

RECENT ADVANCES IN IMMUNOTHERAPY AND TARGETED THERAPIES FOR HEPATOCELLULAR CARCINOMA (HCC): MOLECULAR MECHANISMS, CLINICAL PROGRESS, AND FUTURE DIRECTIONS

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ABSTRACT

Hepatocellular carcinoma (HCC) is an extremely dangerous cancer globally with high molecular heterogeneity, late diagnosis, and treatment inefficacy. Knowingly, the last 10 years have brought about the immunotherapy and targeted therapies that have transformed the way advanced HCC is treated to be replaced with the traditional monotherapy therapy, based on sorafenib. IMbrave150, CheckMate 040, and HIMALAYA deliver valuable results to the domain of immune checkpoint inhibitors (ICIs) and their combinations with anti-angiogenic agents, becoming a new standard of care, with a significant positive impact on overall survival and the quality of life. Simultaneously, the identification of molecular drivers and the explanation of the signaling effectors oncogenic as the Wnt/ β -catenin, PI3K/Akt/mTOR, and MAPK have informed resistance mechanisms and facilitated the advances of precision therapies. Irrespective of these developments, therapeutic resistance, immune evasion and toxicities associated with treatment are significant problems. The current studies focus on the combination of the liquid biopsy, circulating tumor DNA (ctDNA), and multi-omics platforms to determine predictive biomarkers and maximize patient stratification. Also, clinical efficacy and practical applicability are convergent at the point of real-world evidence and adaptive designs of clinical trials, so that precision immuno-oncology is a reality. Future perspectives are on coupled ICIs with epigenetic modulators, metabolic inhibitors, and adoptive cell therapies that can lead to long-term responses and worldwide efforts, which can bolster access and equity in the treatment of HCC. This is an overarching review of the current literature that outlines the molecular pathways, treatment advances, and translation issues that are changing the management of HCC in the context of targeted and immune-based treatment.

Keywords: Hepatocellular carcinoma (HCC); immunotherapy; targeted therapy; immune checkpoint inhibitors; tyrosine kinase inhibitors; tumor microenvironment; biomarkers; ctDNA; multi-omics integration; clinical trials; real-world evidence; personalized medicine; mTOR signaling; VEGF/VEGFR pathway; combination therapy.

INTRODUCTION

The most frequent Hepatocellular carcinoma (HCC) and one of the main health issues in the world is primary liver cancer. Despite its improved

surveillance and treatment, most patients appear with intermediate to advanced disease and largely are linked with high mortality the products of early

diagnosis, within-the-liver cirrhosis and very few systemic choices in the past [1-4]. Over the last five years, however, the fast transition of taking molecular targets and immunotherapy has begun to alter both standards of care during the first-line and subsequent-line treatment, with combination therapy showing a clinically measurable improvement on survival over treatment with tyrosine-kinase inhibitor (TKI) monotherapy [57]. The conclusion in this review is that of the existing knowledge on the molecular origin of HCC, the clinical course of immunotherapy and targeted therapeutics, and the outstanding gaps that reflect the future research agenda.

1.1 Overview of Hepatocellular Carcinoma (HCC)

HCC adds to the monumental figure of primary liver cancer as well as is typically conjoined with the climate of chronic liver disease and cirrhosis. Liver cancer has been rated as one of the most prevalent cancer killers in the globe and it has travelled on one of the greatest effects on the lost year particularly in regions where there are high rates of chronic hepatitis due to exposure to liver viruses and aflatoxin exposures [1,2]. The current practice guideline of the major societies focuses on multidisciplinary care, treatment adjusted to the stage and the need of tracking the activities of the at-risk groups to deliver potentially curative treatment in case it can be done [3,4]. The heterogeneity of the tumor in question, comorbidity between the liver dysfunction and the etiologic heterogeneity of chronic hepatitis B virus (HBV) infection to metabolic dysfunction-related steatotic liver disease (MASLD) complicates the selection of the treatment and identification of its consequences [3,4,8].

1.1.1 Global epidemiology and burden of liver cancer

As per the recent global estimates, liver cancer has come to be among the commonest causes of cancer mortality because of malignancy; GLOBOCAN 2020 showed cases of liver cancer in the world of about 905,700, and deaths of over 830,200 cases in a year, and it is ranked as number 6 best diagnosed cancer and number 3 leading cause of cancer death [1, 2]. The highest average

burden is in East Asia and sub-Saharan Africa, yet the rise is witnessed in the Western countries, which are being blamed on the risk of metabolism and MASLD/MASH (formerly NAFLD/NASH) [2,8]. The overall trends of liver diseases accentuate the scope of the problem: liver diseases lead to Cirrhosis and HCC are the leading causes of mortality that results in the death of a relative 2 million people annually [9]. Despite increased development in the treatment, late presentation and low survival of advanced HCC is widespread within a 5 year period in most facilities [3,4].

1.1.2 Pathogenesis and risk factors of HCC

The HCC pathogenesis is convergent genetic and epigenetic as well as microenvironmental solutions in an opportunity of chronic hepatic injury and inflammation. Persistent HBV or HCV infection, alcohol-associated liver disease, and MASLD/MASH are the major global encouragements with HBV having a singular oncogenic force given virus DNA fusion into the human genome, genome destabilization and pro-tumorigenic alterations in transcription [10]. Aflatoxin diets also put people in danger of cancer, and are synergistic with chronic HBV in the endemic areas [13]. A biologic rationale of immune checkpoint blockade and other immunomodulatory activities is induced by the tissue microenvironment (TME) which helps sustain immune evasion and additional advancement of tumors through immunosuppressive cell populations inside the tumor microenvironment (e.g. regulatory T cells, myeloid-derived suppressor cells) as well as inhibitory cytokine networks [14,15]. The greater contribution of MASLD/MASH, which by 2023 consensus of multisocieties will have the capability to concentrate on metabolic malfunction, highlights the interplay between cardiometabolic danger and hepatocarcinogenesis and highlights fresh etiologic patterns in the majority of nations with high incomes [8].

1.1.3 Limitations of conventional treatments (surgery, radiotherapy, chemotherapy)

These potentially curative therapies as liver transplant, surgical resection and ablation are restricted to the minority of patients discovered at

the very early or early Barcelona Clinic Liver Cancer (BCLC) stages and having enough hepatic reserve [3,4]. The recurrence rates of intrahepatic metastasis and de novo carcinogenesis in a cirrhotic field are high [5070 in resected patients as early as 5 years] [3]. Transarterial chemoembolization (TACE) can also be used to provide survival benefits of an intermediate stage that is selected properly, but results in disease left palliative and recurrent doses can result in worsening the state of the liver [3,4]. The patients with the cirrhosis have a decreased response to and tolerance of traditional cytotoxic chemotherapy and the first generation systemic-TKIs (e.g., sorafenib) have shown very little survival advantage with no small amounts of toxicity and low response rates [4,17]. All these limitations to a greater extent led to the desire to find treatment that would not only be able to regulate the biological processes of tumors but also to understand the limitations of the impaired hepatic system itself.

1.1.4 Emergence of targeted and immunotherapeutic approaches

Since 2020, Immunotherapy-based regimens have already been shown to be a first-line option in unresectable HCC. In a trial (IMbrave150) that proceeded to form a new standard in patients who are eligible and confirmed to have dual interventions with ICI-anti-VEGF drugs capable of remodeling the TME and normalizing the vasculature, there was improved overall survival and progress-free survival with atezolizumab and bevacizumab over sorafenib [5]. Other ICI-based first-line regimen included HIMALAYA (tremelimumab one priming and durvalumab) which has the long-lasting survival benefit and has shown the survival benefit with a long follow-up [6,7]. TKIs such as lenvatinib would be relevant in patients who cannot get immunotherapy or anti-VEGF therapy, and the series in the real-world back the high level of activity and tolerable adverse events profile [18]. TKI combination groups and sequences with ICIs (with nivolumab-ipilimumab) and anti-angiogenic therapies are being optimized and these are guided by new biomarkers, safety concerns (i.e. the risk potential of 10 mg per kg variceal bleeding with anti-VEGF), as well as by

practice-guideline dictate [3,4,19,20]. A drastic turn of this topography brings out a paradigm shift towards the use of single-agent TKIs towards the more profound knowledge of the mechanism based combinations that can magnify the tumor intrinsic pathways as well as the immunologic milieu.

2. MOLECULAR BASIS OF HEPATOCELLULAR CARCINOMA

The hepatocellular carcinoma (HCC) molecular pathogenesis is multifaceted consisting of genetic mutations, epigenetic alterations, and tumor microenvironment (TME) changes. Such malpractices on the part of these molecules cause hepatocyte change, tumor genesis, development and immunoevasion. The intricacy of oncogenic signaling and immune environment of HCC is now being revealed and it is possible to embark on precision-targeted and immunotherapeutic interventions [21], [22] taking into consideration high-throughput sequencing, transcriptomic profiling and multi-omics analyses in the past few years.

2.1 Genetic and Epigenetic Alterations in HCC

The HCC is characterized by a broad set of genomic alterations that involve the signaling pathways of oncogenes, cell cycle, as well as apoptosis and DNA repair route. Recurring mutation of driver genes as in the case of TP53, CTNNB1, AXIN1 and TERT promoter is reported in the next-generation sequencing project and they contribute towards hepatocarcinogenesis [23]. Moreover, deviant epigenetic signaling (such as the DNA methylation, histone, and non-coding RNAs upheaval) constitutes one of the key control parameters of gene expression and cell proliferation in cancer [24]. Global hypomethylation events and hypermethylation of tumor suppressor genes (e.g., CDKN2A, RASSF1A) cause genomic instability as well as activity facilitating histone deacetylase on chromatin accessibility exerting oncogenic transcription activity [25].

2.1.1 Key Oncogenic Signaling Pathways (Wnt/ β -Catenin, PI3K/Akt/mTOR, MAPK)

There are several signaling pathways that play a central role in the tumorigenesis of HCC. The most frequently mutated of them is the Wnt/ β -catenin system, where mutation in CTNNB1 and AXIN1 leads to nuclear accumulation of β -catenin and proliferation regulation gene transcription [26]. It is an intramolecular pathway primarily engaged in non-inflammatory, well-differentiated subtypes of HCC as well as being associated with the immune checkpoint inhibitor (ICI) resistance [27].

PI3K/Akt/mTOR causes metabolic, survival, and angiogenic control of HCC. Loss of PIK3CA/PTEN mutation or amplification is likely to cause dysregulation, which contributes to Akt phosphorylation and the subsequent transmission of the mTOR signal through it [28]. Inappropriate stimulation of this pathway increases hepatocyte growth, anti-apoptotic and hepatocyte redistribution of metabolism that promotes tumor progression [29].

Similarly, the MAPK/ERK cascade facilitated by the RAS/RAF is involved in cell cycle and angiogenesis promotion of HCC [30]. Combination therapy tools are of importance as interaction between PI3K/Akt and MAPK signaling prompts countering signals of resistance, complicating therapeutic targeting, and this underscores the role of combination therapy tools [31].

2.1.2 Tumor Suppressor Gene Mutations and Chromosomal Instability

One of the most ubiquitous changes that have occurred in HCC is the inactivation of TP53 since it has been reported in up to 50 percent of cases and is strongly associated with poor prognosis and resistance to therapy [32]. Mutations in TP53 also impair the process of DNA repair and apoptosis that enables secondary mutations and gain of chromosomal instability to be accumulated [33]. Similarly, ARID1A and SMARCA2 are the members of the chromatin-remodeling complex called SWI/SNF, loss-of-function mutations lead to the impairment of the controls of transcription and motivation of oncogenesis [34].

Chromosomal instability (CIN) is another trait of HCC. Oncogenes and tumor suppressors are imbalanced due to frequent chromosomal disciplines (1q, 8q), loss (8p, 17p) [35]. The resultant amplifications of genes due to structural rearrangements include those of the MYC which are involved in the uncontrolled proliferation and dedifferentiation [36]. Besides, the silencing of the DNA repair genes by epigenetic mechanisms enhances the frequency of mutation and the instability of the microsatellites that bolster the malignant phenotype [37].

2.2 Tumor Microenvironment (TME) and Immune Evasion Mechanisms

The HCC microenvironment refers to an active system of immune, stromal and endothelial cells which all control the progression and response of tumors to therapy. The constant cytokine cascade associated with chronic inflammatory processes caused by viral infection, alcohol or metabolic damage, activates the hepatic stellate cell and the Kupffer cells and generates immunosuppressive conditions [38].

2.2.1 Role of Cancer-Associated Fibroblasts, Kupffer Cells, and Endothelial Cells

This mechanism of remodelling the extracellular matrix and the synthesis of growth factors (e.g., TGF- β , HGF) that facilitate tumour growth and invasion depends on the contribution of the cancer-associated fibroblasts (CAFs) [39]. Another chemokine that CAFs release includes CCL2, CXCL12 that recruit immunosuppressive macrophages as well as regulatory T cells (Tregs), another suppression of antitumor immunity [40]. The Kupffer cells that are chronic liver injury-induced residents of the liver transform to an M2-like state, releasing IL-10 and VEGF in promoting angiogenesis and immunological tolerance [41]. Another role, which is equally important, is that the existence of tumor endothelial cells contributes to the formation of abnormal vasculature, which, in turn, contributes to the occurrence of tumor hypoxia and inhibits the infiltration of immune cells [42]. Anti-angiogenic medicines such as bevacizumab also exploit this vulnerability by restoring normalcy of blood

vessels and augmenting their avenues to the immune system [43].

2.2.2 Crosstalk Between Immune Checkpoints and HCC Progression

HCC tumors signal immune checkpoints which are commonly used to evade immune surveillance. PdL1 tumor and stromal overexpression suppress T cell-PD-1 interaction that inhibits the cytotoxic T lymphocyte (CTL) elicitation of T cell exhaustion [44]. Increased T cell activity of CTLA-4, TIM-3, and LAG-3 upregulates T cells antitumor immunity, and the immunosuppressive action is facilitated by TGF- β and IL-10, which is produced by cytokines [45].

A novel mechanism of immune evasion that has been reported in recent studies is called metabolic reprogramming. Tumor glycolysis reduces glucose in the TME hence inhibiting metabolism of the T-cells, and inhibits the growth of lactate formation, block antigen presentation and dendritic activity [46]. Therapeutic targeting of metabolic pathways combined with ICIs turn out, in turn, to be a promising way to resolve the resistance [47].

2.3 Molecular Classification of HCC Subtypes

The evolving multi-omics has allowed the molecular classification of the HCC into various subtypes, and they solely rely on the expression of the genes and the mutation profile, and immune features. In general, there are two categories of HCC: proliferative and non-proliferative with specific causes (molecular) and consequences of treatment [48]. The high chromosomal instability, vascular invasion, and PI3K/Akt and IGF activation are high in the proliferative subtype that is amplified with TP53 and FGF19 [49]. This form is commonly associated with low differentiation as well as low prognosis.

The non-proliferative subtype, in its turn, is usually associated with the mutations in CTNNB1 and the activation of Wnt/ β -catenin suggesting high differentiation and less aggressive behavior [50]. An additional division that Zimmerman et al. (2018) assigns HCC is an immune-high and immune-low phenotype, with the former one being notable accumulation of immunity, high sensitivity to ICIs, and the latter being immune exclusion and resistance [51]. The synthesis of

transcriptomic, proteomic and metabolomic data has been maintained to complement these domains enabling patient-derived therapeutic methods to adhere to the vision of precision oncology [52].

3. IMMUNOTHERAPY IN HEPATOCELLULAR CARCINOMA (HCC)

Immunotherapy has changed the treatment practice of hepatocellular carcinoma (HCC), and provided the patients with vehement liver cancer with very limited treatment options in the past with a new sense of hope. The immunogenic character of HCC interpreted by a chronic inflammatory response during the liver and infection with the hepatitis b virus is an environment in which immunological checkpoint inhibitors (ICIs) may contribute to the regeneration of antitumor immunity in worn out T cells [53]. With the discovery of ICIs against programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) since 2017, they have led to impressive clinical improvements together with a number of regulatory approvals and combination regimens that have overshadowed patient survival to a significant degree [54], [55]. Nevertheless, heterogeneity of responses, immune-mediated toxicities and resistance mechanisms are the main concerns, which continue to affect the focus of the research in the field.

3.1 Immune Checkpoint Inhibitors (ICIs)

The agents of immune checkpoint are targeted at the tumor microenvironment (TME) T-cell control mechanisms. The two common types of inhibitory pathways used are the PD-1/PD-L1 and CTLA-4 which have been exhaustively studied within the environment of immune tolerance [56] in chronic inflammation of the liver. Such inhibitors are now considered vital components of the modern HCC therapy in both mono and combination with the targeted or locoregional therapies by requiring clinical trial and real-life studies [57].

3.1.1 PD-1/PD-L1 Inhibitors (Nivolumab, Pembrolizumab, Atezolizumab)

PD-1/PD-L1 inhibitors have demonstrated significant clinical efficacy in advanced HCC and were associated primarily with cytotoxic T lymphocytes reactivation and immune suppressive signal regulation [58]. Nivolumab one of the early ICIs that were used in HCC is a fully human IgG4 antibody to PD-1. Phase I / II CheckMate 040 trial mentioned sustained responses and controllable side effects and prompted the early Structure to FDA approval of patients who had been previously treated in 2017 [59]. The second CheckMate 459 phase III trial which was successfully able to compare the use of nivolumab and sorafenib but which could not meet its pre-specified overall survival LTH endpoint achieved clinical meaningful improvements in patient subgroups with positive response of the immune reaction [60].

Pembrolizumab is another PD-1 blocker and it has demonstrated to be equally effective. KEYNOTE-224 trial revealed the objective response rate (ORR) of approximately 17 and that over long durations of disease control in some of the patients [61]. However, according to Both studies KEYNOTE-240 and KEYNOTE-394 slight improvement over placebo is demonstrated in the OS, and there is a possibility that the change can be achieved only with the help of monotherapy in specific populations [62]. Some of the predictive biomarkers at the current research are the expression of PDL-1, the tumor mutational burden (TMB), and the presence of both the act of active invasion of CD8+ T-cells [63].

The IMbrave150 research trial combination of atezolizumab, a PD-L1 and bevacizumab (anti-VEGF antibody) was the first discovery study to greatly prolong the OS and progress-free survival (PFS) compared to the first-line studies with sorafenib [64]. Besides inhibiting angiogenesis, bevacizumab is a tumor vasculature-normalizing immunomodulator which induces immune intrusion together with PD-L1 [65]. This long-lasting effect of atezolizumab in another combination with bevacizumab has demonstrated its efficacy on multiple real-life follow-ups and is now the standard in the form of a first-line monotherapy in advanced HCC [66].

3.1.2 CTLA-4 Inhibitors (Tremelimumab, Ipilimumab)

The CTLA-4 blockers that were operational in the priming stage of T-cell activation have been operational in HCC as a single agent or together with PD-1/PD-L1 blockers [67]. Tremelimumab is a totally human and IgG2 monoclonal antibody targeting CTLA-4 which demonstrated the capacity to suppress tumours in a NCT01008358 trial with a decrease in hepatitis C viral load serving as sign of the total recovery of antiviral immunity [68]. The clinical utility also became solidified on the basis of the HIMALAYA trial: the STRIDE regimen (single priming dose of tremelimumab with maintenance with durvalumab) in terms of a median OS of 16.4 months, more heavily the first time of any immunotherapy-based combination, much better compared to sorafenib, and as a novel combination of immunotherapy-based combinations in the first line of therapy [69].

A second CTLA-4 inhibitor, ipilimumab is being tested with nivolumab in CheckMate 040 cohort 4 study, with the combination of the two having an mouth-watering ORR 32% and a median OS of 22.8 months, one of the highest in advanced HCC [70]. It is established that the dual blockade boosts the antitumor immunity by not only promoting the growth of T-cells (CTLA-4 inhibition) and overcoming exhaustion (PD-1 inhibition), but immune-related adverse events (irAEs), such as colitis, dermatitis, and hepatitis, persist [71]. Additional streamlining of dosage schedules that aim to provide balance between efficacy and acceptability is also being sought particularly among the patients who have pre-existent hepatic dysfunction [72].

3.2 Combination Immunotherapy

Since single agent ICIs can only work when applied to a small number of patients, the combination immunotherapy mode of action has been derived to enhance immune activation so as to overcome resistance and achieve longer term clinical outcomes [73]. Examples of such combinations are dual checkpoint blockade and ICIs with anti-angiogenic agents, targeted therapies or locoregional therapy i.e. transarterial chemoembolization (TACE).

3.2.1 Dual Checkpoint Blockade Strategies

Dual immuno-check blockade uses the independent action of PD-1/PD-L1 and CTLA-4 inhibition of a stronger and sustained antitumor immune response. Nivolumab combined with ipilimumab in CheckMate 040 Cohort settled the rate of improvement as compared to monotherapy and the level of toxicity was manageable when the immunosuppressive interventions were employed effectively [74]. Similarly, there have been active trials of a combination of durvalumab and tremelimumab (including HIMALAYA and EMERALD-3), with more protracted OS and improved quality of life outcomes [75].

Mechanistically, CTLA-4blockade facilitates the activation and priming of naive T cells within the lymphoid tissues and the blockage of PD-1 re-charge the exhausted T cells at the tumor microenvironment [76]. Such a synergistic effect expands the repertoire of effector T-cells and intratumoral infiltration and turns cold tumors to hot immunogenic tumors with a greater chance to react to immune-attack [77].

Even though the possibility of dual blockade is enormous, there is also an augmentation in immune-associated toxicities. This unwanted event is observed between Grade 3 or greater including adverse events like autoimmune hepatitis and gastrointestinal inflammation up to 40, and this should closely select patients and use multidisciplinary treatment [78]. The ongoing clinical trials are focused on dosage regimen, sequencing, and novel checkpoint markers such as LAG-3 and TIGIT to get enhanced outcomes with fewer side effects [79].

3.2.2 Immunotherapy Combined with Targeted Therapy or TACE

One of the changes that have been observed in managing HCC is immunotherapy in conjunction with targeted therapy. The tumor vascularity can be reconfigured by anti-angiogenic agents such as bevacizumab, lenvatinib, and cabozantinib to allow the invasion of T cells and inhibit immunosuppression through hypoxic-mechanisms that can supplement immune checkpoint-based inhibitions [80]. Since the clinical data of LEAP-002 (lenvatinib plus pembrolizumab) and COSMIC-312 (atezolizumab

plus cabozantinib) trials reveals a potential of the synergism effect, it is necessary to maintain the balance between the toxicity so far [81].

The use of ICIs is also actively explored, in association with a locoregional treatment method that can be TACE or radiofrequency ablation, (RFA) besides the explicit use of agents. These reactions will activate immunogenicity cell death and release of tumor-associated antigens, and this is the effect of causing the liver to become a form of an in-situ vaccine to enhance systemic immunity [82]. The preliminary results show that a sequential or concomitant use of ICIs in conjunction with TACE leads to a higher survival rate without relapse and immune activation in the organism [83]. The most appropriate sequencing and timetable of such strategies are still yet to be known as combining randomized controlled trials such as EMERALD-1 and KEYNOTE-937 keep enlightening these treatment methods [84].

Despite these advances, there are still several unresolved questions including finding reliable biomarkers to forecast treatment response concerns, ways to address immune related toxicities in cirrhotic patients, and how to address primary resistance mechanisms. The adoption of multi-omics data, artificial intelligence, and liquid biopsy tools will provide the avenue to diagnose the patient better and introduce real-time monitoring of the immunotherapy efficacy [85].

3.3 EMERGING IMMUNE MODULATION STRATEGIES

The immunotherapy of hepatocellular carcinoma (HCC) development has not merely stopped at immune checkpoint blockade but has engaged in new immunomodulatory modalities, and the goal associated with the development of lasting and tumor specific immunity. Because the liver is immunotolerant and the tumor microenvironment (TME) is heterogeneous in HCC, these ecological innovations have formed a fruitful new approach of using the cancers vaccines, adoptive cell therapy (ACT), and oncolytic virus (OV)-based immunotherapy to complement or obviate resistance to the current checkpoint inhibitors [86]. The foregoing approaches circumvent the distinct immunologic voices -i.e., tumor antigen presentation and cell

manipulations related to immunology and oncolysis by viruses - to augment both the innate and adaptive antitumor immunity [87]. One of the frontiers of the era of precision immuno-oncology of liver cancer is the adoption of novel therapies into the clinical systems.

3.3.1 Cancer Vaccines and Neoantigen-Based Therapies

The vaccines being given in curing cancer are meant to induce a specified type of immune reaction to tumor-related antigens (TAAs) or to tumor-appropriate neoantigens (TSNAs), changing the immune system and restoring it to the quantity of antigens it is intended it can find; the malignant hepatocytes that are neoplastic. TAAs, which have been prioritized in creating vaccines in HCC are alpha-fetoprotein (AFP), glypican-3 (GPC3), telomerase reverse transcriptase (TERT), and NY-ESO-1 that are strongly overexpressed in the tumor cells but very minimal in the normal hepatocyte cells [88].

Preclinical trials with peptide based vaccines targeting either AFP or GPC3 indicate that they are safe and immunogenic, but with poor clinical efficacy as a result of immune suppression in the TME and antigenic heterogeneity [89]. This area has been revitalized in recent times by the development of neoantigen-based vaccines, which use next-generation sequencing to identify the tumor specific-mutation upon which it is based. Immunotherapeutic therapy with individual mutagenic antigens can result in robust responses of both CD4+ and CD8+ T-cells, which are specific to the mutational topography, and are an accurate predictive response to immunotherapy [90].

Incidentally, pilot study has shown that a multi-epitope GPC3 peptide vaccine per se induced quantifiable tumor therapies and prolonged recurrence-free recurrence following surgery in patients with early-waged hepatocellular carcinoma [91]. Similarly, outdoor outcomes have been indicated of GPC3 or AFP peptide-loaded dendritic cell (DC)-based vaccines with enhanced and augmented T-cell reactions and cytokine discharge in phase I/II studies [92]. The other type of vaccine is the mRNA based vaccines that are quick to achieve and have multiple antigens

coding capacity. Compared to peptide vaccines, the preclinical trials against mRNA-LNP GPC3 vaccines have shown maximum antigen presentation and triggering of immune response [93].

ICI Cancer vaccines used in combination with ICIs have been found to be synergistic in countering immunosuppressive signaling in the TME. In one case, GPC3 and TERT vaccines have been found to be more efficient together with PD-1 inhibitors since vaccines cause T-cell exhaustion [94]. Moreover, adjuvant agonists prime in the form of agonists to toll-like receptor (TLR) and STING pathway are being added to boost procedures of native immune priming and ameliorate the vaccine [95]. The application of personalized mRNA and neoantigen vaccines based on peptides is a paradigm shift on its own and can offer an opportunity to achieve sustained tumor-specific control of immune memory in HCC [96].

3.3.2 Adoptive Cell Transfer (CAR-T, TILs, NK-Cell Therapy)

Another new and rapidly emerging frontier in HCC immunotherapy is adoptive cell transfer (ACT) therapy that aims at directly expanding cytotoxic immune effector populations that can individually death target tumor cells selectively. Methods of ACT are chimeric antigen receptor (CAR) T cell, tumor-infiltrating lymphocyte (TIL) and natural killer (NK) cell based therapies, they offer different mechanisms of tumor cell destruction through cytotoxicity [97].

Hematologic malignancies which are transformed by CAR-T cell therapy have been a base in the development of CAR-T in solid tumors including HCC. The socially engineered CAR-T cells may be generated with artificial receptors of the surface antigens of the malignant cells. GPC3, AFP, and MUC1 have proven to be favorable targets of CAR in HCC because it is restricted in tumor tissue [98]. Car-T cells targeted to GPC3 have demonstrated effective tumor control in xenografts models, and were capable of inducing tumor infiltration of T cells and production of IFN- release cytokines [99]. Firstly, it was actively described that the activity of anti-GPC3 CAR-T on the treatment of advanced HCC patients is partial

response and disease control, and its use is possible and safe as demonstrated by clinical trials, including NCT02905188 [100].

However, CAR-T therapy in the treatment of HCC faces several impediments, including but not limited to the expression of the immunosuppressive TME, poor sizing of tumor hepatoma trafficking, and potentials of on-target off-tumor toxicity [101]. The answer to those problems involves armored CAR-T cells (co-expressing IL-15 or PD-1 dominant-negative receptors) and locoregional delivery of CAR-T cells via hepatic artery infusion that has been shown beneficial in preclinical models [102]. There is also development of a form of CAR-T cells, so-called dual-targeting, which destroys GPC3 and AFP simultaneously in order to eliminate antigen escape and increase clearance of the tumor [103].

Besides the CAR-T TIL therapy uses autologous lymphocytes that are isolated after tumor unlike the lymphocytes that are drawn off ex vivo and recirculated to boost the immune system. Although HCC is still at an early stage of its development, the TIL therapy demonstrated a potential and an ability of long-term reactions in small randomized clinical trials [104]. Similarly, NK-cell immunotherapy. NK-cells have a non MHC-restricted killing capacity that facilitates adaptive immunity. Clinical grade NK cells that have been expanded ex vivo or are isolated with cord blood have demonstrated antitumor activity, as well as, good safety in the treatment of HCC [105]. Furthermore, CAR-engineered NK cells (CAR-NK) are being studied with improvements in their efficiency, GPC3-CAR NK cells are highly cytotoxic and have reduced cytokine release syndrome (CRS) in comparison with CAR-T treatment [106]. These developments are a development in the direction of even more personalized, even cell-based immunotherapy platforms that can be tailored to HCC heterogeneity.

3.3.3 Oncolytic Virus Immunotherapy

Oncolytic virus (OV) is a new type of treatment which consists of direct tumor killing and subsequent activation of the immune system. OVs are viruses that have been genetically engineered

or identified in nature and which selectively replicate in the cancer cells but not in the normal hepatocytes [107]. This causes immunogenic cell death that releases tumor antigens and danger-associated molecular patterns (DAMP) changing the TME into a proinflammatory condition that facilitates immune stimulation [108].

A number of viral vectors including adenoviruses, vaccinia viruses, herpes simplex viruses (HSV) and reoviruses have been demonstrated in preclinical and clinical premodels of HCC. In one case, JX-594 (Pexastimogene devacirepvec) GMC-stimulating factor is a genetically engineered vaccinia virus demonstrated to have significant tumor regression in phase II studies [109]. This virus leads to oncolysis and priming of the immune system resulting in increased incursion by CD8 + T cells into the tumor microenvironment and NK cells [110].

Recent studies have more aggressive efforts into the implantation of OVs with immune-stimulatory genes such as IL-12, PD-1 antagonists or costimulatory ligands to enhance their antitumor potential [111]. Indicatively, mini antibodies (PD-L1) oncolytic adenoviruses have been shown to be highly effective as compared to the traditional viruses in the inhibition of local immunosuppression and subsequent systemic immunity following the administration of the virus [112]. Also, preclinical trials indicate synergies with combination treatment combining OVs with either anti-angiogenic agents or ICIs. JX-594 with nivolumab has increased the T-cell infiltration and prolonged the survival in test HCC models, which provides a foundation to proceed with clinical testing [113].

The capability of OV therapy to assist in reworking of the immunologic landscape of a generally tolerogenic organ, the liver, is a significant attribute of OV therapy. OVs can potentially turn tumors with cold immune phenotype to hot immune lesions by causing local cytokine storm and type I interferon signaling [114]. In order to maximize the localization model of viruses and decreasing system toxicity, innovative routes of delivery like intra-arterial, and ultrasound guided intratumoral are in study now [115].

Even though the promising results were achieved, the issues of antiviral immunity, low rates of

intratumoral spreading, and hepatic inflammation should still be addressed. Correspondent strategies are being developed using carrier cell delivery (e.g. MSCs or monocytes) and nanoparticle encapsulations to increase viral persistence and targeting specificity [116]. OV therapy with other immunodegrading agents thus constitutes a bright future as far as next-generation immunotherapy of HCC is concerned.

4. TARGETED THERAPIES FOR HEPATOCELLULAR CARCINOMA (HCC)

Hepatocellular carcinoma (HCC) is the cancer that has traditionally been chemotherapy resistant to cytotoxics but with the evolution of the molecularly targeted therapy approach to the disease, the treatment mode has changed dramatically. The genetic and molecular heterogeneity of HCC has predetermined the contribution of tyrosine kinase inhibitors (TKIs) and anti-angiogenic drugs to the systemic treatment staple as the single agent or in combination with immunotherapies [117]. They act by disrupting the signaling pathways that are notable in the development of tumors, cancer angiogenesis and metastasis, such as, the vascular endothelial growth factor (VEGF), the fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and the Ras / Raf / MEK cascades [118]. Despite the positive change in the initial results of survival, the recent decade has seen the continuous improvement of dosing, sequencing and combination therapy prove to be effective at providing the patients with the advanced stage of HCC with a better opportunity [119].

This comes as a result of the fact that the enzyme has four tyrosine-binding sites capable of regulating other proteins that turn out to have the tyrosine kinase activity (Charles et al., 2007).

The therapeutic effect of the HCC occurs primarily as a result of the inhibition of tumor cell angiogenesis and growth, which is the first successful family of targeted therapeutic drugs of the first promising and effective drug, TKIs. They behave by preventing in an inhibitory manner in the tyrosine kinases ATP-binding sites that prevent downstream signaling during oncogenesis. The appearance of the TKIs began with sorafenib, then lenvatinib, regorafenib, and cabozantinib

completely changed the situation with the advanced disease, extending the survival and providing clinically significant results [120].

4.1 Tyrosine Kinase Inhibitors (TKIs)

A benefit in survival in advanced hepatocellular carcinoma was demonstrated overall with Sorafenib, a multikinase inhibitor of Raf kinases and angiogenic receptors, such as VEGFR-2 VEGFR-3, and PDGFR-b. The historic SHARP trial demonstrated that sorafenib extended the median life span of 7.9 months to 10.7 months compared to placebo hence creating a significant stride in the therapy of HCC [121]. Mechanically, Raf/MEK/ERK tumor cell proliferation and angiogenesis inhibition in the VEGF and PDGF methodical stimulation are repressed by sorafenib [122]. It also controls the tumor microenvironment (TME) by suppressing the generation of pro-inflammatory cytokines and promoting immune survey [123].

The use of sorafenib is, however, limited by early resistance development including activation of other pro-survival signals like PI3K/Akt; induction of epithelial-to-mesenchymal transition (EMT) [124]. The hypoxia has been related to activation of HIF-1alpha and reorganization of the metabolism of the TME that reduces drug penetration and cancer infiltration by immune cells [125]. In order to resolve these drawbacks, combination therapies using immune checkpoint discouraging agents, or metabolic discouraging agents are under consideration [126].

Lenvatinib is the more recent treatment of the type that has been introduced to serve as an alternative first-line treatment basing on the results of the REFLECT trial according to which both drugs have no reduced outcomes, and lenvatinib has a superior progression-free survival (PFS) and objective response rate (ORR) [127]. Lenvatinib inhibits VEGFR1-3, FGFR1-4, PDGFRalpha, RET and KIT in combination not only to deal with angiogenesis but also with fibroblastic supported tumor development [128]. It was also preclinically proven that lenvatinib can modify tumor cabinets by a model of tumor vasculature, enhance T-cell infiltration, and enhance antitumor immunity, providing it with a rationale

to be combined with PD-1 blockers, such as pembrolizumab [129].

There was accumulated clinical evidence to demonstrate the superior ORR, which ranges between 24 and 9 percent (lenvatinib versus sorafenib) and superior disease control in patients with good hepatic conditions (Child-Pugh A) [130]. Another factor that should be noted is that lenvatinib is working in the FGF mediated resistance, which puts it in an excellent position in patients who are already resistant to sorafenib [131]. However, there are other common side effects such as hypertension, proteinuria, fatigue, which need close observation of the patient and decrease the dosage [132].

4.1.2 Second-Line TKIs (Regorafenib, Cabozantinib)

Second-line TKIs, such as regorafenib and cabozantinib, have been shown to be significant therapeutic agents with robust phase III trial data with sorafenib evolution.

Regorafenib structurally related to sorafenib, but with increased specificity and inhibition of VEGFR1-3, TIE2, RET and FGFR1-2, and oncogenic kinases, which is proliferative and metastatic [133]. RESOURCE trial has demonstrated the efficacy of the trial in the form of the median OS (10.6 months vs. 7.8 months in placebo) and PFS which substantiates its efficacy in the patients who have been successfully using sorafenib before [134]. The broader spectrum of effects of regorafenib on targeting the angiogenesis inhibition allows the drug to not only disrupt the mentioned escapes but also regulate the polarization state of the macrophages in TME, which further enhances antitumor immunity [135]. In addition, regorafenib was reported to have a synergetic effect with the PD-1 blockade, the effect of restoring cytotoxic T- cell-mediated immunotherapy, and reversing immunosuppression by sorafenib [136].

Cabozantinib is another second-line agent that not only inhibits MET, AXL, RET and VEGFR2, but also applies the pathways resulting in resistance to the other TKIs [137]. The CELESTIAL trial established the efficacy of cabozantinib in the situation when there was enhancement of OS of 10.2 months over 8.0 months placebo and massive

disease control in sorafenib pre-treated individuals [138]. Cabozantinib is also not only an anti-angiogenic agent but also has a mechanistic effect in its ability to disrupt the tumor-stroma interaction as well as the epithelial-to-mesenchymal signaling, which is a necessary process in invasion and metastasis [139].

It can be stated that there is empirical evidence that suggests that cabozantinib is capable of processing immune suppression by reducing regulatory T cells (Tregs) and myeloid-derived cells (MDSCs) and thereafter by exposing them to immune checkpoint inhibitors [140]. Combination regimens, such as cabozantinib and atezolizumab (COSMIC-312), are promising with their evidence regarding the extension of the PFS and the interference with the immune response, but toxicity must also be considered [141].

In total, sequential and combination strategies including first- and second-line TKIs are continuously being refined, depending on the parameters of biomarkers resistance biomarkers and liver functions that make it possible to optimize patient outcomes [142].

4.2 Anti-Angiogenic Therapies

The pathogenesis of HCC is defined by angiogenesis which is instigated by the overexpression of VEGF and receptor overexpression prime in the first place. The process of tumor vascularization contributes to the delivery of nutrients and avoidance of immunities due to the creation of a hypoxic and immunosuppressive microenvironment [143]. Therefore, the anti-angiogenic agents of VEGF/VEGFR signaling now form an integral component of the HCC treatment, either as a single therapy or in conjunction with immunotherapy regimens.

4.2.1 VEGF/VEGFR Inhibitors (Bevacizumab, Ramucirumab)

A deficiency of vitamin D is an immune checkpoint blockade complied with by bevacizumab, a monoclonal antibody against VEGF-A, which suppresses the process of tumor neovascularization, screens the vascular vasculature to normal, and increases immune cell penetration and drug delivery [144]. Combined

with atezolizumab (anti -PD-L1, IMbrave150) bevacizumab sets a new standard of care, as it enhances both OS and PS compared to sorafenib administered as the first line of treatment, which proves that this method of treatment is superior to the previous one [145]. Bevacizumab is also able to enhance the normalization of tumor vasculatures that favors cytotoxic T-cell trafficking, and suppressed immunosuppressive cytokines such as IL-10 and TGF- β that have been reported to inhibit T-cell growth and tumor immunity [146]. Besides combination regimens, bevacizumab monotherapy did not prove to be effective and Synergistic immune modulation is especially essential. The long-term benefits of atezolizumab /bevacizumab when used in combination therapy, however, is still being proven in practical research but particularly among the people who had the viral-associated HCC and had not lost their liver functionality [147]. However, caution should be observed due to the gastrointestinal bleeding, blood pressure due to portal hypertension and the varices of the esophagus [148].

The other evidence-based intervention of patients undergoing a progression after sorafenib is Ramucirumab, which is a human IgG1 monoclonal antibody targeting VEGFR2. There is evidence using alpha-fetoprotein (AFP) ≥ 400 ng/mL as second-line treatment has better OS in the REACH-2 trial [149]. VEGFR2 selective blockage with ramucirumab allows its use in hepatic impaired patients compared to TKIs, which inhibit a range of different kinases and are therefore better tolerated [150].

Mechanistically, ramucirumab prevents endothelial growth and angiogenic interactions and reinvigorates cellular immune infiltration in the TME [151]. Preclinical models have shown that VEGFR2 block will improve the activity of PD-1 blockers by inhibiting hypoxia and increasing the maturation of the dendritic cells [152]. The current clinical trials cover combinations of ramucirumab with the checkpoint inhibitors such as pembrolizumab which is showing the initial synergy in refractory HCC [153].

The anti-angiogenic therapies put together continue to play an important role in the management of HCC be it in monotherapy or in

combination with immunotherapy. The fact that they have the ability to remodel their tumor vasculature and can overcome immunosuppression gives them a powerful foundation on which to enhance the efficacy of therapy in advanced disease [154].

4.3 SIGNAL PATHWAY INHIBITORS

The development of hepatocellular carcinoma (HCC) also requires the involvement of numerous aberrant intracellular signaling cascades that regulate proliferation, apoptosis, angiogenesis and metabolism. These include the PI3K/Akt/mTOR, MAPK /ERK and epigenetic regulation signatures which play a role in sustaining an oncogenic transformation and drug resistance [155]. The attack of these signaling nodes is now an attractive mode of therapy particularly in patients who have already developed a response to mentions of tyrosine kinase inhibitors (TKIs) or immune checkpoint inhibitors (ICIs). Recent studies have determined that the intracellular signaling could be blocked to reprogram the immune microenvironment instead of simply inhibiting tumor cell growth to enhance immunotherapy efficacy [156]. The next part presents the argument regarding the new advances concerning mTOR inhibitors, epigenetic modular, and combination targeted-immunotherapy plans that all define the new paradigm of precision oncology of HCC.

4.3.1 mTOR Inhibitors and Downstream Signaling Modulation

Mammalian target of rapamycin (mTOR) is a major cell metabolism, cell growth and survival controller which acts as an integrator of various stimuli, such as nutrient availability, energy and growth factor -cues. The HCC disregard of the PI3K/Akt/mTOR signaling is the most frequent molecular occurrence reported in approximately 40-50% of patients [157]. Mutations of PIK3CA, PTEN loss and Rheb overexpression are constitutive activations of mTOR complex 1 (mTORC1) leading to unregulated proliferation and anti-apoptotic properties [158].

First animal clinical experiments of rapamycin analogs (rapalogs) everolimus and temsirolimus demonstrated that pharmacologic mTORC1

sequestration could prevent HCC progression and angiogenesis [159]. The EVOLVE-1 trial initially compared everolimus to those patients who had already progressed with HCC following sorafenib, and with adjustment of stabilization of the disease, but not with elevation of overall survival (OS). Nevertheless, the increased translational data demonstrated that mTOR inhibition could precondition tumours to the other treatment forms by adjusting autophagy and reducing the survival signals that were induced by hypoxia [160].

The recent advance has focused on dual mTORC1/mTORC2 inhibitors and PI3K/mTOR dual inhibitors, which provide the pathway with more restricted inhibition and surpass resistance to rapalog monotherapy. As an example, vistusertib and BEZ235 were presented as potent antitumor effects in models of the treatment of HCC at the same time blocking mTOR phosphorylation and downstream signaling proteins (i. e. the S6K1 and 4E-BP1) leading to inhibition of protein synthesis and growth [161]. Besides, the inhibition of mTOR was proved to strengthen the generation of T-cells, cut short the development of regulatory T-cells and has been shown to be additive to the immune checkpoint inhibition [162].

The therapeutic implications of the metabolic reprogramming created by means of inhibition of mTOR are also exclusive to the present study. The glycolysis and glutamine metabolism processes stimulate the growth of HCC cells, which is an activity of the mTORC1 signaling. These catabolic dependencies are disrupted through t-learning the mTOR resulting in a reduction of lactate synthesis, restoring the immune cell functionality of the tumor micro-environment [163]. There are also clinical trials of combined mTOR blockers alongside the anti-PD-1/PD-L1 (e.g., everolimus and nivolumab), where hopefully the immunometabolic synergies can be utilized to furnish durable responses [164].

4.3.2 Epigenetic Modulators and Histone Deacetylase Inhibitors

As a key factor in the pathogenesis of hepatocarcinogenesis, epigenetic regulation, including DNA methylation, histone alterations,

and noncoding RNAs, is involved. The epigenetic modifications are able to silence tumor suppressor genes, activate oncogenes and enable immune evasion without affecting the DNA sequence [165]. The given mechanisms are especially applicable to HCC where chronic inflammation, a viral infection, and oxidative stress may stimulate extensive epigenetic remodelling which promotes the further multiplying of tumours [166].

One of the most important enzymes of epigenetics, histone deacetylases (HDACs), deacetylate histone tails, which partially causes the condensation of chromatin and transcriptional repression. HDAC1, HDAC2 and HDAC3 have been found to be highly overexpressed in HCC and have been associated with poor prognosis, metastasis, and resistance to treatment [167]. The HDACis (vorinostat, belinostat, panobinostat) have been able to reopen silenced tumor suppressors, induce apoptosis and suppress angiogenesis [168].

As an example, the panobinostat therapy of HCC cell lines achieved the upregulation of p21, BAX and E-cadherin, and growth inhibitor and reverted epithelial-to-mesenchymal transition (EMT) [169]. Besides, HDACis may alter the immune microenvironment of tumors by amplifying the major histocompatibility complex (MHC) and tumor-associated antigens expression, improving T-cells recognition [170]. The use of the combination therapies of HDACis and ICIs has demonstrated good synergies in preclinical models, where the HDAC blockade amplified the expression of PD-L1 and antigen presentation [171].

Other epigenetic targets are being actively pursued besides HDACs and include DNA methyltransferases (DNMTs) and bromodomain and extraterminal domain (BET) proteins. Two DNMT inhibitors, azacitidine and decitabine, have shown effectiveness in the inhibition of silencing of tumor suppressors such as CDH1 and SOCS1 mediated by methylation and the reestablishment of normal hepatocyte gene expression patterns [172]. Likewise, BET inhibitors, including JQ1 and OTX015, disrupt chromatin rearrangement and transcriptional elongation of oncogenes like MYC that cause a decrease in tumor growth [173].

The future of epigenetic modulators combined with TKIs or immunotherapies is bright. It has been demonstrated that HDAC inhibition and BET inhibition can be used to boost the effects of PD-1 blockade and sorafenib sensitivity, respectively, by inhibiting autophagy and angiogenic signaling/enhancing immune gene transcription [174]. Dual inhibition of HDAC and PD-1 pathways in the treatment of HCC is in clinical trials, which is a major advance towards combined epigenetic-immunotherapy approaches [175].

4.4 Combination of Targeted and Immunotherapies

Implementation of both guided therapy and immunotherapy is a contradiction of progressive HCC therapy. The synergistic balance of these modalities is followed by the fact that the mechanisms are complementary i.e. particular agents to control tumor vasculature, metabolism and immune inhibition, whilst ICIs recover adaptive immune surveillance [176]. All of these factors together result in the transformation of an immunologically cold HCC microenvironment to a hot microenvironment that is more infiltrated and with less inhibitory signaling by immune cells. The combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) is the most successful and has proved its superiority in terms of survival benefits in the IMbrave150 trial and is the first example of immunotherapy-based standard of care in advanced HCC [177]. Bevacizumab mechanically replicates abnormal vasculature, reduces immunosuppression of permeability of hypoxia, and enhances the delivery of immune effector cells into the tumors [178]. Also, the combination of lenvatinib/pembrolizumab (LEAP-002 trial) and cabozantinib/ atezolizumab (COSMIC-312) combinations were good at clinical activity, but the latter at a cost-benefit of toxicity, is one of the central criteria [179].

It is also known that FGF, MET and AXL inhibitors are also good immunomodulators with VEGF blockade. The pathways lead to the immune escape by facilitating Tregs infiltration and augmented PD-L1. One such action is the suppression of MET signaling by cabozantinib that

has been shown to decrease MDSCs and enhance the action of dendritic cells, and in particular the actions of the PD-1 inhibitors [180].

The association between the desired treatment-administering practice and immunologic subduction becomes enduring to the PI3K/Akt/mTOR pathway, known to exert an influence on tumor metabolism and immune trials. It has been discovered that the mTOR-PD-1 co-inhibition elevates the survival of cytotoxic T-cells and reduces the Treg-mediated inhibition in preclinical models of HCC [181]. Besides this, the integration of epigenetic agents and ICIs is becoming so promising; the HDAC and DNMT can also be used to drive up the neoantigen and therefore, clear of tumor under immunotherapy [182].

Despite these developments, there exist challenges. It is significant that biomarkers, which have predictive characteristics, like angiogenic gene signature, immune infiltrate pattern, and circulating tumor DNA, need to be created to choose the patients and track the treatment activity [183]. There would also be a need to counter the consequences of overlapping poisoning including immune-related vascular damage and hepatitis on this universal utilization of combination plans [184]. These trends are more concerned with the creation of triplets of targeted agents, ICIs, and metabolic or epigenetic microcontrollers, which would crack the tumors with immune response being adopted long term [185].

5. Biomarkers of Therapeutic Response.

The emerging selective and immunotherapeutic technology of hepatocellular carcinoma (HCC) has intensified the need to discern useful and reliable biomarkers in forecasting such treatments, treatment efficacy, and personalizing therapy. Despite the impressive clinical response in immune checkpoint inhibitors (ICIs) and tyrosine kinase inhibitors (TKIs), the response of cells to treatment by HCC remains nonhomogeneous due to their complex molecular and immunologic landscape [186]. Predictive biomarkers should therefore be discovered to allow maximum patient selection, toxicity reduction, and resistance to overcome. Biomarkers in HCC are discovered

under the multi-omics technologies as in combination with the application of sophisticated bioinformatics /liquid biopsy systems to acquire precision oncology [187]

.5.1 Predictive Biomarkers for Immunotherapy Efficacy

Systemic treatment It has been established that immunotherapy has revolutionized treatment of HCC at an advanced stage but all of them do not achieve long term responses. When making decisions regarding therapy, stratifying patients and identifying predictive biomarkers that can indicate immune activation, tumor antigenicity, and microenvironment context are required [188]. Some of the commonly used biomarkers which have been studied to determine immunotherapy responsiveness in HCC include programmed death-ligand 1 (PD-L1) expression, tumor mutational burden (TMB), or microsatellite instability (MSI) and immune gene signatures or gut microbiome composition.

PD-L1 Expression

On tumor energies as well as immune cells, PD-L1 is a popular predictive biomarker on diverse cancers. Its predictive value, though, is not always constant in HCC. When comparing T-cells, it has been shown that a high concentration of PD-L1 correlates with their infiltration and better treatment outcomes in patients taking treatment with ICIs, such as nivolumab and pembrolizumab [189]. Conversely, PD-L1-negative tumors too respond, and it implies that it does not make PD-L1 a full predictor [190]. Even more is complicated by the fact that the heterogeneity of PD-L1 expression owing to intratumor heterogeneity, different detection methods, and effects of hepatitis-inflammatory inflammation make it heterogeneous [191]. Nonetheless, PD-L1 will remain an important component of immunosignature-composite-based and other molecular variable biomarkers [192].

Tumor Mutational Burden and Neoantigen Load

Tumor mutational burden (TMB) is a neoantigen load/tumor immunogenicity surrogate, suggesting counting the number of somatic mutations in a

megabase scale. TMB tumors with high TMB are also likely to present new epitopes that can be identified by cytotoxic T lymphocytes that increases the likelihood of immunotherapy action [193]. In HCC, however, the mean TMB is significantly lower than in either melanoma or lung cancer and limits its prediction ability independently [194]. Nevertheless, some subsets of HCC that contain malfunctioning DNA repair systems or APOBEC-based mutagenesis exhibit heightened TMB, in addition to responsive effect to ICIs [195]. Neoantigen load generated by whole-exome sequencing is an enhanced fine parameter and certain studies have related neoantigen elevated presentation as a lasting reaction in ICI-treated HCC patients [196].

Microsatellite Instability and DNA Repair Deficiency

Microsatellite instability (MSI) and mismatch repair deficiency (dMMR) have been denoted as the immunotherapy responsiveness biomarkers of solid tumors. In HCC, tumors with MSI-high tendencies are very uncommon (less than 3per cent), but have an increased lymphocytic infiltration and are more highly expressed with immune checkpoint molecules [197]. MSI look is linked to genomic unsteadiness, neoantigen creation and good outcomes with PD- 1 blockage. Diagnosis and dMMR through polymerase chain reaction (PCR) or next-generation sequencing (NGS) can therefore be useful in prediction even in a smaller population of patients [198].

Immune Gene Signatures and Tumor Microenvironment

Transcriptional profiling has helped to subdivide HCC into immune-hot Whole and immune-cold based on the expression of cytokines and immune cell infiltrations. There is strong interferon-gamma (IFN- 3)-signaling in immune hot tumors, great densities of CD8 + T-cells, and immune checkpoint molecules, which comprise PD-1, LAG-3, and TIGIT, associated with enhanced ICI responses [199]. Consideratively, immune-cold tumors exhibit the occurrence of T-cell exclusion and Wnt/B-catenin sign pathway activation and consequently immune resistance [200]. Immunotherapy efficacy has been found to be

predicted through combination of immune gene expression signature i.e. CXCL9, CXCL10, GZMB into predictive models [201].

Gut Microbiome and Systemic Immune Modulation

Gut-giver axis emerges as a potential good biomarker that can determine the efficacy of immunotherapy. The control of the microbial metabolites and pattern recognition receptors (PRRs) under the control of the gut microbiota leads to the process of hepatic immunity. The genera of bacteria that have been enriched in the intestine of HCC and have been associated with increased responses to ICIs and it has been found that immune tolerance has been facilitated by the functions of dysbiosis [202]. It has been proven that the ischemic microbiota transplantation (ICI responder) resumes Immunotherapy sensitivity in non-responder patients in early clinical studies meaning that the modification of the microbiome can potentially serve as a new biomarker as well as a bio-therapeutic agent [203].

Circulating Biomarkers and Liquid Biopsy

Well-known non-invasive biomarkers currently under development as immunotherapy response monitoring include circulating tumor DNA (ctDNA), exosomes and soluble PD-L1. Quantitative changes in the number of ctDNA during ICI therapy are related to radiologic reactions and improved survival [204]. In addition, exosomal PD-L1 is a form of immune questioning and it can be applied as a dynamic biomarker of tracking the treatment process [205]. The future of the development of the latter is in the combination of real-time evaluation of immunotherapy efficacy by liquid biopsy with radiomic data and artificial intelligence-driven algorithms [206].

5.2 Molecular Biomarkers for TKI Sensitivity

TKIs continue to play a central role in systemic therapy in end-stage HCC, although the response to therapy also differs considerably across different patients. Countless insights into the molecular determinants of TKI sensitivity can help optimize the efficacy and avoid unjustifiable toxicity. VEGF/FGF pathways activation, FGF19

amplification, RAS/RAF mutations and c-MET overexpression as well as the presence of circulating angiogenic factors are biomarkers predictive of TKI response [207].

VEGF and Angiogenic Pathway Signatures

The major angiogenic and vascular permeability mediators are VEGF and its receptors (VEGFR13). An increased VEGF level at baseline is linked to improved patient response to sorafenib, as well as lenvatinib, indicating the predictive value of the VEGF pathway [208]. Moreover, angiopoietin-2 and placental growth factor (PlGF) are angiogenic factors that have been confirmed as the pharmacodynamic biomarkers of anti-angiogenic TKIs treatment [209]. Measurement at serial intervals of these markers gives information on efficacy in the treatment and the development of resistance.

FGF19 Amplification and FGFR4 Activation

Fibroblast growth factor 19 (FGF19), fibroblast growth factor receptor 4 (FGFR4) overactivation is a type of oncogenic driver in about 1015% of HCCs. Mitogenic and anti-apoptotic signaling are augmented by FGF19/FGFR4 signaling, which augments resistance to standard TKIs. According to recent insights of clinical research, patients that possess FGF19 amplification respond positively to lenvatinib, which has strong FGFR inhibition, but less responsive to sorafenib [210]. Further FGFR4 selective inhibitor agonists, like fisogatinib (BLU-554) serve to corroborate the application of this biomarker in directing selective therapy [211].

c-MET Overexpression and HGF Signaling

The HGF/c-MET axis is a vital factor in proliferation, invasion and metastasis of the hepatocyte. The upregulation of c-MET or HGF ligand has been associated with bad prognosis and resistance to sorafenib [212]. On the contrary, cabozantinib which has a great potency as an MET and AXL inhibitor has shown better efficacy in c-MET over-expressing tumours implying that MET status may be utilized as a predictive biomarker with regard to treatment choice [213]. New clinical decision-making compositions are also in development using immunohistochemical and

transcriptomic tests of MET expression to guide the TKI therapy [214].

RAS/RAF Pathway Mutations

RAS/RAF/MEK/ERK cascade is involved in cell survival and cell proliferation of HCC. The upregulation or mutations of this pathway confers TKI resistance by inducing compensatory signaling. KRAS mutations, NRAS mutations, and RAF1 amplification alleviate low sensitivity to sorafenib and tumor aggression, respectively [215]. The inhibition of parallel pathways (PI3K/ Akt or mTOR) in addition to TKIs can help overcome those in this subset [216].

Hypoxia-Induced and Metabolic Biomarkers

The expression of hypoxia-inducible factor-1 alpha (HIF-1 α) indicates intratumoral hypoxia and plays a role in the angiogenic evasion and resistance to TKI. Large HIF-1 levels are an indicator of poor response to sorafenib and regorafenib [217]. There are also metabolic biomarkers of TKI efficacy such as lactate dehydrogenase (LDH) and glutamine synthetase (GS), showing metabolic reprogramming when under treatment pressure [218].

Circulating Biomarkers for TKI Monitoring

The dynamic exercise of using biomarkers like circulating VEGF-A, soluble VEGFR-2 and angiopoietin-2 in blood has served as a biomarker to indicate the treatment response. Reductions in the levels of VEGF-A and angiopoietin-2 during treatment are associated with favourable outcomes in patients using sorafenib or regorafenib [219]. Besides, the ctDNA profiling is used to identify therapy-resistant-related gene mutations such as TP53, CTNNB1, and AXIN1 early, making it possible to make timely therapeutic considerations [220].

Combining molecular and metabolic with circulating biomarkers would allow for a more sophisticated stratification of HCC patients who are put under TKIs. The resulting approach, based on creating such biomarkers, is not only more precise in the therapy but also preconditions the rationality of combinations of regimens with immunotherapies or epigenetic modulators [221].

5.3 LIQUID BIOPSY AND CIRCULATING TUMOR DNA (CTDNA)

The liquid biopsy surface has transformed into an innovative idea in the treatment of hepatocellular carcinoma (HCC) and has become a less-invasive, dynamic, and real-time view of the tumor pathophysiology. Unlike conventional tissue biopsies, when one snap-on of the spatial and time association of the tumor is taken, the liquid biopsies allow longitudinal results of the disease, therapy reaction, and resistance in case of treatment [222]. Among the various products of blood analysis which have become dominant, such as circulating tumor cells (CTCs), extracellular vesicles (EVs), cell-free RNA (cfRNA), and circulating tumor DNA (ctDNA), the latter has been identified to be sensitive and specific in identifying changes in genes and epigenetics that are right about HCC [223].

Clinical Utility of ctDNA in HCC

ctDNA consists of small fragments of tumor pushed DNA to the blood either through necrosis, apoptosis or by an active leakage. Tumor-mutations, methylation, and copy-number changes detected in the fragments are clinically meaningful since they are essential clinical data on the disease burden and the heterogeneity of the tumor, especially in advanced HCC when the tumor may be in an inaccessible location or the liver have limited and scarce reserves [224]. ctDNA quantification and discovery are clinically significant since they give important clinical data on the disease burden and the heterogeneity of tumor as well as in advanced stages of HCC where the location of the tumor may not be easily accessible and the

As it was demonstrated, ctDNA level is closely related to tumor stage, size, and vascular invasion (Severini et al., 2017). Negative prognosis has been linked to high levels of ctDNA preferential to treatment and declines during treatment may be considered a favorable response [226]. One such biomarker of real-time therapeutic response is the dynamic decline in ctDNA mutant allele frequency (MAF), which (along with more traditional radiographic parameters of treatment response) has been proposed as a proficient biomarker of the therapeutic response [227].

ctDNA for Early Detection and Minimal Residual Disease (MRD)

CtDNA demonstrates a large potential of early HCC diagnosis as well as in the post-operative treatment of HCC. Conventional screening technology such as alpha-fetoprotein (AFP) is not sufficiently sensitive particularly in cases of small or early tumors. ctDNA methylation assays, which encompass loss or repression of methylated TERT, RASSF1A, and GSTP1, have been highly effective in distinguishing between patients having HCC and healthy groups of people as well as the situation of cirrhosis [228]. Also, minimal residual disease (MRD) that is the existence of low levels of tumor DNA after curative resection or ablation can be recognized and is capable of foreseeing recurrence months before radiologic confirmation [229]. Such characteristics will grant the option of customized adjuvant therapy and increased surveillance of the high-risk patients.

Genomic Profiling and Resistance Mechanisms via ctDNA

It is possible to characterize noninvasively actionable processes of mutant/resistance to systemic therapy in terms of genomic profiling of ctDNA. The list of the most prevalent mutations in the ctDNA of patients with HCC consists of TP53, CTNNB1, AXIN1 and TERT promoters [230]. It is worth noting that ctDNA sequencing can also suggest that the patient might be having a resistance to TKIs or ICIs, such as MET amplification, FGF19 gain, and PI3KCA mutations to make timely modifications to the treatment [231].

Innovative technologies including digital droplet PCR (ddPCR) technologies and next-generation sequencing (NGS) have been used to improve the analytical sensitivity of ctDNA detection to the level of identifying variant allele frequencies smaller than 0.1 percent, which is considering enough to trace the impacts of low-burden disease and mixed reactions to treatment [232]. Intra tumor heterogeneity is also measured by multi-region ctDNA sequencing and proves to be particularly significant in HCC where the degree of clonal diversity and evolution in the response to therapy (25) is high.

Integration of ctDNA with Radiogenomics and AI

The intersection of ctDNA technologies with imaging biomarkers is revolutionizing the process of overcoming the problem of accurate monitoring of liver cancer, which is referred to as radiogenomics. Since it has chances of predicting both biological aggressiveness and approach to treatment with high accuracy, clinicians have been able to predict mutational pattern and image beauty [234]. Currently, AI and machine learning algorithms are used to combine the data of ctDNA with radiomics and clinical parameters to make early relapse, immunotherapy responsiveness, and drug resistance predictions [235]

The ctDNA based assays have a great deal of promise in them, but possess numerous technical and biological limitations preventing their extensive use in clinics. Problems with low concentration of ctDNA at early-stage HCC, the confounding of the assay results with non-tumor-related cfDNA, and lack of a generalized assay system are the most apparent issues [236]. Besides, genetic heterogeneity of HCC complicates the decoding of ctDNA profiles and involves the standardization of panels and reference databases. Nevertheless, recent clinical trials (LIVERA and HEPALIQ) are utilising ctDNA as a predictor in order to use the data in clinical choices when faced with the surveillance and forecasting therapeutic reaction [237].

5.4 MULTI-OMICS INTEGRATION FOR PRECISION ONCOLOGY

Hepatocellular carcinoma is a complex disease because of the multi-layered molecular heterogeneity, which involves the dysregulation of the genome, epigenome, transcriptome, proteome, metabolome and microbiome. The single-omics studies have proved helpful, but not extensive enough to be able to determine how the dynamic interactions which are at play elucidate initial development of tumors, their progression and resistance to treatment therapies [238]. Therefore, the adoption of multi-omics (consisting of genomics, epigenomics, transcriptomics, proteomics, and metabolomics) systems have

become an important paradigm of precision oncology of the HCC [239].

Functional Genomics and Transcriptomics.

Much genomic evidence has been used to define HCC with obvious molecular subtypes using specific driver mutations and transcriptional signatures such as The Cancer Genome Atlas (TCGA) and ICGC studies [240]. Through the combination of the genomics and transcriptomic data, they are in a position to establish the expression patterns of the gene in regard to the oncogenic pathways including Wnt/ β -catenin, PI3K/ AKT/ mTOR and the TGF- β signaling. Provisionally, through the simultaneous analysis of CTNNB1 mutation status and transcriptomic immune signature, immune-cold phenotype type defiant to PD-1 blockade can now be predicted [241].

More deregulated non-coding RNAs (lncRNAs, miRNAs and circRNAs) deregulated by high-throughput RNA sequencing (RNA-seq) can be found to be involved in immune escape, angiogenesis and metastasis. As it is noted, the poor response to sorafenib is linked to the expression of lncRNA HOTAIR and miR-21 in particular, implying that they are the promising predictive biomarkers [242].

Epigenomic and Proteomic Profiling.

Epigenetic regulation, including DNA methylation, histone modeling and chromatin remodeling is another dimension of tumor biology which is of importance. It has been shown through integrative investigation that aberrant DNA methylation of respective promoters on CDKN2A, SOCS3 and GSTP1 genes are associated with tumor aggressiveness and resistance to treatment regimens [243]. The multi-omics technology, which describes both the methylome and transcriptome data, enables the articulation of the networks of the gene expression that may be forecasted to determine their prognosis and sensitivity to a certain drug [244]. Proteomic profiling adds information on post-translational modification and activation status of pathways to that of the genomic and transcriptomic information. Quantitative phosphorylation of mass spectrometry based

quantitative proteomics has identified treatment responsive phosphorylation patterns of kinases(s) (e.g., mTOR, EGFR, MEK) [245]. Moreover, phosphoproteomic signature has been used to stratify the patients who are likely to respond to combination TKI-ICI therapies [246].

Metabolomics and Microbiome Integration

Given that the liver plays an immensely essential role in metabolism, metabolomics can be an incompressible resource of information about the restructuring of the energy and nutrient utilization in HCC. It has been reported that the pathways of glycolysis process, glutamine metabolism as well as lipid synthesis are the determinants of TKI and ICI response in metabolomic and transcriptomic studies [247]. Such a high level of acylcarnitines and sphingolipid compared to the miserable prognosis of lenvatinib-delivered therapy are signs of oxidative stress and mitochondrial dysfunction [248].

The gut microbiome is a multifaceted and hepatic metabolism and immunity network, which is now viewed as a worldwide controller of therapeutic response. Co-sequencing of microbiomes of microbes with immune and metabolomic hypotheses that the microbial dysbiosis could mediate the metabolism of bile acids, immune tolerance, and result in immunotherapy are altered [249].

Artificial Intelligence and Systems Biology Approaches

Systems biology and artificial intelligence (AI) systems are currently being deployed in order to deal with the tremendous data complexity of integrating multi-omics. Predictive of therapeutic response, recurrence or toxicity are multi-dimensional signatures of a prescriptive response, recurrence, or toxicity and can be determined using machine learning models [250]. AI-based algorithms were already demonstrated as more accurate predictors of survival and ICI rates in advanced HCC compared to traditional single-omic algorithms [251]. Even for the cases in which the identification of a druggable interaction of molecules could not have been observed with single-layers, such modes enable it to be found [252].

Clinical Translation and Future Prospects

The oncology approach of HCC is evolving at a faster rate through initiatives such as HEPAMAP and OncoOmics-HCC which integrate atomic level of data with clinical metadata in the development of personalized therapeutic algorithms on patients [253]. On the one hand, the multi-omics approach with the liquid biopsy technology, such as ctDNA and exosome proteomics and the latter, has a high likelihood of making molecular monitoring continuous and non-invasive [254]. Further, the pharmacogenomic findings are encompassed into the omic models and can guide dose-adjustment and drug-selection policies to diminish hepatotoxicity and magnify the advantages of therapeutic intervention [255].

It is already predetermined by the integrative omic-based modeling, which will unite the accuracy of molecular data and commercial determination systems against the difficulties of the standardization of data, computational and financial structures, and expense [256]. Omics technology is now more affordable and scalable, so will someday no longer be in the research category but would be more directly integrated into every day clinical management, and this shall be a new era of personalized treatment of hepatocellular carcinoma.

6. CLINICAL TRIALS AND REAL-WORLD OUTCOMES

Systemic treatments of hepatocellular carcinoma (HCC) have achieved extraordinary advances during the past decade because of various major phase II and phase III clinical trials demonstrating the efficacy of immune checkpoint inhibitors (ICIs), targeted therapy, and combinations of them. The re-consideration of these studies has reformed the treatment regimens of HCC and it is not a disease with limited systemic options, there are a wide number of treatment options that could be used successfully at any stage of the disease [222]. However, the heterogeneity of the patients, liver failure, and a reverse situation of the patient adopting the controlled trial setting against the real-life population remain clinical translation issues. It is also imperative to comprehend the results of the trials and practical results with the

aim of streamlining the procedure of therapeutic sequencing and improving the rate of survival in this complex disease [223].

6.1 Major Phase II/III Clinical Trials in HCC Immunotherapy

6.1.1 IMbrave150 and CheckMate Studies

The IMbrave150 trial was a historical phase III trial which acknowledged a combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) as the new adjunct to the first line of unresectable HCC. It was demonstrated that the overall survival (OS) and progression-free survival (PFS) improved significantly compared to sorafenib which had been the long-standing systemic therapy [224]. This was the initial trial to document a greater than one-year survival advantage over sorafenib in the atezolizumab - bevacizumab arm [225]. Combining mechanistic effects of simultaneous blockade of VEGF-promoted angiogenesis and PD-L1-promoted immune evasion was able to remodel the tumor microenvironment (TME) into lasting immune responses [226].

Survival benefit was additionally found using follow-ups of IMbrave150 long observation suggesting sustained prolonged benefit of survival with longer observation as well as the 3-year OS rates of over 30 that had not been previously realized in advanced HCC [227]. It is interesting to note that this combination also increased the quality of life indicators, including reduced physical fatigue and a good physical functioning as an indicator of holistic clinical outcomes other than survival per se [228].

Like IMbrave150, a program named CheckMate (that is, CheckMate 040 and CheckMate 459), studied nivolumab, an anti-PD-1 agent, as a single-agent (sequentially and in combination) with ipilimumab (anti-CTLA-4). Nivolumab was given the accelerated FDA approval process in 2017 due to findings of the CheckMate 040, an enduring objective response (ORR of 15-20), and long-term disease management in patients with heavy history of previous interventions [229]. That is however not what was observed of the phase III CheckMate 459 trial, which compared nivolumab versus sorafenib as a first-line intervention, which did not meet its primary OS endpoint despite recording a

favoring hazard ratio (HR = 0.85) and a better safety profile [230].

This was succeeded by a rise in response rates to the nivolumab/ipilimumab dual immune blockade regimen used in the refractory HCC with ORR, and the median OS of 32 and 22.8 months, respectively [231]. This was cited as the reason for dual ICI regimens and the motivation for additional research tackling the combination of ICIs with TKIs or anti-angiogenic agents.

In other phase III trials such as KEYNOTE-224 and KEYNOTE-240, secondary use of pembrolizumab was suggested following the collapse of sorafenib. Although KEYNOTE-240 only failed to become statistically significant in terms of the improvement of OS pre-specified by a set of criteria, pooled analyses revealed a consistent advantage across many sub-groups [232]. These historic trials have seen the foundation of immunotherapy-based management using advanced HCC.

6.2 Success Rates and Limitations in Clinical Translation

The effectiveness of these trials is a paradigm change of the HCC management but its clinical implementation is restrained by a series of biological, logistical, and methodological limitations. First of all, the etiology (HBV, HCV, or malfunction of the liver), liver functioning, and genetic heritage of the patients population makes the patient population heterogeneous, which limits the applicability of the trial result into the real-world practice [233]. In fact, in the IMbrave150 case, the dominant Cancer type to recruit had been the Child-Pugh A patients, however in clinical practice, a significant proportion of patients had been recorded to have Child-Pugh B or C liver disorder, which influences drug metabolism and tolerability [234].

The other problems associated with the application of the anti-VEGF therapy are immune-related adverse events (irAEs) and the risk of bleeding particularly in cirrhotic patients with varices. Even though IMbrave150 also included pre-treatment variceal screening carried out through the use of endoscopy, in real-life conditions, the latter is less intense resulting in higher bleeding rate [235]. Moreover, the pricing

and infrastructure to center immune-oncology treatments remain costly in low- and middle-income countries and create disparity in access and results [236].

ICIs response of unselected HCC groups is usually 15-25 percent; therefore, there is a need to identify predictor biomarkers and the best way to select patients. WNT/ beta-catenin-activated tumors including them, *C. perfringens* exhibit immune exclusion, and no ICI responsiveness, hence the quality of molecular stratification [237]. Similarly, robust and adaptive resistance pathways like resistance by enhancing alternate immune checkpoints (e.g. LAG-3, TIM-3) and TME-remodeling are already issues [238].

Although combination strategies are quite effective, some complexity is introduced in terms of dose optimization as well as toxicity control. To illustrate, ICI or ICI-TKI regimens are prone to hepatic transaminase and hypertension necessitating a narrow margin of efficacy and safety [239]. In addition, OS and PFS, trial outcomes, do not necessarily represent clinical benefit due to pseudoprogression and slow reactions to immunotherapy [240]. These limitations demonstrate that adaptive trial designs and real-life validation are still necessary in order to optimize therapeutic algorithms.

6.3 Real-World Data on Combination Therapy Outcomes

RWE augments clinical trial evidence since it depicts outcomes of therapy in more varied and heterogeneous populations of patients including those with comorbidities and with compromised liver function of patients who are typically not enrolled in trials. Studies on massive international registries such as REFLECT-R, IMREAL and HepatoLife have provided crucial perceptions of safety, efficacy, and sequencing of immunotherapy-based mixes in the actual practice [241].

The latest multicenter trials of the atezolizumab-bevacizumab treatment regimen offered evidence of the overall survival which is more than 17 months even in the patients with reduced liver functioning or portal hypertension [242]. Importantly, real-life cohorts have shown that the discontinuation rates have improved due to

gastrointestinal bleeding, which makes it necessary to make certain about pre-emptive variceal management [243].

The registries in Asian-Pacific and European disclose that practically lenvatinib/pembrolizumab mixture has objective reaction rates in 35-40 percent of the information in the trial validates the findings of the trial, which also indicates specific dissimilarities in the sensitivity by area, which can be traced to viral etiology [244]. Equally, the results of empirical studies regarding the association between cabozantinib and atezolizumab and regorafenib and nivolumab have revealed that these combinations have synergies in particular patients who have high baseline VEGF and hepatic functioning in good condition [245].

Sequential post progression therapy too possesses an impact, which is introduced in longitudinal real-world studies. Patients who receive immunotherapy and then administer TKIs or the opposite, the median OS values contain more than 30 months that confirms this theory of immunotherapy inducing tumor sensitization to later agents [246]. Also, the RWE endorses the fact that biomarker-driven treatment, grounded on ctDNA profiling, PD-L1 expression, or angiogenic passive indicators, is a noteworthy reason behind the satisfactory degree of the therapeutic precision and reaction rates [247].

Nonetheless, there remains a discrepancy in practice between real world and clinical values due to inconsistent levels of adherence, dose, and access to the resources. Moreover, post-marketing pharmacovigilances studies pose problems of tight follow-ups of immune-mediated hepatitis and Renal poisoning in combination drugs [248].

Overall, the real-life implementation may assist in the clinical usefulness of combination immunotherapy and targeted therapy in advanced HCC and therefore be generalizable in other settings not a controlled trial. A mixture of trial data combined with clinical findings and molecular biomarker evidence is already delivering the second generation of personalized immuno-oncology, that is maximization of patient selection, drug dosing, and long-term safety [249].

7. Challenges and Limitations

Despite the significant breakthrough done by the immunotherapy and targeted therapy approaches to hepatocellular carcinoma (HCC), there are numerous challenges and limitations that still hinder the desire to achieve the best clinical outcomes and use it in the whole population. One of the most challenging issues is the biological heterogeneity of HCC because many different etiological factors cause it, among which are the infection of hepatitis B and C, alcohol-related liver disease, and steatohepatitis because of the malfunction of the metabolism (MASH). It is this heterogeneity that generates variability in the behavior of tumors, immune microenvironment and response to therapy as well as it is difficult to develop universal regimens of treatment [250]. Moreover, intratumoral and intertumoral heterogeneity is at least one of the factors to cause primary and acquired resistance to immune checkpoint inhibitors (ICIs) and tyrosine both inhibitors (TKIs) that demand adaptive therapy and precision in medicine techniques [251].

The other severe limitation is the non-optimal response to immunotherapies. Despite the fact that immune checkpoint blockade (PD-1/PD-L1 and CTLA-4 inhibitors) has entirely revolutionized the paradigms, few patients (20-30 percent) respond durably and that is why most of them are treatment resistant [252]. Part of this poor efficacy is due to the immune-murdering tumor microenvironment (TME) which is characterized by the regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs) as well as the tumor-associated macrophages (TAMs) that repress the cytotoxic T-cell response [253]. Additionally, the prevention of immune exclusion in genetic mutations such as the CTNNB1 (What is referred to as β -catenin) activation also does not grant the process of T-cell infiltration and responsiveness to ICIs [254].

The obstacles are also connected with the toxicity and safety concern. Immune-related (irAEs) such as hepatitis, colitis, pneumonitis, and endocrine dysfunctions are frequent and this may lead to immunosuppressive action forcing them to discontinue any therapeutic action [255]. The use of ICIs with anti-angiogenic or targeted therapies is more effective but most likely leads to high

blood pressure, blood in the urine, and liver decompensation, especially in people with cirrhosis [256]. Such combinations of toxicities in a patient with compromised liver functions is a sensitive clinical issue to treat.

Other factors that are limiting are discovery and validation of biomarkers. Even though some of the potential biomarkers, such as PD-L1 expression, tumor mutational burden (TMB), and circulating tumor DNA (ctDNA) have shown promise, none has been shown to be available in all clinical environments and all with predictive power that can be reliably predicted, and none has been standardized across studies [257]. Further, it is complicated by the inability of data to be simplified, its high cost, and lack of coordinated computational pipelines to bespoke biomarker discovery [258] in the multi-omics platform environment.

Problems still exist in clinical design and patient selection. The most significant two phase III trials IMbrave150 and CheckMate 459 have only enrolled patients who have preserved liver function (Child-Pugh A), and few comorbidities, which rules out a great portion of real-world patients, who are usually older, frail, or have Child-Pugh liver disease B/C [259]. In that way, the external validity of such trials is restricted and increment in the real world may not entirely depict the effectiveness of such studies in clinical trials.

Lastly, ADTs do not exist in every part of the world based on the cost and accessibility barriers. The high cost of both immunotherapy and targeted therapy as well as insufficient healthcare infrastructural facilities in the developing countries worsen the disparity in the differences in accessibility and survival opportunities of the treatment [260]. They need international collaboration, making policies favorable, and adequate distribution of resources to go through these barriers so that the therapeutic advancements are made internationally in the interest of patients.

8. FUTURE PERSPECTIVES AND DIRECTIONS

The current tendencies toward the management of hepatocellular carcinoma are firmly moving towards precision immuno-oncology, including

the molecular profiling, artificial intelligence (AI), and multi-omics technologies in the delivery of customized treatment options. Clinicians will be able to identify actionable mutations, immune subsets, and resistance mechanisms as well as personal combinations of treatment regimens that become individual tumor biology in individual patients when genomic and transcriptomic sequencing is improved [261].

One of the promising directions, which will result in overcoming immune resistance, is the use of rational combination therapy that integrates the use of ICIs, TKIs, epigenetic modulators, and metabolic regulators. Several other inhibitory checkpoints, such as LAG-3, TIM-3, TIGIT, VISTA, are undergoing development through novel agents to circumvent the adaptive immune responses that arise during PD-1 inhibition [262]. Besides, the forthcoming generation of immunotherapy is embodied by bispecific antibodies and antibody-drug conjugates (ADCs) immunotherapeutics that are very specific and possess high cytotoxic ability [263].

Oncolytic virus integration, neoantigen vaccines, adoptive cell therapies such as CAR-T, CAR-NK and tumor infiltrating lymphocytes (TILs) have also a bright future. The methods are supposed to increase the tumor-specific immunity and reorganize the TME into an immunoreactive one [264]. An example of these is the CAR-T cells targeting the glypican-3 (GPC3) that is an antigen associated with HCC and has undergone promising preclinical and early clinical responses [265].

Application of the technology of liquid biopsy and non-invasive imaging biomarkers will keep on redefining disease tracking and forecasting of reactions. In combination, the synthesis of ctDNA sequencing and proteomic data and metabolomic information will yield real-time data on tumor evolution and treatment modification [266]. Moreover, the multi-omics integration and digital pathology based on AI will probably be more precise in the quality of diagnosis and identification of new prognostic models and predict therapeutic events more accurately than the standard procedures of histopathology [267].

In order to facilitate clinical development, adaptive and basket trial designs will become

increasingly relevant to evaluate targeted agents and immunotherapies in biomarker-selected subpopulations [268]. The implementation of real-world evidence (RWE) through registries across the world, and electronic health records will complement the traditional clinical trials to ensure that the data on the efficacy and safety could be applicable to diverse populations and healthcare systems [269].

Finally, it is also important to change the world in terms of access to new treatment modalities. This will require increasing infrastructure that will facilitate biomarker testing and increasing regulatory framework, in addition to reducing costs of treatment by constructing biologics in the area in order to bridge the gap between the high-income and low-income nations [270]. This requires HCC to institute a justifiable and speedy translational innovation through the collections of co-existence in research in Asia, Africa and Latin America.

9. Conclusion

Hepatocellular carcinoma is an overwhelming cancer challenge of massive concern throughout the entire globe, but with the combination of immunotherapy, targeted therapy, and precision medicine, a new promising age has come. There have been partnerships and long-term clinical study programs such as IMbrave150 and CheckMate 040 to change the treatment paradigm to render immunotherapy derivations as the new advanced disease gold standard combination. However, the problems of tumor heterogeneity, resistance to drugs and availability are still in existence and it implies that more molecular knowledge and equal measures in all parts of the world are necessary.

Recent technologies, in particular, liquid biopsy, ctDNA profiling, and application of multi-omics are altering the opportunities to monitor the disease course, anticipate the reaction to treatment, and take corrective measures in the framework of a real-time mode. To an even greater extent, artificial intelligence and systems biology applied to clinical oncology will eventually allow refining the predictive algorithms and offering a chance of completely individualized treatment algorithms.

The future must be envisioned forward to more biomarker-based therapies, the rational combination regimes, and the cooperation on international level so that the scientific findings could be enhanced to the actual patient enhancements. With the therapeutic environment continuing to improve, the final outcome has remained unchanged, that is to turn advanced HCC into a manageable and chronic disease with improved survival and quality living by the patients all over the world.

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