and Povlsen, 1969), was first established for hepatocellular carcinoma (HCC) in 1996 (Sun et al., 1996). Despite initial slow and complex development, recent advancements have significantly enhanced the role of PDX models in liver cancer research. Today, the HCC PDX model is recognized as a highly accurate and clinically predictive tumor model, as it preserves the genetic complexity of human tumors and closely mirrors the in vivo interactions between tumor cells and surrounding tissues, providing a valuable tool for advancing HCC research and therapeutic strategies (Brown et al., 2018).

Two critical aspects in developing HCC PDX models are the selection of suitable host animals and the choice of transplantation site. Subcutaneous transplantation, the simplest in vivo method, enables efficient measurement of tumor growth and response to therapies but lacks the authentic tumor microenvironment, making it suboptimal for fully replicating HCC. Conversely, orthotopic transplantation, which involves placing the tumor in the liver itself, provides a tumor microenvironment that is more similar to human HCC, with relevant blood supply and microenvironmental factors. However, this approach is technically challenging and has a lower success rate, which limits its broader application in research (Hernandez-Gea et al., 2013).

The most commonly used PDX model for HCC involves implanting patient-derived tumor samples into immunodeficient mice to avoid immune rejection. While effective, this approach cannot be used for studying immunotherapy, as the immunodeficient hosts lack a functional human immune system. To address this, researchers are developing "humanized" mouse models with implanted human immune cells, allowing for the evaluation of immune response and the efficacy of immunotherapies in HCC. Although promising, these humanized models still require extensive refinement to closely match each patient's unique immune characteristics (Brown et al., 2018; Zhao et al., 2018).

PDX models are proving to be a mature and invaluable platform for individualized treatment research in HCC. Their use in preclinical studies has yielded promising insights into drug efficacy, biomarker discovery, and the testing of therapeutic combinations. PDXliver, the first publicly accessible database of HCC PDX models, catalogs extensive drug response data, which reflects the heterogeneity of HCC and aids in identifying biomarkers for targeted therapies (He et al., 2018). For example, a recent study by Jin et al. identified significant antitumor activity of lenvatinib and gefitinib in HCC PDX models with high expression of the epidermal growth factor receptor (EGFR), supporting EGFR as a biomarker for stratifying patients in clinical trials.

While the HCC PDX model is a powerful tool in precision oncology, challenges remain. The creation of PDX models is resource-intensive, time-consuming, and has a relatively low success rate, which limits its widespread use. Nonetheless, the ongoing development of PDX models holds considerable promise for advancing HCC research, particularly in areas such as drug screening, biomarker identification, and therapeutic customization. As techniques continue to evolve, the PDX model is expected to become an even more essential component in the quest for precision medicine in HCC.

Patient-derived organoids

To address the limitations of traditional two-dimensional (2D) cell cultures for studying tumor biology, researchers have shifted toward three-dimensional (3D) tumor cultures, including liver slices and mechanical 3D culture devices. However, these models still face challenges, such as short culture durations and limited ability to fully preserve the original characteristics of the tumor. A primary objective of 3D tumor cultures is to replicate the in vivo tumor environment accurately, preserving the genetic and histological characteristics of the parent tumor to allow for effective individualized treatment models.

A breakthrough in 3D culture came in 2012 with the development of tumor organoids derived from intestinal tumors (Sato et al., 2011). Tumor organoids introduced a new level of precision by forming self-organizing 3D structures that mimic the in vivo structure and complexity of human tissues, with applications across a range of tissue types (Fan et al., 2019; Tuveson and Clevers, 2019). Liver organoids, developed by Huch et al. in 2015, demonstrated that liver-specific organoids could differentiate into biliary or hepatic cells, providing a versatile model for liver-specific studies. In another approach, Takebe et al. (2013) created liver organoids by combining human induced pluripotent stem cells (iPSCs) with endothelial and mesenchymal cells, showing that iPSC-derived organoids could recapitulate more of the liver's multicellular architecture, especially in advanced systems like perfusion microcolumn chips.

In liver cancer research, patient-derived organoids (PDOs) hold significant potential for studying carcinogenesis, such as hepatitis B virus-induced tumorigenesis, and developing individualized treatments. PDOs are generally derived from either needle biopsies or surgical specimens, though the complex culturing process results

Table 1. Overview of Approved Clinical Trials for Targeted Therapies in Advanced Hepatocellular Carcinoma

Name	Study type	Drug	Target	Line
SHARP/NCT00105443	Phase III	Sorafenib	Multikinases	First-line
REFLECT/NCT01761266	Phase III	Lenvatinib	Multikinases	First-line
RESORCE/NCT01774344	Phase III	Regorafenib	Multikinases	Second-line
RESORCE/NCT01774344	Phase III	Cabozantinib	Multikinases	Second-line
REACH-2/NCT02435433	Phase III	Ramucirumab	VEGFR2	Second-line
AHELP/NCT02329860	Phase III	Apatinib	Multikinases	Second-line

Table 2. Clinical trials of combination therapy for advanced HCC

Name	Study type	Drug	Target	Primary endpoint	Line
IMbrave150/NCT03434379	Phase III	Atezolizumab Bevacizumab	PD-L1	19.2 months (mOS)	First-line treatment
			VEGF	29.8 (ORR)	
NCT03794440	Phase II/ III	Sintilizumab IBI305	PD-1	-	-
			VEGFR		
RESCUE/NCT03463876	Phase II	Camrelizumab Apatinib	PD-1	-	-
			VEGFR2	-	
NCT03764293	Phase III	Lenvatinib Pembrolizumab	Multikinases	-	-
KEYNOTE524/NCT03006926	Phase Ib		PD-1	-	-
LEAP-002/NCT03713593	Phase III				-

Table 3. Relative advantage related to PDX and PDO

Name	PDX	PDO	3DP
Establishment success rate	-	-	++
Cost	-	+	++
Recapitulates tumoural heterogeneity	+	+	+
Multi-Cell culture conditions	+	+	++
Microenvironment construction	++	+	++
High-throughput screening	-	+	+

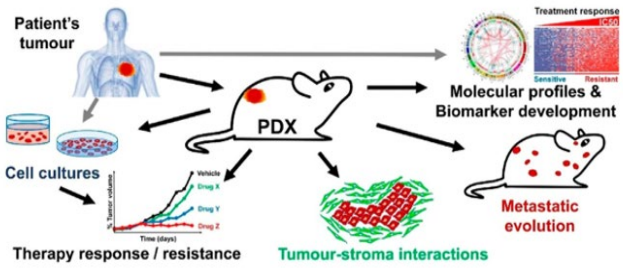

Figure 1. Patient-derived tumors are initially implanted in mice, where they undergo a growth phase, after which they can be transplanted and expanded to form PDX cohorts. These cohorts are ideal for preclinical research, including drug efficacy testing and molecular profiling. Additionally, tumor samples from PDX models can be collected to establish tissue biobanks, which are valuable for sustaining ongoing preclinical research efforts. This figure is cited from Invrea et al. (2019) and is part of an open-access article distributed under the Creative Commons Attribution License (CC BY 4.0), allowing unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

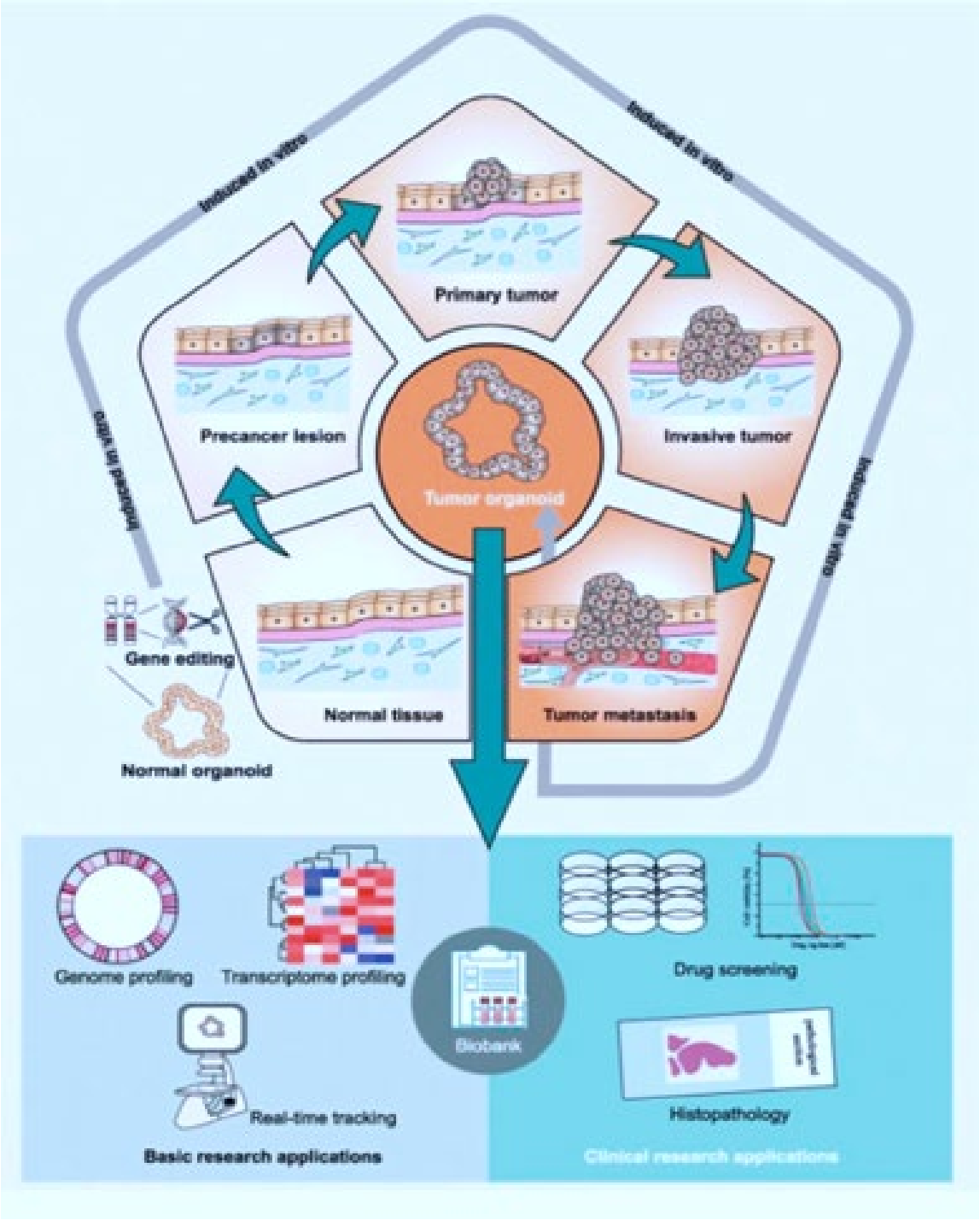

Figure 2. Patient-derived tumor organoids offer significant value across both basic and clinical research, serving as versatile models for various preclinical applications. This figure is cited from Fan et al. (2019) and is part of an open-access article distributed under the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

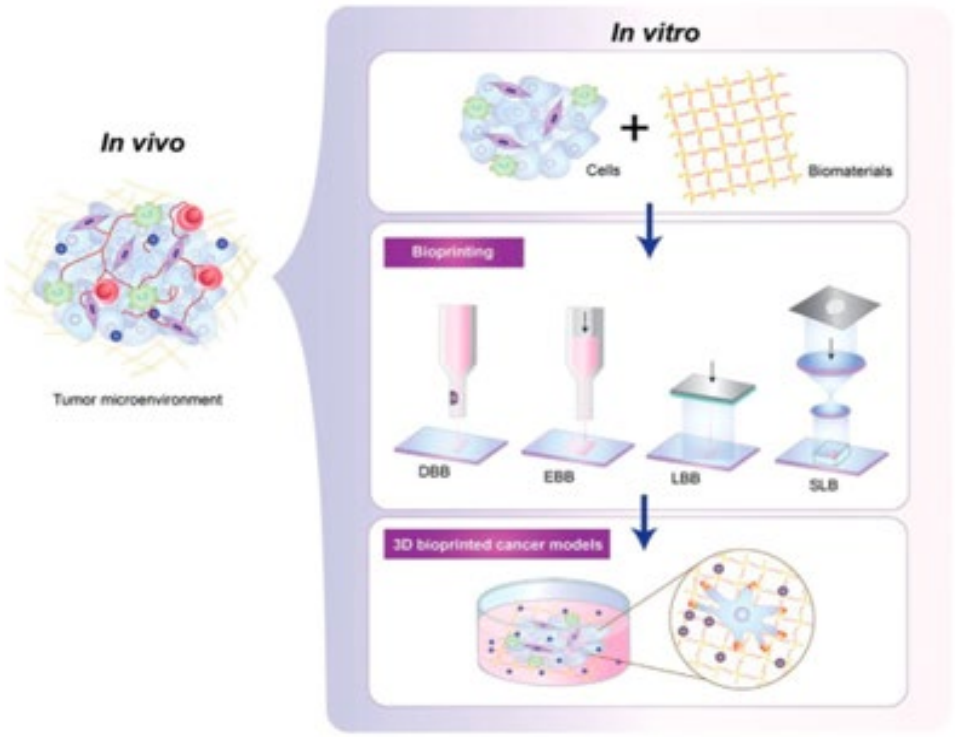

Figure 3. To create 3DP tumor models, tumor cells are encapsulated in biocompatible bio-inks and processed through high-precision bioprinters. These models can be generated using various bioprinting techniques, including droplet-based (DBB), extrusion-based (EBB), laser-based (LBB), and stereolithography (SLB) methods. This figure is cited from Jung et al. (2019) and is part of an open-access article distributed under the Creative Commons Attribution License (CC BY 4.0), allowing unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

in relatively low establishment success rates (37.5% and 26%, respectively) (Broutier et al., 2017; Nuciforo et al., 2018). Despite this, PDOs faithfully replicate key tumor characteristics, including growth patterns, cellular differentiation, HCC-specific marker expression, and genomic alterations. These features make PDOs valuable for precision medicine, allowing for targeted therapy studies, drug screening, and treatment response prediction (Li et al., 2019).

Recent studies demonstrate PDOs' utility in understanding drug resistance mechanisms in HCC. For example, Bagrodia et al. 2012 found that PDOs could reveal sorafenib resistance pathways linked to Hedgehog signaling reactivation and receptor tyrosine kinase-induced MEK/ERK and AKT signaling pathways. Additionally, a recent technique that uses hydrogel capsules to culture HCC PDOs has shown promise in replicating the tumor microenvironment, thereby preserving tumor heterogeneity and providing a robust platform for targeted therapy applications (Perche et al., 2012). Clinical trials are underway (NCT05384184 and NCT02436564) to evaluate HCC PDOs in patient-specific treatment contexts.

Looking forward, research on HCC PDOs is moving towards better simulation of the in vivo tumor microenvironment (TME) to enhance therapeutic relevance. For instance, Loh et al. developed an HCC PDO model cultured in conditioned medium to simulate the TME, allowing exploration of hepatocyte resistance mechanisms to sorafenib. Similarly, Li et al. (2019) introduced a hydrogel-based co-culture system to replicate pro-angiogenic signaling between hepatoma and endothelial cells, enhancing the model's ability to study TME-driven drug resistance and identify novel therapeutic targets.

The integration of PDOs with TME components promises to refine our understanding of HCC heterogeneity and drug resistance, offering a more accurate and robust platform for liver cancer research. This next-generation PDO-TME model could play a pivotal role in the future of precision oncology, providing an invaluable tool for drug discovery, biomarker identification, and the development of tailored therapies.

3D bioprinting of HCC

Recent advancements in additive manufacturing, or 3D printing, have paved the way for its application in biomedicine, particularly in 3D bioprinting (3DP). This technology has ushered in a new era of bioengineered medicine by allowing for the creation of complex, highly customized biological structures (Murphy and Atala, 2014). In 3DP, structures are created using methods such as inkjet, micro-extrusion, and laser-assisted bioprinting, with micro-extrusion being the most widely adopted (Mandrycky et al., 2016). Central to 3DP is bio-ink a material critical to the technology's clinical potential. A variety of bio-inks, such as alginate, gelatin, collagen, chitosan, and decellularized extracellular matrix, are tailored to different bioprinting technologies and tissue applications (Kulkarni et al., 2000).

3D bioprinting has gained traction in cancer research, providing models that replicate the complex 3D structure of tumors with physiologically relevant cell-cell and cell-matrix interactions (Almela et al., 2018; Wang et al., 2018). A 3DP model of HepG2 liver cancer cells using a gelatin-sodium alginate bio-ink demonstrated enhanced levels of liver function-related proteins and genes, as well as indicators of cell proliferation, metastasis, drug resistance, and epithelial-mesenchymal transition properties that reflect the unique 3D microenvironment of liver tumors (Munaz et al., 2016). This enhanced physiological relevance makes 3D bioprinted tumor models promising for preclinical research, particularly for drug testing and development, offering a more accurate platform than traditional two-dimensional cultures.

Additionally, we have shown that 3DP models made with primary HCC cells derived from patients can maintain high cell viability over extended periods. This feature is advantageous for liver cancer-targeted therapy drug testing and could enhance the predictive accuracy of personalized therapies (Hospodiuk et al., 2017). Compared to other preclinical models, such as patient-derived xenografts (PDX) and patient-derived organoids (PDO), 3DP models demonstrate cost efficiency, reproducibility, and precision, thanks to computer-aided design. These models are particularly promising for personalized medicine applications and require less time and resources than PDX or PDO models, making them a viable option for wider preclinical use.

3D bioprinting allows for the reproduction of the tumor microstructure, preserving the tumor's original features. This quality not only makes 3DP an ideal model for cancer research but also lends it a unique advantage in drug testing, where its high-resolution capability can replicate the intricate tumor microenvironment, including vascular structures (Zhou et al., 2016; Heinrich et al., 2019), liver cancer research still requires further exploration to validate the model's utility fully, particularly when combined with tumor microenvironment elements in liver cancer.

Further research into the integration of 3DP with cancer chip technology shows potential to advance liver cancer modeling. This combination enables researchers to simulate key microenvironmental features of tumors, which could significantly aid in understanding drug resistance mechanisms and tumor evolution in hepatocellular carcinoma (Shoulders et al., 2009). The growing adoption of 3DP in cancer modeling, with the ability to create complex, patient-specific structures, is anticipated to drive major advancements in the development of precision and personalized treatment strategies.

Conclusion

The development of targeted therapies for hepatocellular carcinoma (HCC) has shown great promise but also faces significant challenges due to the tumor's molecular heterogeneity. While advancements in genomic profiling have uncovered key genetic alterations and signaling pathways involved in HCC, only a small percentage of cases present targetable mutations. Anti-angiogenic therapies, particularly those targeting VEGF, have proven beneficial, but the complexity of HCC necessitates continued exploration of novel therapeutic targets. Furthermore, the integration of cutting-edge technologies such as next-generation sequencing, 3D bioprinting, and patient-derived models holds great potential for advancing precision medicine in HCC. To overcome the limitations of current treatments, it is essential to refine biomarker-driven strategies, enhance drug efficacy, and develop personalized therapeutic approaches that account for the dynamic nature of HCC and its tumor microenvironment.

Author contributions

T. and M.M.H.S. conceptualized and developed the methodology. B.A., M.M.R. S.A.A.A. and prepared the original draft and reviewed and edited the writing. A.A.N. and M.S.A. analysed the data and reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

References

Abou-Alfa, G. K., Meyer, T., Cheng, A. L., El-Khoueiry, A. B., Rimassa, L., Ryoo, B. Y., ... & Madoff, D. (2018). Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. New England Journal of Medicine, 379(1), 54–63. https://doi.org/10.1056/NEJMoa1717002

Almela, T., Al-Sahaf, S., Brook, I. M., Khoshroo, K., Rasoulianboroujeni, M., Fahimipour, F., ... & Ejeian, F. (2018). 3D printed tissue-engineered model for bone invasion of oral cancer. Tissue and Cell, 52, 71–77. https://doi.org/10.1016/j.tice.2018.03.009

Al-Salama, Z. T., Syed, Y. Y., & Scott, L. J. (2019). Lenvatinib: A review in hepatocellular carcinoma. Drugs, 79(6), 665–674. https://doi.org/10.1007/s40265-019-01116-x

Bagrodia, S., Smeal, T., & Abraham, R. T. (2012). Mechanisms of intrinsic and acquired resistance to kinase-targeted therapies. Pigment Cell & Melanoma Research, 25(6), 819–831. https://doi.org/10.1111/pcmr.12007

Boyault, S., Rickman, D. S., de Reynies, A., Balabaud, C., Rebouissou, S., Jeannot, E., ... & Bioulac-Sage, P. (2007). Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology, 45(1), 42–52. https://doi.org/10.1002/hep.21467

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394–424.

Broutier, L., Mastrogiovanni, G., Verstegen, M. M., Francies, H. E., Gavarro, L. M., Bradshaw, C. R., ... & Lemoine, N. R. (2017). Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nature Medicine, 23(12), 1424–1435. https://doi.org/10.1038/nm.4438

Brown, Z. J., Heinrich, B., & Greten, T. F. (2018). Mouse models of hepatocellular carcinoma: An overview and highlights for immunotherapy research. Nature Reviews Gastroenterology & Hepatology, 15(9), 536–554. https://doi.org/10.1038/s41575-018-0033-6

Bruix, J., Qin, S., Merle, P., et al. (2017). Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet, 389(10064), 56–66. https://doi.org/10.1016/S0140-6736(16)32453-9

Bruix, J., Qin, S., Merle, P., Granito, A., Huang, Y. H., Bodoky, G., ... & Meyer, T. (2017). Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomized, double-blind, placebo-controlled, phase 3 trial. The Lancet, 389(10064), 56–66. https://doi.org/10.1016/S0140-6736(16)32453-9

Bruix, J., Raoul, J. L., Sherman, M., Mazzaferro, V., Bolondi, L., Craxi, A., ... & Saltzman, D. (2012). Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: Subanalyses of a phase III trial. Journal of Hepatology, 57(4), 821–829. https://doi.org/10.1016/j.jhep.2012.06.014

Chau, I., Peck-Radosavljevic, M., Borg, C., Malfertheiner, P., Seitz, J. F., Park, J. O., ... & Hamdy, S. (2017). Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib: Patient-focused outcome results from the randomized phase III REACH study. European Journal of Cancer, 81, 17–25. https://doi.org/10.1016/j.ejca.2017.05.001

Cheng, A. L., Kang, Y. K., Chen, Z., Tsao, C. J., Qin, S., Kim, J. S., ... & Zhou, Y. (2009). Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomized, double-blind, placebo-controlled trial. The Lancet Oncology, 10(1), 25–34.

Cheng, A. L., Kang, Y. K., Chen, Z., Tsao, C. J., Qin, S., Kim, J. S., ... & Wang, L. (2009). Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomized, double-blind, placebo-controlled trial. The Lancet Oncology, 10(1), 25–34. https://doi.org/10.1016/S1470-2045(08)70285-7

Collins, F. S., & Varmus, H. (2015). A new initiative on precision medicine. New England Journal of Medicine, 372(9), 793–795. https://doi.org/10.1056/NEJMp1500523

Edge, S. B., & American Joint Committee on Cancer, A. C. S. (2010). AJCC cancer staging handbook: From the AJCC cancer staging manual (Vol. 19, p. 718). Springer.

El-Khoueiry, A. B., Sangro, B., Yau, T., Crocenzi, T. S., Kudo, M., Hsu, C., et al. (2017). Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. The Lancet, 389(10088), 2492–2502. https://doi.org/10.1016/S0140-6736(17)31046-2

European Association for the Study of the Liver. (2018). EASL clinical practice guidelines: Management of hepatocellular carcinoma. Journal of Hepatology, 69(1), 182–236. https://doi.org/10.1016/j.jhep.2018.03.019

Fan, H., Demirci, U., & Chen, P. (2019). Emerging organoid models: Leaping forward in cancer research. Journal of Hematology & Oncology, 12(1), 142. https://doi.org/10.1186/s13045-019-0832-4

Finn, R. S., Merle, P., Granito, A., Huang, Y. H., Bodoky, G., Pracht, M., et al. (2018). Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESORCE trial. Journal of Hepatology, 69(2), 353–358. https://doi.org/10.1016/j.jhep.2018.04.010

Fisher, R., Pusztai, L., & Swanton, C. (2013). Cancer heterogeneity: Implications for targeted therapeutics. British Journal of Cancer, 108(3), 479–485. https://doi.org/10.1038/bjc.2012.581

Gilabert, M., & Raoul, J. L. (2018). Potential of ramucirumab in treating hepatocellular carcinoma patients with elevated baseline alpha-fetoprotein. Journal of Hepatocellular Carcinoma, 5, 91–98. https://doi.org/10.2147/JHC.S157413

Harding, J. J., Nandakumar, S., Armenia, J., Khalil, D. N., Albano, M., Ly, M., et al. (2019). Prospective genotyping of hepatocellular carcinoma: Clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. Clinical Cancer Research, 25(7), 2116–2126. https://doi.org/10.1158/1078-0432.CCR-18-2293

He, S., Hu, B., Li, C., Lin, P., Tang, W. G., Sun, Y. F., et al. (2018). PDXliver: A database of liver cancer patient-derived xenograft mouse models. BMC Cancer, 18(1), 550. https://doi.org/10.1186/s12885-018-4459-6

Heinrich, M. A., Bansal, R., Lammers, T., Zhang, Y. S., Michel, S. R., & Prakash, J. (2019). 3D-bioprinted mini-brain: A glioblastoma model to study cellular interactions and therapeutics. Advanced Materials, 31(14), e1806590. https://doi.org/10.1002/adma.201806590

Hernandez-Gea, V., Toffanin, S., Friedman, S. L., & Llovet, J. M. (2013). Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology, 144(3), 512–527. https://doi.org/10.1053/j.gastro.2013.01.002

Hiraoka, A., Kumada, T., Kariyama, K., Takaguchi, K., Atsukawa, M., Itobayashi, E., et al. (2019). Clinical features of lenvatinib for unresectable hepatocellular carcinoma in real-world conditions: Multicenter analysis. Cancer Medicine, 8(1), 137–146. https://doi.org/10.1002/cam4.1909

Hoshida, Y., Nijman, S. M., Kobayashi, M., Chan, J. A., Brunet, J. P., Chiang, D. Y., et al. (2009). Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Research, 69(18), 7385–7392. https://doi.org/10.1158/0008-5472.CAN-09-1089

Hospodiuk M., Dey M., Sosnoski D., Ozbolat I. T. (2017). The Bioink: A Comprehensive Review on Bioprintable Materials. Biotechnol. Adv. 35, 217–239. 10.1016/j.biotechadv.2016.12.006

Hu, B., Cheng, J. W., Hu, J. W., Li, H., Ma, X. L., & Tang, W. G., et al. (2019). KPNA3 confers sorafenib resistance to advanced hepatocellular carcinoma via TWIST-regulated epithelial-mesenchymal transition. Journal of Cancer, 10(17), 3914–3925. https://doi.org/10.7150/jca.31448

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M. M., et al. (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell, 160(1-2), 299–312. https://doi.org/10.1016/j.cell.2014.11.050

Iavarone, M., Invernizzi, F., Czauderna, C., Sanduzzi-Zamparelli, M., Bhoori, S., Amaddeo, G., et al. (2019). Preliminary experience on safety of regorafenib after sorafenib failure in recurrent hepatocellular carcinoma after liver transplantation. American Journal of Transplantation, 19(11), 3176–3184. https://doi.org/10.1111/ajt.15551

Ikeda, M., Sung, M. W., Kudo, M., Kobayashi, M., Baron, A. D., Finn, R. S., et al. (2019). A phase Ib trial of lenvatinib (LEN) plus pembrolizumab (PEMBRO) in unresectable hepatocellular carcinoma (uHCC): Updated results. Submitted Abstracts Gastrointestinal Tumours, Non-Colorectal, 30, V286–V287. https://doi.org/10.1093/annonc/mdz247.073

Karlovich, C. A., & Williams, P. M. (2019). Clinical applications of next-generation sequencing in precision oncology. Cancer Journal, 25(4), 264–271. https://doi.org/10.1097/PPO.0000000000000385

Knowlton, S., Onal, S., Yu, C. H., Zhao, J. J., & Tasoglu, S. (2015). Bioprinting for cancer research. Trends in Biotechnology, 33(9), 504–513. https://doi.org/10.1016/j.tibtech.2015.06.007

Kudo, M. (2018). Cabozantinib as a second-line agent in advanced hepatocellular carcinoma. Liver Cancer, 7(2), 123–133. https://doi.org/10.1159/000488542

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. The Lancet, 391(10126), 1163–1173. https://doi.org/10.1016/S0140-6736(18)30207-1

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., Baron, A., Park, J. W., Han, G., Jassem, J., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. The Lancet, 391, 1163–1173.

Kulkarni P., Marsan A., Dutta D. (2000). A Review of Process Planning Techniques in Layered Manufacturing. Rapid Prototyp. J. 6, 18–35. 10.1108/13552540010309859

Li, L., Knutsdottir, H., Hui, K., Weiss, M. J., He, J., Philosophe, B., et al. (2019). Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. JCI Insight, 4(2), e121490. https://doi.org/10.1172/jci.insight.121490

Li, W. et al. LncRNA SNHG1 contributes to sorafenib resistance by activating the Akt pathway and is positively regulated by miR-21 in hepatocellular carcinoma cells. J. Exp. Clin. Cancer Res. 38, 183 (2019)

Llovet, J. M., Montal, R., Sia, D., & Finn, R. S. (2018). Molecular therapies and precision medicine for hepatocellular carcinoma. Nature Reviews Clinical Oncology, 15(10), 599–616. https://doi.org/10.1038/s41571-018-0073-4

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., ... & Moscovici, M. (2008). Sorafenib in advanced hepatocellular carcinoma. New England Journal of Medicine, 359(4), 378–390.

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. New England Journal of Medicine, 359(4), 378–390. https://doi.org/10.1056/NEJMoa0708857

Mandrycky, C., Wang, Z., Kim, K., & Kim, D. H. (2016). 3D bioprinting for engineering complex tissues. Biotechnology Advances, 34(4), 422–434. https://doi.org/10.1016/j.biotechadv.2015.12.011

Mcgranahan, N., & Swanton, C. (2017). Clonal heterogeneity and tumor evolution: Past, present, and the future. Cell, 168(4), 613–628. https://doi.org/10.1016/j.cell.2017.01.018

Meyer, T., Fox, R., Ma, Y. T., Ross, P. J., James, M. W., Sturgess, R., et al. (2017). Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): A randomised placebo-controlled, double-blind, phase 3 trial. The Lancet Gastroenterology & Hepatology, 2(8), 565–575. https://doi.org/10.1016/S2468-1253(17)30156-5

Montal, R., Andreu-Oller, C., Bassaganyas, L., Esteban-Fabro, R., Moran, S., Montironi, C., et al. (2019). Molecular portrait of high alpha-fetoprotein in hepatocellular carcinoma: Implications for biomarker-driven clinical trials. British Journal of Cancer, 121(4), 340–343. https://doi.org/10.1038/s41416-019-0513-7

Munaz A., Vadivelu R. K., St. John J., Barton M., Kamble H., Nguyen N.-T. (2016). Three-dimensional Printing of Biological Matters. J. Sci. Adv. Mater. Devices 1, 1–17. 10.1016/j.jsamd.2016.04.001

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature Biotechnology, 32(8), 773–785. https://doi.org/10.1038/nbt.2958

Nuciforo, S., Fofana, I., Matter, M. S., Blumer, T., Calabrese, D., Boldanova, T., et al. (2018). Organoid models of human liver cancers derived from tumor needle biopsies. Cell Reports, 24(5), 1363–1376. https://doi.org/10.1016/j.celrep.2018.07.001

Parikh, N. D., Singal, A. G., & Hutton, D. W. (2017). Cost effectiveness of regorafenib as second-line therapy for patients with advanced hepatocellular carcinoma. Cancer, 123(19), 3725–3731. https://doi.org/10.1002/cncr.30863

Park, J. W., Chen, M., Colombo, M., et al. (2015). Global patterns of hepatocellular carcinoma management from diagnosis to death: The BRIDGE study. Liver International, 35(9), 2155–2166.

Perche, F., & Torchilin, V. P. (2012). Cancer cell spheroids as a model to evaluate chemotherapy protocols. Cancer Biology & Therapy, 13(12), 1205–1213. https://doi.org/10.4161/cbt.21353

Pinyol, R., Montal, R., Bassaganyas, L., Sia, D., Takayama, T., Chau, G. Y., et al. (2019). Molecular predictors of prevention of recurrence in HCC with sorafenib as adjuvant treatment and prognostic factors in the phase 3 STORM trial. Gut, 68(6), 1065–1075. https://doi.org/10.1136/gutjnl-2018-316408

Qu, X. D., Chen, C. S., Wang, J. H., Yan, Z. P., Chen, J. M., Gong, G. Q., et al. (2012). The efficacy of TACE combined with sorafenib in advanced stages of hepatocellular carcinoma. BMC Cancer, 12, 263. https://doi.org/10.1186/1471-2407-12-263

Raoul, J. L., Bruix, J., Greten, T. F., Sherman, M., Mazzaferro, V., Hilgard, P., et al. (2012). Relationship between baseline hepatic status and outcome, and effect of sorafenib on liver function: SHARP trial subanalyses. Journal of Hepatology, 56(5), 1080–1088. https://doi.org/10.1016/j.jhep.2011.12.009

Ricard-Blum S. (2011). The Collagen Family. Cold Spring Harb. Perspect. Biol. 3, a004978. 10.1101/cshperspect.a004978

Ricard-Blum S. (2011). The Collagen Family. Cold Spring Harb. Perspect. Biol. 3, a004978. 10.1101/cshperspect.a004978

Rygaard, J., & Povlsen, C. O. (1969). Heterotransplantation of a human malignant tumour to "Nude" mice. Acta Pathologica et Microbiologica Scandinavica, 77(4), 758–760. https://doi.org/10.1111/j.1699-0463.1969.tb04520.x

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van den Brink, S., et al. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology, 141(5), 1762–1772. https://doi.org/10.1053/j.gastro.2011.07.050

Schulze, K., Imbeaud, S., Letouzé, E., Alexandrov, L. B., Calderaro, J., Rebouissou, S., et al. (2015). Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nature Genetics, 47(5), 505–511. https://doi.org/10.1038/ng.3252

Shoulders M. D., Raines R. T. (2009). Collagen Structure and Stability. Annu. Rev. Biochem. 78, 929–958. 10.1146/annurev.biochem.77.032207.120833

Soto-Perez-De-Celis, E., Aguiar, P. N., Cordon, M. L., Chavarri-Guerra, Y., & Lopes, G. L. (2019). Cost-effectiveness of cabozantinib in the second-line treatment of advanced hepatocellular carcinoma. Journal of the National Comprehensive Cancer Network, 17(6), 669–675. https://doi.org/10.6004/jnccn.2018.7275

Sun, F. X., Tang, Z. Y., Lui, K. D., Ye, S. L., Xue, Q., Gao, D. M., et al. (1996). Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopic implantation of histologically intact tissues. International Journal of Cancer, 66(2), 239–243. https://doi.org/10.1002/(SICI)1097-0215(19960410)66:2<239::AID-IJC17>3.0.CO;2-7

Swaminathan, S., Hamid, Q., Sun, W., & Clyne, A. M. (2019). Bioprinting of 3D breast epithelial spheroids for human cancer models. Biofabrication, 11(2), 025003. https://doi.org/10.1088/1758-5090/aafc49

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature, 499(7459), 481–484. https://doi.org/10.1038/nature12271

Teufel, M., Seidel, H., Kochert, K., Meinhardt, G., Finn, R. S., Llovet, J. M., et al. (2019). Biomarkers associated with response to regorafenib in patients with hepatocellular carcinoma. Gastroenterology, 156(6), 1731–1741. https://doi.org/10.1053/j.gastro.2019.01.261

Tharehalli, U., Svinarenko, M., & Lechel, A. (2019). Remodelling and improvements in organoid technology to study liver carcinogenesis in a dish. Stem Cells International, 2019, 3831213. https://doi.org/10.1155/2019/3831213

Topalian, S. L., Drake, C. G., & Pardoll, D. M. (2015). Immune checkpoint blockade: A common denominator approach to cancer therapy. Cancer Cell, 27(4), 450–461.

Tuveson, D., & Clevers, H. (2019). Cancer modeling meets human organoid technology. Science, 364(6444), 952–955. https://doi.org/10.1126/science.aaw6985

Villanueva, A. (2019). Hepatocellular carcinoma. The New England Journal of Medicine, 380(15), 1450–1462. https://doi.org/10.1056/NEJMra1713263

Wang, X., Zhang, X., Dai, X., Wang, X., Li, X., Diao, J., et al. (2018). Tumor-like lung cancer model based on 3D bioprinting. 3 Biotech, 8(12), 501. https://doi.org/10.1007/s13205-018-1519-1

Weis, S. M., & Cheresh, D. A. (2011). Tumor angiogenesis: Molecular pathways and therapeutic targets. Nature Medicine, 17(11), 1359–1370. https://doi.org/10.1038/nm.2537

Wong, R., & Corley, D. A. (2008). Racial and ethnic variations in hepatocellular carcinoma incidence within the United States. American Journal of Medicine, 121(6), 525–531.

Wree, A., Broderick, L., Canbay, A., Hoffman, H. M., & Feldstein, A. E. (2013). From NAFLD to NASH to cirrhosis—New insights into disease mechanisms. Nature Reviews Gastroenterology & Hepatology, 10(11), 627–636.

Yoo, C., Park, J. W., Kim, Y. J., Kim, D. Y., Yu, S. J., Lim, T. S., et al. (2019). Multicenter retrospective analysis of the safety and efficacy of regorafenib after progression on sorafenib in Korean patients with hepatocellular carcinoma. Investigational New Drugs, 37(3), 567–572. https://doi.org/10.1007/s10637-018-0707-5

Zhao, Y., Shuen, T., Toh, T. B., Chan, X. Y., Liu, M., Tan, S. Y., et al. (2018). Development of a new patient-derived xenograft humanised mouse model to study human-specific tumour microenvironment and immunotherapy. Gut, 67(10), 1845–1854. https://doi.org/10.1136/gutjnl-2017-315201

Zhao, Y., Zhu, X., Wang, H., Dong, D., Gao, S., Zhu, X., et al. (2019). Safety and efficacy of transcatheter arterial chemoembolization plus radiotherapy combined with sorafenib in hepatocellular carcinoma showing macrovascular invasion. Frontiers in Oncology, 9, 1065. https://doi.org/10.3389/fonc.2019.01065

Zhou, X., Zhu, W., Nowicki, M., Miao, S., Cui, H., Holmes, B., et al. (2016). 3D bioprinting a cell-laden bone matrix for breast cancer metastasis study. ACS Applied Materials & Interfaces, 8(44), 30017–30026. https://doi.org/10.1021/acsami.6b10673

Zhu, A. X., Baron, A. D., Malfertheiner, P., Kudo, M., Kawazoe, S., Pezet, D., et al. (2017). Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma: Analysis of REACH trial results by child-pugh score. JAMA Oncology, 3(2), 235–243. https://doi.org/10.1001/jamaoncol.2016.4115

Zhu, A. X., Kang, Y. K., Yen, C. J., Finn, R. S., Galle, P. R., Llovet, J. M., et al. (2019). Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): A randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet Oncology, 20(2), 282–296. https://doi.org/10.1016/S1470-2045(18)30937-9

Zhu, A. X., Kang, Y. K., Yen, C. J., Finn, R. S., Galle, P. R., Llovet, J. M., et al. (2019). Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): A randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet Oncology, 20(2), 282–296. https://doi.org/10.1016/S1470-2045(18)30937-9

Zhu, A. X., Park, J. O., Ryoo, B. Y., Yen, C. J., Poon, R., Pastorelli, D., et al. (2015). Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): A randomised, double-blind, multicentre, phase 3 trial. The Lancet Oncology, 16(7), 859–870. https://doi.org/10.1016/S1470-2045(15)00050-9

Zschäbitz, S., & Grüllich, C. (2018). Lenvatinib: A tyrosine kinase inhibitor of VEGFR 1-3, FGFR 1-4, PDGFRα, KIT and RET. Recent Results in Cancer Research, 211, 187–198. https://doi.org/10.1007/978-3-319-91442-8_13

Zucman-Rossi, J., Villanueva, A., Nault, J. C., & Llovet, J. M. (2015). Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology, 149(5), 1226–1239. https://doi.org/10.1053/j.gastro.2015.05.061