Novel therapeutics in hepatoblastoma

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Abstract
Hepatoblastoma is the most common primary liver malignancy in children less than 5 years of age. The incidence of this cancer is increasing but therapy for hepatoblastoma has not significantly changed and it is primarily managed with combinations of surgery and chemotherapy. Conventional chemotherapy regimens include cisplatin, as it is currently the most effective agent for this tumor. Unfortunately, with the current modalities, the prognosis for advanced-stage disease remains poor due to the high rate of recurrence and/or the development of chemoresistance. As the understanding of the pathophysiology of hepatoblastoma deepens, there is a burgeoning interest in developing novel therapeutic agents for hepatoblastoma, including targeted therapies. In the current review, we discuss the use of major signaling pathways and their effector molecules such as β-catenin, c-MET, mTOR, PIM kinases, and PLK1 as therapeutic targets. In addition, we explore various alternative pre-clinical approaches that have been described in hepatoblastoma, including immunotherapy, differentiation agents, micro-RNAs, and targeted drug delivery. Finally, we outline the therapies currently under investigation in pre-clinical and clinical settings that show promising preliminary results for hepatoblastoma treatment.

Keywords: hepatoblastoma, chemotherapy, targeted therapy
Introduction

Hepatoblastoma (HB) accounts for 91% of primary hepatic malignancies diagnosed in children less than 5 years of age. The incidence has been increasing over the past several years, but the causative factors for HB have not yet been clearly elucidated. Several associations including preterm birth, low birth weight, Beckwith-Wiedemann syndrome and germ-line mutations of genes such as adenomatous polyposis coli (APC) have been described. Despite the apparent ambiguity in etiology, there have been significant advances in the treatment of HB, which has evolved from being primarily surgical to the current multidisciplinary management. In this review, we aim to discuss the novel therapeutic strategies that are currently in use in the preclinical and clinical arenas for the treatment of HB.

Need for chemotherapy

The anatomy and regenerative potential of the liver makes it amenable for surgical resection of tumors, rendering surgery a cornerstone of HB management. The International Childhood Liver Tumor Strategy Group of International Society of Pediatric Oncology (SIOPEL) analyzed 154 cases of HB and found pretreatment extent of disease (PRETEXT) was the only significant predictive factor for overall survival (OS), resulting in the widespread adoption of PRETEXT classification in HB. The PRETEXT staging system utilizes Couinaud’s description of liver segmentation to describe the extent of the tumor prior to treatment. Annotation factors including involvement of the inferior vena cava, hepatic veins, or portal veins, extrahepatic abdominal disease and distant metastases are also considered when determining tumor resectability. Therefore, PRETEXT aids in the evaluation of patients as surgical candidates at diagnosis. Complete surgical resection without any additional therapy has been shown to provide definitive cure for HB patients with well-differentiated tumors at early stages, especially with pure fetal histology.

For children with advanced stage HB, upfront surgical resection may not be possible. In 1982, Children’s Cancer Study Group and the Pediatric Oncology Group conducted a nonrandomized trial to assess response to combination therapy of vincristine, cyclophosphamide, adriamycin, and 5-fluorouracil in pediatric liver malignancies. This study provided one of the early evidences of the chemosensitive nature of HB. Since then, several trials have been conducted by Children's Oncology Group (COG) and SIOPEL to assess the efficacy of cisplatin combined with other agents in HB. Additional studies have demonstrated that in tumors that are not amenable to resection at diagnosis, administration of neo-adjuvant chemotherapy results in reduction of tumor volume making subsequent resection feasible. Hence, chemotherapy is currently pivotal in HB management.

Current role of chemotherapy

Currently, chemotherapy is offered to most patients with HB. The timing and regimen are dependent on PRETEXT stage. Patients with early stage HB are considered for adjuvant chemotherapy, whereas those diagnosed with high risk, refractory, and/or metastatic HB are offered neoadjuvant chemotherapy to reduce tumor volume and decrease the likelihood of micro-metastasis.

Regardless of the regimen, cisplatin provides the backbone of treatment as it remains one of most effective agents in HB. The results from COG trial, AHEP0731, recommends cisplatin, 5-fluorouracil, and vincristine (CSF) for low-risk HB and CSF along with doxorubicin for intermediate and high risk patients.

These current regimens have attributed to an increase in OS by approximately 20% in
advanced stage HB by increasing surgical resection rates and decreasing recurrences.\textsuperscript{5,11,17,18} However, the OS for advanced stage disease remains poor, due to the development of chemoresistance and recurrence. Cancer stem cells (CSC) and clonal selection have been cited as reasons for treatment failure and development of multi-drug resistance (MDR).\textsuperscript{1,19} Regimens need to be optimized to prevent MDR and also decrease treatment associated toxicities such as cisplatin-induced ototoxicity and renal toxicity. In view of increasing incidence of HB, there is an immense scope for novel agents to overcome these challenges. We outline some of the novel therapeutic strategies that have been evaluated in preclinical and clinical settings.

1. Preclinical novel approaches

HB has a relatively low rate of genetic mutations.\textsuperscript{20,21} Despite this finding, there is deregulation of downstream and/or collateral pathways that result in tumor formation. As the cellular pathophysiology in HB is further explored, there has been considerable increase in the understanding of cellular signaling pathways and identification of drug targets (Figure 1).

1.1 Canonical Wnt/\(\beta\)-catenin signaling

The canonical Wnt signaling plays a major role in hepatic development through its effect on hepatocyte nuclear factor (HNF)-1\(\beta\), forkhead box (Fox) A1, FoxA2, and GATA binding protein 4 (GATA4).\textsuperscript{22} This pathway is also essential in maintaining liver homeostasis and zonation, which is demonstrated by pericentral-specific expression of Wnt/\(\beta\)-catenin targets such as axin2, glutamine synthetase (GS), and cytochrome CYP2E1 and CYP1A2.\textsuperscript{22} Disturbances in canonical Wnt signaling have been implicated as molecular signatures of HB.\textsuperscript{22,23} 

\(\beta\)-catenin is the effector molecule of the Wnt signaling pathway. The most common mutation in HB affects the gene CTNNB1, which causes \(\beta\)-catenin to be perpetually active.\textsuperscript{24} \(\beta\)-catenin is a cytoplasmic protein but is also present on the cytosolic surface of the cell membrane where it forms a complex with E-cadherin and alpha-catenin.\textsuperscript{22} Upon activation, \(\beta\)-catenin translocates to the nucleus to associate with transcription factors like T-cell factor/lymphoid enhancer factor (TCF/LEF), resulting in cell growth, proliferation and survival.\textsuperscript{22} The sequence of events that results in HB formation following \(\beta\)-catenin activation remains unclear, but \(\beta\)-catenin activity has been linked to HB clinical prognosis.\textsuperscript{25,26} Park and colleagues found increased nuclear staining coupled with decreased membrane and cytosolic staining for \(\beta\)-catenin, indicative of the active form, in advanced stages of HB.\textsuperscript{26} Additionally, Tang et al. showed that \(\beta\)-catenin induces the tumor initiating phenotype and CD133 expression in HB.\textsuperscript{27}

Because considerable data substantiate \(\beta\)-catenin’s role in HB, various agents have been explored to target it.\textsuperscript{28} These molecules include celecoxib which decreases nuclear translocation of \(\beta\)-catenin and decreases DNA binding, etodolac which decreases activated and total \(\beta\)-catenin and ICG001, a small molecule that antagonizes Wnt/\(\beta\)-catenin/TCF-mediated transcription.\textsuperscript{28} In vitro studies evaluating these three agents showed decreased cell viability and proliferation when used in human HB cell lines.\textsuperscript{28} When celecoxib and ICG001 were combined with cisplatin, there was greater inhibition of tumor cells in vitro than cisplatin alone.\textsuperscript{28} Additionally, ICG001 decreased expression of the cancer stem cell marker CD133.\textsuperscript{27}

These studies provided evidence that targeting \(\beta\)-catenin may affect tumor growth in HB, but it remains to be determined whether these effects will translate clinically. The Wnt/\(\beta\)-catenin/TCF pathway is important in development and mostly inactive in adult tissues, but investigations for the extrahepatic off-target effects will be necessary.
Figure 1. Targeted therapeutic approaches for hepatoblastoma. Major signaling pathways are highlighted along with their effector molecules and the therapies discussed in this review. Arrows depict activation, either directly (solid arrows), or indirectly through multiple intermediates (dashed arrows), and blocked lines depict inhibition. † Therapies with effects in vitro, * Therapies with effects in vivo.

SP, Substance P; NK1R, Neurokinin-1 receptor; HGF, Hepatocyte growth factor; GSK-3β, Glycogen synthase kinase 3β; CK1, Casein kinase 1; Dvl-2, Disheveled-2; TCF/LEF, T-cell factor/lymphoid enhancer factor; CDK, Cyclin-dependent kinase; mTORC1, Mammalian target of rapamycin complex 1; PI3K, Phosphatidylinositol-3 kinase; GPCR, G-protein coupled receptor; RTK, Receptor tyrosine kinase; IGF1R, Insulin-like growth factor 1 receptor; EGFR, Epidermal growth factor receptor; mAB, monoclonal antibody; EpCAM, Epithelial cell adhesion molecule; GPC3, Glypican 3; CAR-T, Chimeric antigen receptor T cells; oHSV, Oncolytic Herpes Simplex virus; MV, Measles virus; DX-NC, Doxorubicin nanocapsule; SF-GNP, Sorafenib gold nanoconjugate particle; miRNA, Micro-RNA; mRNA, messenger RNA; RISC, RNA-induced silencing complex; PLK-1, Polo-like kinase 1; PIM kinases, Proviral Integration site for Maloney murine leukemia virus kinases; PP2A, Protein phosphatase 2A; RAR/RXR, retinoic acid/rexinoid receptor.

1.2 β-catenin degradation complex

Cytosolic concentration of β-catenin is regulated by a protein complex composed of adenomatous polyposis coli (APC), axin, glycogen synthase...
kinase 3β (GSK), casein kinase 1α (CK1α), and disheveled (Dvl) proteins. In absence of Wnt-induced activation of β-catenin, this complex modulates β-catenin activity through phosphorylation and ubiquination. Axin and Dvl are also downstream targets of activated β-catenin, implying self-regulation of β-catenin in normal cells.

Disturbances in the β-catenin degradation complex may contribute to HB pathophysiology. Koch and colleagues reported mutations affecting axin2 in 37 HB cases. These tumors displayed high levels of activated β-catenin despite an absence of β-catenin gene mutations. They also found higher levels of axin2 mRNA in specimens exhibiting activating β-catenin mutations. Huang et al. showed presence of Dvl-2 overexpression in HB samples. Knockdown of Dvl-2, or use of niclosamide to decrease Dvl-2, reduced β-catenin expression and HB cell proliferation in vitro and in vivo. In these cancers, β-catenin activation and subsequent tumorigenesis was not inhibited, despite high levels of degradation complex proteins in the tumor cells, raising the question of a potential dual nature of these molecules. Although this mechanism is not clearly elucidated, therapeutic approaches targeting degradation complex molecules showed decreased tumorigenesis and represent potential for targeted therapies.

1.3 Hepatocyte growth factor (HGF)/c-met axis

C-Met is a tyrosine kinase receptor leading to Wnt-independent activation of β-catenin. Binding of hepatocyte growth factor (HGF) ligand to c-Met, phosphorylates membrane bound β-catenin, which then translocates to the nucleus to exert its effects. The HGF/c-Met axis is important in controlling epithelial-mesenchymal morphogenesis, angiogenesis and cell-cell adhesion.

C-Met-induced β-catenin activation is hallmarked by phosphorylation of tyrosine 654 (Y654). Purcell and colleagues showed that Y654-β-catenin was present in 83% of HB samples and cell lines suggesting that c-Met or tyrosine kinase inhibitors may be used to target β-catenin. SU11274, a MET kinase inhibitor, was used to target β-catenin resulting in decreased HB viability.

1.4 Phosphatidylinositol-3- kinase (PI3K)/AKT/mTOR pathway

Aberrations of PI3K/AKT/mTOR pathway are commonly implicated in cancer. Receptor tyrosine kinases activate PI3K, which activates AKT resulting in phosphorylation of downstream substrates controlling apoptosis, cell cycle and gene expression.

Mammalian target of rapamycin (mTOR), an AKT substrate, is important in HB pathophysiology. Hartmann et al. showed high levels of phosphorylated AKT and mTOR in HB cases, implying pathway activation. Activated mTOR complexes with proteins mtor and rictor to form the mTORC1 and mTORC2 complexes, respectively. Michael and colleagues showed that p-mTOR-S2448, a marker of mTORC1 activation, was localized to the peri-central hepatocytes in a normal liver, which exhibit high β-catenin activity at baseline. Furthermore, they demonstrated that disrupting the β-catenin axis through knockout or small interfering RNA (siRNA) silencing of the CTNNB1 gene resulted in decreased p-mTOR-S2448, implying that β-catenin promotes mTORC1 activation. Liu et al. showed that raptor knockdown inhibited β-catenin-induced HB formation in mice through downregulation of mTORC1 signaling. These findings provided evidence that mTORC1 activation is important in promoting tumorigenesis in HB.

Different strategies have been employed to target the PI3K/AKT/mTOR pathway in HB. PI3K inhibitor LY294002 has been shown to decrease tumor cell viability and sensitize HB cell lines to cisplatin and doxorubicin.

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viability and increasing apoptosis. Rapamycin, an mTOR inhibitor, decreased HB tumor growth both in vitro and in vivo.

The PI3K/AKT/mTOR pathway also affects chemoresistance. Grotegut and colleagues showed that PI3K/AKT pathway was upregulated in HB cells exposed to cisplatin and subsequent treatment with PI3K inhibitor, wortmannin, led to apoptosis, highlighting the potential role of PI3K/AKT signaling in refractory and recurrent HB.

1.5 Kinases and phosphatases

Kinases and phosphatases transmit and amplify intracellular oncogenic signaling and represent potential drug targets. Proviral Integration site for Maloney murine leukemia virus kinases, or PIM kinases have been found to promote tumorigenesis in HB. PIM kinases are serine/threonine kinases that act as oncoproteins by inhibiting apoptosis and activating proliferation and cell survival. There are three PIM family members, PIM1, PIM2 and PIM3, and PIM3 has been shown to be the kinase involved with HB proliferation and survival. PIM3 expression correlated with worse OS in HB patients, irrespective of clinical stage. Stafman et al. demonstrated that siRNA PIM3 knockdown resulted in decreased proliferation and migration in HuH6 cells. Studies showed that PIM kinase inhibition with AZD1208 resulted in significantly decreased HB stemness and acted synergistically with cisplatin to increase survival of HB bearing animals. These preclinical data provided evidence for the potential utility of targeting PIM3 in HB.

Polo-like kinase-1 (PLK-1) has also been targeted in HB. PLK-1 is a serine/threonine kinase important for cell cycle progression and apoptosis. PLK-1 regulates G2/M transition by controlling spindle formation and activation of cyclin-dependent kinases and affects apoptosis by phosphorylating and inactivating p53 and p63 gene products. Yamada and colleagues showed higher expression of PLK-1 in 74 HB specimens compared to normal liver tissue which correlated with clinical prognosis. Komatsu reported the over-expression of PLK-1 in HB cancer stem cells. Investigators used volasertib to inhibit PLK-1 function in HB cells, leading to decreased viability and proliferation. Volasertib also showed synergistic effects on tumor growth inhibition in mice when combined with irinotecan.

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase that has been explored in HB. PP2A, a tumor suppressor, has been shown to be inactivated in a number of cancers. PP2A is involved in the regulation of Wnt, mTOR and MAP kinase pathways. FTY720 (fingolimod) has been used in HB to activate PP2A, resulting in decreased tumor growth and potentiating cisplatin activity both in vitro and in vivo.

These findings emphasized the tumor-promoting roles of kinases and phosphatases and illustrated their potential for therapeutic targeting in HB. Additionally, the pre-clinical data highlighting efficacy when combined with standard chemotherapeutic agents provides evidence for a potential clinically effective strategy.

1.6. Immunotherapy

Immunotherapy has been a breakthrough in pediatric cancer management, representing an alternative therapeutic strategy to overcome chemotherapeutic resistance. Various approaches have been employed to harness the immune system or its effector molecules, including immuno-stimulatory agents, monoclonal antibodies, adoptive therapy, and oncolytic virotherapy.

Epithelial cell adhesion molecule (EpCAM) has been identified for immunotherapy in HB. EpCAM is a transmembrane glycoprotein that functions in adhesion, proliferation and migration. Elevated EpCAM expression has been observed in up to 80% cases of HB, contrasted to normal liver tissue. EpCAM has
been targeted using monoclonal antibodies (mAb) to induce antibody or complement-dependent cytotoxicity.\textsuperscript{56} Armeanu-EBinger and colleagues showed that humanized antibodies directed against EpCAM resulted in increased HB cell lysis via activation of gamma delta T-cells.\textsuperscript{56}

Glypican 3 (GPC3) is another molecule explored as a HB immunotherapeutic target. GPC3 is a cell surface proteoglycan that promotes cell growth and inhibits differentiation through downstream activation of Wnt signaling, FGF and Hedgehog pathways.\textsuperscript{57} It has also been shown to increase chemoresistance in gastrointestinal tumors by promoting multi-drug resistance associated protein expression.\textsuperscript{55,58,59} Studies have found that GPC3 is highly expressed in up to 97\% of HB.\textsuperscript{58,60} Wenpeng et al. demonstrated engineered T-cells expressing GPC3-specific chimeric antigen receptors (CARs) resulted in HB cell lysis \textit{in vitro} and \textit{in vivo}.\textsuperscript{61} A clinical trial is currently underway to evaluate GPC3-specific CARs in high risk HB (NCT04093648).

Oncolytic virotherapy has also been investigated in HB. This therapy utilizes genetically engineered viruses that specifically target tumor cells to induce cell lysis and invoke a subsequent immunogenic response through the release of damage-associated molecular cytokines and chemokines.\textsuperscript{53} Kuroda et al. described the use of an oncolytic herpes simplex virus (oHSV), bM24-TE, to induce cell death in HepG2 cells.\textsuperscript{62} The oHSV incorporated β-catenin/Tcf responsive promoters which allowed gene transcription and selective viral replication in HB cells with strong β-catenin/Tcf signaling.\textsuperscript{62} Megison and colleagues reported that M002, another oHSV genetically engineered to only replicate in tumor tissue and to express murine IL-2, decreased \textit{in vitro} and \textit{in vivo} tumor growth in HuH6 cells.\textsuperscript{63} Lastly, another oHSV, T01, resulted in similar cytotoxic effects \textit{in vitro} and \textit{in vivo} models of HB.\textsuperscript{64} Attenuated measles virus has also been used to target HB cells.\textsuperscript{65}

These preclinical studies show promising results for the use of immunotherapy in HB. EpCAM and GPC3 are highly expressed in tumor cells compared to normal liver making them targets with an adequate therapeutic window.\textsuperscript{56,57,60} However, use of monoclonal antibodies and CAR-T cell therapy have been associated with adverse effects due to ‘target’ expression on normal tissues as well as cytokine release and immunomodulation,\textsuperscript{53} which may serve as potential barriers to their widespread application for HB.

1.7. Differentiation agents

Retinoids have been employed to induce differentiation in acute promyelocytic leukemia (APL) and high-risk neuroblastoma.\textsuperscript{66,67} The rationale entails the premise that differentiation arrests proliferation followed by eventual apoptosis and decreased tumor burden.\textsuperscript{67} Their effect is mediated via retinoic acid receptors in the nucleus that bind to specific DNA sequences upon activation and modulate gene expression.\textsuperscript{67} Waters et al. demonstrated that UAB30, a synthetic rexinoid, significantly decreased proliferation and motility and increased apoptosis \textit{in vitro} as well as decreased tumor growth and increased animal survival in animals bearing HuH6 tumors,\textsuperscript{68} providing preclinical evidence supporting the potential for retinoids as a therapeutic adjunct for HB.

1.8 Substance P/Neurokinin-1 Receptor (NK1R)

Substance P (SP) is a tachykinin that binds to a G-protein coupled receptor, Neurokinin-1 Receptor (NK1R), activating downstream effectors protein kinase C, NF-kB, MAPK and ERK.\textsuperscript{69,70} Inhibition of SP-induced NK1R activation with aprepitant has been used to treat chemotherapy-induced nausea and vomiting (CINV).\textsuperscript{70} The role of SP/NK1R in cancer therapeutics has recently been shown to extend beyond CINV. SP/NK1R signaling accentuated tumor growth and proliferation by activating cellular calcium mobilization and MAPK and by
promoting a stimulatory tumor microenvironment.\textsuperscript{69-71} The relevance of SP/NK1R signaling in HB has been demonstrated in a number of studies. Garnier and colleagues found overexpression of the truncated splice variant, NK1R-tr, in the tumor cells from 47 HB cases as compared to normal liver.\textsuperscript{72} This truncated isoform shows resistance to desensitization and internalization leading to amplified oncogenic signaling.\textsuperscript{69,72} Berger et al. showed that inhibition of NKRI with antagonists aprepitant, L-733,060, and L-732,138 led to significantly decreased expression of stemness markers Sox2, Oct4, and Nanog, increased apoptosis and decreased tumor growth.\textsuperscript{73} When combined with cisplatin and doxorubicin, aprepitant synergistically inhibited HB proliferation.\textsuperscript{73}

In HB, NK1R interacts with the mTOR and β-catenin pathways.\textsuperscript{74} Ilmer et al. reported that aprepitant decreased phosphorylation of p70S6K and 4E-BP1/2, known downstream targets of mTORC1.\textsuperscript{74} Aprepitant-induced NKRI inhibition prevented nuclear accumulation of β-catenin due to downregulation of FOXM1 protein.\textsuperscript{74} Therefore, targeting the SP/NK1R pathway may inhibit HB tumor cells via multiple molecular targets.

1.9 Proteasome inhibitors

Proteasome inhibitors impair the degradation of tumor suppressor proteins resulting in decreased tumor growth.\textsuperscript{75} Their utility has been proven in myeloma and mantle-cell lymphoma.\textsuperscript{75} These agents act primarily by increasing apoptosis.\textsuperscript{76,77} One compound, bortezomib, increased the pro-apoptotic factors of Bcl-2 family, activated caspase-8 and enhanced receptor-mediated apoptosis in HB.\textsuperscript{78,79} Bortezomib, alone and in combination with cisplatin, inhibited growth \textit{in vitro} and \textit{in vivo}.\textsuperscript{76} Another proteasome inhibitor, MG132, has shown similar effects.\textsuperscript{77} MG132 induced apoptosis by modulating the expression of Bcl proteins and increasing the activation of caspase 3.\textsuperscript{77}

Proteasomes also promote HB oncogenesis through Gankyrin (Gank) signaling.\textsuperscript{80,81} Valanejad et al. showed increased expression of Gank in 31 clinical HB specimens as compared to normal tissue and Gank expression correlated with stem cell activity and prevented differentiation.\textsuperscript{81} Gank interacts with the 26S proteasomal subunit promoting proteasomal degradation of tumor suppressor proteins.\textsuperscript{81,82} The small molecule inhibitor, cjc42, has been shown to prevent Gank 26S binding and has led to decreased \textit{in vitro} proliferation in HB along with increased mRNA levels of tumor suppressors.\textsuperscript{80}

1.10 Micro-RNA (miRNA) based therapeutics

MiRNAs are non-coding RNAs that regulate gene expression.\textsuperscript{83} MiRNAs have been primarily employed as investigational tools, but have recently been investigated as potential therapeutics in HB.\textsuperscript{84} Indersie et al. showed that miR-624-5p was effective in decreasing cell growth and inducing senescence through decreased β-catenin activity.\textsuperscript{85} They also reported decreased \textit{in vivo} tumor growth.\textsuperscript{85} Other investigators showed that miR-4510 inhibited \textit{in vitro} and \textit{in vivo} HB tumor growth by downregulating GPC3 expression.\textsuperscript{86} However, optimization of miRNA stability and delivery will be required prior to clinical application.\textsuperscript{83}

1.11 Targeted drug delivery systems

Most tumors exhibit characteristics that may be targeted with nanosystems and bioconjugates to increase specificity and efficiency of chemotherapy agents.\textsuperscript{87} Liu and colleagues developed a doxorubicin nanocapsule that preferentially released the drug in the acidic tumor microenvironment of HB along with decreased release in normal tissues such as the heart.\textsuperscript{88} These nanocapsules significantly prolonged doxorubicin half-life and led to increased tumor inhibition in mice xenografts.\textsuperscript{88} Vishwakarma demonstrated that sorafenib gold
nanoconjugate particles (SF-GNP) allowed improved drug delivery and retention leading to enhanced anti-proliferative activity and circumvention of sorafenib resistance in HB cells.\textsuperscript{89}

2. Clinical novel approaches

The following developments have been undertaken in the clinical setting to overcome the limitations of currently used agents.

2.1 Alternative platin-based agents

Cisplatin is incorporated in treatment regimens for nearly all HB stages but otic and renal toxicities continue to pose challenges.\textsuperscript{5,12} Alternative platin-based agents have been evaluated to address these issues. COG conducted a phase-II trial to assess the efficacy of a third generation platin compound, oxaliplatin, for refractory pediatric solid tumors, including HB.\textsuperscript{90} Although associated with less adverse effects, oxaliplatin did not show similar potency as cisplatin in HB.\textsuperscript{90}

2.2 Regimens for high risk HB

Novel chemotherapy combinations have been evaluated to improve efficacy in high risk disease.\textsuperscript{5} The SIOPEL-4 trial was a prospective, single arm study evaluating the use of SuperPLADO (cisplatin alternating with carboplatin-doxorubicin) with higher dose of cisplatin in high risk HB.\textsuperscript{91} This regimen led to higher OS and resection rates compared.\textsuperscript{91} Recently, a topoisomerase I inhibitor, irinotecan, was shown to be effective in high risk HB. Zhang et al. conducted phase-II study to evaluate the efficacy of irinotecan in combination with vincristine in relapsed HB and reported an OS of 71.4% with the combination.\textsuperscript{92} Qayed and colleagues described the use of irinotecan as maintenance therapy in three patients with high risk HB with complete remission reported up to 6 years.\textsuperscript{93} In another COG study (AHEP0731), the combination of vincristine and irinotecan resulted in clinical responses in 14/30 patients with high risk HB and a 3-year OS of 62%.\textsuperscript{94} Sorafenib, a kinase inhibitor, has been used in in two isolated HB case reports.\textsuperscript{95,96} Marsh reported slowing of HB progression and decreased size of lung metastasis after a 4-week course of sorafenib and bevacizumab.\textsuperscript{95} In another case study, Shanmugam et al. reported a complete resolution of HB lung metastasis and disease stabilization with sorafenib.\textsuperscript{96} Randomized trials are needed to evaluate these agents.

Conclusion

Although HB has limited mutational diversity, the tumors demonstrate aberrations resonating across different pathways. As a consequence, multiple therapeutic targets may be available including the Wnt/β-catenin and mTOR pathways, PIM kinases, NK1 and EpCAM. As the pre-clinical landscape of novel therapeutics evolves, clinical trials will be necessary to evaluate the efficacy of these agents as well as their adverse effects.
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