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Chapter

Perspective Chapter: Critical Role of Hedgehog in Tumor Microenvironment

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Abstract

Hedgehog (Hh) signaling is a highly conserved pathway that plays a pivotal role during embryonic development. Mounting evidence has implicated Hh signaling in various types of cancer. Accordingly, inhibition of aberrant Hh signaling continues to be pursed across multiple cancer types -with some success in certain malignancies. In addition, with the renaissance of antitumor immunotherapy, an in-depth understanding of the molecular mechanisms underlying how the multifaceted functions of Hh signaling shape immunologically suppressive tumor microenvironment might be the key to unlocking a new era of oncological treatments associated with a reduced propensity for the development of drug resistance. Here, we focus on the latest advances regarding the immunological effects of misregulation of Hh signaling on tumor immunity. We also review the current status of clinically approved Hh inhibitors and dissect the mechanisms of drug resistance. Finally, we discuss the potential clinical applications that harness the immunomodulatory effects of Hh signaling not only to circumvent drug resistance, but also to achieve durable efficacy following immunotherapies, thus ultimately resulting in improved patient outcomes.

Keywords: hedgehog signaling, tumor microenvironment, immune cell, smoothened inhibitors

1. Introduction

The Hedgehog (Hh) signaling pathway was discovered as a key regulator of organ development in *Drosophila melanogaster* by Christiane Nüsslein-Vollhard and Eric Wieschaus in the 1980s [1]. It was named after the gene locus associated with a spiky appearance of "hedgehog" phenotype in mutant *Drosophila* larve, findings based on which both investigators were awarded the Nobel Prize in Physiology or Medicine in 1995 "for their discoveries concerning the genetic control of early embryonic development," together with Edward B. Lewis [2]. Since then, the Hh signaling has been extensively studied as a highly conserved evolutionary pathway to orchestrate embryonic development, cell growth and differentiation, homeostasis [3]. Unlike other classical signaling cascades, Hh signaling is almost silent in the adult organisms but reactivated in a few tissues such as the skin, during tissue regeneration and wound

healing [3]. Not surprisingly, aberrant activation of this pathway has been demonstrated as a potent oncogenic driver to promote numerous hallmarks of cancer [4]. Therefore, the multifaceted role of Hh signaling may allow exploitation of this key pathway for novel and more effective cancer therapy [5].

Activation of Hh signaling is dependent on the primary cilium, a highly specialized organelle found on most vertebrate cells. Three Hh ligands, sonic hedgehog (Shh), desert hedgehog (Dhh), and Indian hedgehog (Ihh), are known to actuate the Hh pathway during embryonic and tissue development [6]. Whereas the expression patterns for Dhh and Ihh are tissue-specific, Shh has a broader expression pattern in various compartments and in multiple developmental stages [6]. In general, the Hh signaling is activated through either canonical or non-canonical mechanisms. In the canonical pathway, Hh ligands bind to the surface receptor Patched 1 (PTCH1), which alleviates the inhibitory effect of PTCH1 on a G-protein-coupled receptor (GPCR)-like protein, Smoothened (SMO), leading to migration of SMO to the tip of the cilium, which in turn signals suppressor of fused (SUFU) to release glioma-associated oncogene homolog proteins (GLIs). Finally, GLIs translocate into the nucleus, resulting in a signaling cascade through transcriptional regulation of Hh target genes [6] Alternatively, GLI transcription factors can be activated through non-canonical mechanisms, which can be independently of PTCH1, SMO, or both [6]. Of note, mounting evidence has demonstrated that the signaling pathways that can induce non-canonical Hh signaling have been of known significance in oncogenesis, providing the mechanistic basis of the cross talk between Hh signaling and other signaling pathways to promote tumorigenesis, as well as the rationale for development of potential combination therapeutics [7–10].

The discovery of PTCH mutations in basal cell nevus syndrome (BCNS, or Gorlin syndrome, or nevoid basal cell carcinoma [BCC] syndrome), a hereditary form of BCC, provides the first link between the Hh signaling and tumorigenesis [11, 12]. Other than BCC, emerging evidence has involved abnormal activation of Hh signaling in a variety of cancer types, such as medulloblastoma, breast cancer, pancreatic cancer, and lung cancer [13].

So far, three models have been proposed to elucidate the role of Hh signaling in oncogenesis where Hh signaling is over-activated through ligand production, autocrine, juxtacrine, or paracrine reception of the ligand, as well as cross talk between Hh signaling and complex intracellular signaling cascades [13]. First, in BCC and medulloblastoma, activating mutations of Hh pathway have been identified, such as inactivating mutations in PTCH or SUFU, and activating mutations in SMO, as shown in 85% of sporadic BCC or 30% of medulloblastoma, respectively [11, 12, 14, 16]. In this scenario, the autonomous activation of Hh signaling is independent of Hh ligands.

Second, Hh signaling is aberrantly activated through autocrine or juxtacrine ligand-dependent manner, where Hh is secreted and responded by the same or adjacent cells [13]. This category of cancers includes breast cancer, pancreatic cancer, lung cancer, prostate cancer, colorectal cancer, stomach and esophageal cancer, ovarian and endometrial cancer, melanomas, and gliomas [13]. Finally, in pancreatic cancer, prostate cancer, and colon cancer, Hh signaling is activated through a paracrine-dependent manner, where Hh ligands are secreted by tumor cells, whereas the PTCH receptor is expressed on stromal cells in the tumor microenvironment (TME). In this last model of Hh signaling activation, a feedback loop is generated, which allows the transmit of the growth-promoting signals from tumor cells to stromal cells and then back to tumor cells, leading to sustained tumor progression [17].

In the following sections, we will first highlight the key cellular components of TME involved in oncogenic Hh signaling to promote tumor progression. We will then

review the current status of the FDA-approved and non-approved inhibitors of Hh signaling, as well as the molecular mechanisms of drug resistance. Finally, we will provide a critical evaluation of recent studies on the treatments combining immunotherapeutic strategies with approved Hh inhibitors and will propose potential strategies that could be applied to harness existing knowledge to overcome the drug resistance.

2. The role of Hh signaling in the TME

Emerging evidence has suggested that TME is not just a silent bystander, but rather an active player of tumor progression [18, 19]. The composition of TME not only varies between tumor types, but also is continuously evolving in the different stages of tumorigenesis. Hh signaling has been intensively studied with respect to the classical hallmarks of cancer [3–6]. In contrast, its role in the modulation of TME has only become evident in recent studies [20, 21].

2.1 Immune cells

The adaptive and innate immune systems cooperate to form a highly proficient immune surveillance machinery that can identify and eradicate genetically altered cells to prevent tumorigenesis. Tumor-infiltrating leukocytes (TILs), including T and B lymphocytes, monocytes and macrophages, myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), and natural killer (NK) cells, play diverse roles in tumor progression through interactions and production of cytokines, chemokines, and growth factors to support or suppress tumor growth and metastasis [20, 21]. There is increasing evidence from multiple experimental models that demonstrate an important and multifaceted role of Hh signaling in the modulation of immune cell functions. Aberrant Hh signaling induces a hostile, immunosuppressive microenvironment to dampen an effective antitumor immune response.

Regulatory T cells (Tregs) control the activity of effector immune cells such as granzyme B-expressing $CD8^+$ T cells and NK cells by secreting anti-inflammatory cytokines such as TGF-β and interleukin-10 (IL-10) [24]. The immune modulatory role of Hh signaling in T cells is evidenced by recent studies demonstrating that Hh signaling may directly regulate the expression and activity of TGF-β. Treg infiltration has been described for Hh-associated tumors, such as BCC [23], and medulloblastoma [26–29].

Intriguingly, elevated Treg infiltration is accompanied by an increase of TGF-β within intra- and peri-tumoral skin in a human UV-exposed facial BCC model [25]. In line with the putative immunosuppressive phenotype of Hh signaling, genetic abrogation of T-cell TGF-β signaling mitigated tumor progression in a transgenic medulloblastoma mouse model overexpressing smoothened A1 (SmoA1), an obligatory and conserved Hh signal transducer [26]. In this study, TGF-β signaling blockade led to nearly abolishment of Tregs and licensing of CD8 cytotoxic T lymphocytes for antitumor immunity [24].

Mechanistically, GLI2, an Hh effector, has been shown to directly activate the expression of TGF-β in human Tregs [30]. Thus, it has been proposed that Hh signaling may help generate a feed-forward loop where TGF-β induces the inversion of $CD4^+$ T cells to Tregs, which in turn secrete high levels of TGF-β, leading to

enforcement of the continued presence of immunosuppressive Tregs in the tumor microenvironment [31].

Myeloid cell infiltration has been described in multiple cancer entities where tumors may benefit from myeloid cells-mediated immunosuppression. The role of Hh signaling in the tumor-promoting function of myeloid cells has been postulated based on observations in multiple models of Hh-induced tumors. First, in a murine SMO-induced BCC model, tumor growth appears to be enhanced by the recruitment of immunosuppressive myeloid derived suppressor cells (MDSCs), accompanied by a reduction of effector lymphocytes in the tumor lesions [32]. This is mediated by the TGF-β-CCL2 (C-C motif chemokine ligand 2) axis secreted by oncogenic SMO-expressing keratinocytes and the CCL2 receptor expressed by MDSCs. *In vivo*, pharmacological suppression of the CCL2 receptor expression decreased infiltration of MDSCs and resulted in reduced tumor growth, indicating an immunosuppressive phenotype by the oncogenic Hh signaling [33]. Likewise, there is also strong evidence for immunosuppressive function of myeloid cells in Hh-associated medulloblastomas, which are characterized by high myeloid infiltration. For example, gene expression profiling of human Hh medulloblastoma tumors showed enrichment for an M2-like gene expression profile, consistent with immunosuppressive functions of myeloid cells [34]. Moreover, an inverse correlation has been observed between expression of M2-like markers (such as CD163) and survival of human Hh medulloblastoma patients [34].

Along these lines, the notion of an immunosuppressive function of Hh signaling was further affirmed by two recent studies in Hh-induced medulloblastomas. In a mouse model of Hh medulloblastoma (*Ptch1*$^{+/-}$; *Tp53*$^{-/-}$), Dang et al. showed decreased T-cell proliferation in a co-culture system of tumor-infiltrating myeloid cells and *ex vivo* stimulated T cells [35]. Mechanistically, the immunosuppressive phenotype appears to be mediated by CCL2. Genetic knockout of CCL2 receptor not only decreased infiltration of monocyte-derived macrophages but also increased levels of CD8$^+$ T cells in tumors [35]. Likewise, in another mouse model of Hh-induced medulloblastoma (Atoh1-SmoM2), pharmacological inhibition of colony stimulating factor 1 receptor (CSF1R) depleted macrophages and microglia, resulting in delayed tumor growth and prolonged mouse survival [36]. These recent studies support the notion of a tumor- promoting function of macrophages, which are consistent with an early study in another Hh-associated medulloblastoma tumor model, where the presence of MDSCs increases infiltration of Tregs and reduces the number of effector T cells [37]. Interestingly, infiltrating myeloid cells have been described as the predominant source of PD-L1 expression in a mouse model of Hh-induced medulloblastoma where the binding of PD- L1 to PD-1 on effector T cells resulted in T-cell exhaustion and immune escape of tumor cells [38]. Furthermore, an analysis of an immunocompetent breast cancer xenograft mouse model showed that inhibition of Hh signaling (SMO inhibitor vismodegib) led to reduced infiltration of immunosuppressive myeloid cells in the tumors, accompanied by an increase of effector CD8$^+$ T cells and M1 macrophages [39].

2.2 Tumor-associated astrocytes (TAAs)

Astrocytes, the most abundant type of glial cells in the brain, are integral partners with neurons in the regulation of neuronal development and brain function. Hh signaling has emerged as a critical player to support astrocyte-mediated modulation of neuronal activity [40–42]. A recent series of elegant work supports a key role of

tumor-associated astrocytes (TAAs) in promoting tumor growth and metastasis through distinct signaling, including Hh pathway [43–46]. First, TAAs were shown to secret the ligand Shh, which is required for maintaining cell proliferation of Hh-activated medulloblastoma through a Smo-dependent, but Gli1-independent manner, despite the absence of its primary receptor Ptch1. Of note, ablation of TAAs blocked tumor growth [43]. Furthermore, a recent study at single-cell resolution demonstrated that Hh-induced medulloblastoma cells can transdifferentiate into interleukin-4 (IL-4)-secreting TAAs, which in turn stimulates tumor-associated microglia to release insulin-like growth factor 1 (IGF1) to promote tumor progression [44]. Similarly, medulloblastoma-associated astrocytes have recently been shown to produce high levels of CCL2, a tumor-promoting cytokine shown to drive stemness maintenance and proliferation of disseminated tumor cells [45] and to promote metastasis [47]. Moreover, using single-cell RNA sequencing and lineage tracing analyses, Guo et al. investigated cellular origin of TAAs in a mouse model for relapsed Hh-activated medulloblastoma driven by *Ptch1* knockout [46]. This study has elegantly demonstrated that TAAs are predominantly derived from the transdifferentiation of tumor cells in relapsed MB, but not in primary MB, thus establishing the distinct cellular sources of astrocytes [46]. Interestingly, this study revealed that such transdifferentiation of medulloblastoma cells to TAAs depends on bone morphogenetic proteins (BMPs) and that pharmacological inhibition of BMP signaling repressed transdifferentiation and suppressed tumor relapse [46]. It remains to be determined what drives these transdifferentiation events and what intrinsic and extrinsic mechanisms, beyond Hh and BMP signaling, regulate the potential cooperation of TAAs and microglia in promoting the immunosuppressed state of medulloblastoma.

2.3 Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs), the most abundant stromal cells in TME, have emerged as a central player in cancer progression and metastasis [48]. Through diverse phenotypes, origins, and functions, CAFs modulate the cross talk between inflammation and tumorigenesis and contribute to therapeutic resistance by producing various cytokines, chemokines, growth factors, and matrix-degrading enzymes [49].

There is increasing evidence indicating that CAF populations that support or suppress tumor growth and progression through stroma-specific Hh activation have been detected in multiple tumor types, including pancreatic cancer, colon cancer, and bladder cancer [50]. Recent advances in single-cell technologies have enabled detailed characterization of the heterogeneity and plasticity of differential CAF subsets, supporting a new therapeutic strategy in which tumor-supporting CAFs are reprogrammed into tumor-suppressing CAFs [50]. In pancreatic ductal adenocarcinoma (PDAC), Hh signaling pathway is activated in CAFs via a paracrine mechanism and has been associated with pancreatic tumorigenesis [49]. Initial studies indicated that inhibition of Hh pathway impaired tumor growth and sensitized tumors to chemotherapy in multiple PDAC models [51–56]. However, recent studies have challenged the concept of tumor-promoting CAFs. In the context of an oncogenic Kras-driven mouse PDAC model, conditional deletion of *Shh*, the predominant Hh ligand expressed in pancreas, led to cachexia and to poorly differentiated and highly vascularized tumors [57].

Moreover, by using three distinctly genetically engineered mouse PDAC models, another study showed that pharmacologic inhibition of Hh pathway activity

accelerated rather than delayed progression of oncogenic Kras-driven disease by affecting the balance between epithelial and stroma elements, leading to suppression of stromal desmoplasia but accelerated growth of pancreatic intraepithelial neoplasia [58]. These contradictory findings indicate that Hh signaling may play pleiotropic roles in PDAC progression. Interestingly, by using a combination of pharmacologic inhibition, gain- and loss-of-function genetic experiments, cytometry by time-of-flight, and single-cell RNA sequencing, a more recent study defines dosage-dependent effects of Hh signaling on the composition and function of CAFs in PDAC microenvironment [59]. Hh signaling is uniquely activated and differentially elevated in CAFs, with higher levels in myofibroblastic CAFs (myCAF) compared with inflammatory CAFs (iCAF) in both mouse and human PDAC. Driving high levels of Hh signaling promotes tumor growth, whereas Hh pathway inhibition alters the ratio of myCAF/iCAF populations, which is accompanied by a decrease in cytotoxic T cells and an expansion in regulatory T cells, thus altering the composition of CAFs, and shifting the inflammatory response toward a more immunosuppressive phenotype [59]. Given the differential functional implications for CAF subpopulations, changes in the ratio of CAF subtypes may lead to distinct antitumor outcomes. Consistent with, recent studies demonstrated a possible negative impact of current Hh pathway inhibitors on antitumor response in clinical trials, which were largely unsuccessful or even detrimental to patient health [60, 61]. Further understanding of the roles of Hh signaling in CAFs may open the possibility for more effective combination cancer therapies.

3. Therapeutic targeting Hh signaling in cancers

Given the multifaceted role of Hh signaling in cancer, inhibitors of Hh pathways have emerged as an important class of anticancer agents. These compounds fall into three main categories: Hh ligand inhibitors, SMO inhibitors, and GLI inhibitors [62]. Despite extensive efforts devoted to the discovery of Hh signaling inhibitors, so far only three drugs have been approved by the Food and Drug Administration (FDA), all targeting the upstream receptor of Hh signaling SMO, a membrane protein of the GPCR protein family [62].

3.1 FDA-approved inhibitors

To date, three SMO inhibitors, vismodegib, sonidegib, and glasdegib, have been FDA approved in 2012, 2015, and 2018, respectively, for cancer treatment. Cyclopamine, the first SMO antagonist, is a naturally occurring alkaloid found in the corn lily [63] later proved to bind to SMO and to inhibit activation of downstream Hh target genes [64].

Extensive efforts have been made to develop alkaloid derivatives to increase the bioavailability, sensitivity, and specificity of cyclopamine to target SMO [65]. Vismodegib (GDC-0449 or Erivedge), the first cyclopamine derivative and Hh pathway–targeting drug, is currently approved for the treatment of patients with locally advanced or metastatic BCC (US FDA). Compared to cyclopamine, vismodegib shows a higher potency and more favorable pharmacological properties [62]. The approval of vismodegib was based on results from the pivotal phase II ERIVANCE trial (ClinicalTrials.gov, NCT00833417) showing that vismodegib substantially shrank tumors or healed visible lesions (objective response rate, ORR) in 43% of patients with locally advanced BCC and 30% of patients with metastatic BCC, at 21 months, with a median

progression-free survival (PFS) duration of 9.5 months for both metastatic and locally advanced BCC patients [66, 67]. Up to the completion of this manuscript, there have been 86 clinical trials for vismodegib, both monotherapy and combination, in various cancer types (ClinicalTrials.gov).

Sonidegib (Erismodegib, NVP-LDE-225, LDE-225, Odomzo) is another cyclopamine-derived SMO antagonist discovered in 2010 through an *in vitro*, high-throughput screen, showing high tissue penetration and bioavailability, as well as the ability to cross the blood-brain barrier [68]. In 2015, sonidegib became the second SMO inhibitor approved for patients with locally advanced or recurrent BCC (US FDA). The approval of sonidegib was based on results from a multicenter, randomized, double-blind phase II BOLT trial (ClinicalTrials.gov, number NCT01327053), which showed the objective response rates of 38% and 43% in the 800 and 200 mg dosage groups, respectively after 30 months in patients with locally advanced BCC and the objective response rates of 17% and 15%, respectively in those with metastatic BCC [69]. Up to August 2022, there are 46 clinical trials for sonidegib in cancer treatment (ClinicalTrials.gov).

A third FDA-approved inhibitor of Hh signaling is glasdegib (PF-04449913, Daurismo), a benzamide derivative discovered in 2012 with high potency and oral bioavailability [70]. In 2018, glasdegib was approved for combination treatment with low-dose cytarabine arabinoside (LDAC) for patients with acute myeloid leukemia unsuitable for intensive chemotherapy. The approval of glasdegib was based on the results of the phase II BRIGHT 1003 trial (ClinicalTrials.gov, NCT01546038) showing the median overall survival of 8.8 months with glasdegib/LDAC as compared to 4.9 months with LDAC. Furthermore, 17.0% and 2.3% of patients in the glasdegib/LDAC and LDAC arms, respectively, achieved complete remission [71]. Up to this point, there have been 26 clinical trials for glasdegib in various cancer types (Clinica lTrials.gov).

3.2 Resistance mechanisms to FDA-approved inhibitors

The first retrospective study on drug resistance to SMO inhibitor therapy was reported in 2012 where 21% of BCC patients treated with vismodegib developed drug resistance, with a mean tumor recurrence time of 56.4 weeks in clinical examination [72]. Ever since, resistance to SMO antagonists has been observed in patients who never respond to SMO inhibitor therapy (primary resistance), as well as in those who initially respond but later develop resistance to SMO inhibitors (acquired resistance) [73]. Mechanistically, a number of models have been proposed to explain the basis of drug resistance to SMO inhibitor therapy. First, genetic analysis of resistant tumors has revealed mutations of SMO, loss of SUFU, and amplification of GLIs or Hh target genes, such as CCND1 and GLI1 [5, 10]. Second, accumulating evidence supports the notion that the resistance can be driven through the non-canonical Hh signaling, accompanied by the concurrent activation of other oncogenic signaling pathways, such as AP-1 and TGF-β signaling [74], RhoA signaling [75], and RAS-MAPK signaling [76]. Finally, a new mechanism has recently been uncovered to contribute to drug resistance through loss of primary cilia [77, 78]. This was supported by both preclinical and clinical evidence. In Hh-dependent medulloblastoma, recurrent mutations in oral facial digital syndrome 1 (OFD1), a culprit gene led to loss of cilia, and thereby caused resistance to SMO inhibitors [78]. Importantly, sequencing data analysis from resistant BCC patients showed recurrent mutations in ciliary genes, providing clinical relevance of this new mechanism [77]. Therefore, a better understanding of cilia-

regulating signaling pathways in resistant cancer may open up a new route to reintroduce cilia to sensitize resistant cancer cells to SMO inhibitors. Taken together, several strategies have been proposed to overcome the drug resistance through targeting the underlying mechanisms. These approaches include: (1) develop second-generation SMO inhibitors to retain anticancer activities that are not affected by the resistance-inducing mutations [5]; (2) target downstream components of SMO, such as GLIs (see below, non- approved inhibitors), or signaling molecules involved in the non-canonical Hh signaling pathway [8].

3.3 Non-FDA-approved inhibitors

Multiple novel inhibitors targeting SMO have been shown to be effective in preclinical models [5] and are now in active clinical trials, either monotherapy or combination for various cancer types. These compounds include saridegib (patidegib, IPI-926), taladegib (LY2940680), and BMS-833923 (XL139) (ClinicalTrials.gov). On the other hand, even though GLI1 antagonists are not as extensive as those targeting SMO, mounting evidence has shown that targeting the Hh signaling at the level of its final effector, GLI1, is a promising strategy to overcome resistance to currently available SMO inhibitors [79, 80]. In this regard, the promising pharmacological potential of direct and indirect GLI inhibitors, as well as GLI antagonists derived from natural products, has been in active investigation at the preclinical or clinical phase. It is anticipated that future study on these compounds will help develop new strategies tackling resistant mechanisms and tumor heterogeneity [81].

4. Hh signaling and antitumor immune response

In 2018, James P Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine "for their discovery of cancer therapy by inhibition of negative immune regulation" [82]. Although this breakthrough in cancer immunotherapy has revolutionized cancer treatment, only a subset of patients elicit favorable responses and most immunologically cold solid tumors are not responsive [83]. Given the immunosuppressive function of Hh signaling, inhibitors of Hh signaling pathway may hold promise in converting nonresponsive cold tumors into responsive hot ones, which may subsequently allow nonresponders to benefit from immunotherapies. Notably, clinically approved Hh inhibitors, as well as non-approved inhibitors, have been in active preclinical and clinical trials for combined therapies, including immunotherapies.

The first clinical trial with Hh inhibitors in combination with immune checkpoint inhibitors was conducted in 16 patients with advanced BCC (clincialtrial.gov, NCT02690948). This trial showed that pembrolizumab (PD-L1 inhibitor) is active against BCCs. Although the two groups of pembrolizumab with or without vismodegib were not directly compared, the response rate for the combination group was not superior to the monotherapy group [84]. Of note, most patients with advanced BCC progress on or are intolerant to Hh inhibitor therapy despite objective response rates of 30–60% [66–69, 85]. Until Feb 9, 2021, when cemiplimab, a PD-1 antibody, was approved by the US FDA fully for patients with locally advanced BCC, and accelerated for patients with metastatic BCC, after treatment with Hh inhibitors, or for whom Hh inhibitors are not appropriate [86], there was no standard second-line treatment option for these BCC patients [72]. A recent clinical trial study provides the

first report to show clinically meaningful antitumor activity of cemiplimab in patients with BCC after Hh inhibitor therapy ([87], clinicaltrials.gov, NTC03132636). In this trial, the efficacy and safety of cemiplimab were evaluated in patients with locally advanced BCC or metastatic BCC who had previously been treated with an Hh inhibitor. Among the efficacy population (n = 121), centrally reviewed objective response was observed in 31% of patients with estimated duration of response exceeding 1 year in 85% of responders [87].

Importantly, this study also showed that the safety profile was consistent with what is known for immune checkpoint class of drugs, even considering the advanced age of the patient population in the present study [87]. These findings demonstrate the efficacy of immune checkpoint blockade in treating BCC in patients who had previously received Hh inhibitor therapy, thus opening a new horizon for treatment of the many patients who discontinue Hh inhibitor therapy due to disease progression, toxicity, or drug resistance. Moreover, a recent case report demonstrated an impressive response to cemiplimab in a sonidegib-resistant giant basosquamous carcinoma, one form of BCC [88]. Finally, a dozen of clinical trials have been initiated to investigate the combination treatment of anti-PD-1, PD-L1, and CTLA-4 monoclonal antibody therapy with first-line Hh inhibitors in patients with a variety of cancer types (see **Table 1**). The outcome of these trials will not only inform about whether combinatorial treatments can increase the efficacy and duration of antitumor response, but also provide insights into the optimal customized regimen to circumvent resistance to Hh inhibitors.

Comparatively a few recent studies have indicated possible negative effects of the current Hh inhibitor therapy on antitumor immunity [89]. For instance, blockade of SMO signaling may inhibit formation of the immunological synapse [90]. Administration of SMO inhibitors caused the functional disruption of the immunological synapse, leading to the loss of T-cell effector activity [90]. Even though it remains unclear whether Hh inhibitor therapy may impede cytotoxic T-cell killing in cancer patients, a pilot clinical trial study of vismodegib in combination with pembrolizumab did not suggest additive clinical activity [84]. In the clinical context, there is an emerging paradigm that immunotherapy may show the greatest activity when administered early in the natural history of cancers. Further studies are warranted to evaluate the efficacy and duration of immune checkpoint blockade before Hh inhibitor therapy.

5. Conclusions

The Hh signaling pathway has attracted extensive research attention as a key player to contribute to the progression of a variety of human cancer types. With an in-depth understanding of the molecular mechanisms underlying the role of Hh signaling in tumorigenesis, enormous efforts have been made to develop specific inhibitors targeting molecular components of this pathway. Consequently, cancer therapy has undergone a paradigm shift from eradicating tumor cells to multidimensional targeting and normalizing tumor cells and TME. Herein, we reviewed the multifaceted function of Hh signaling in shaping immunologically suppressive TME to promote tumor progression, provided an up-to-date status of active clinical trials of FDA approved Hh inhibitors, and finally, highlighted possible therapeutic interventions that harness the immunomodulatory effects of Hh signaling not only to overcome drug resistance, but also to achieve durable efficacy following immunotherapies.

SMO inhibitor	Combination	Cancer Type	Enrollment	Phase	Status	NCT #
Vismodegib (GDC-0449 or Erivedge)	+ VEGF-A antibody and chemotherapy	Metastatic Colorectal Cancer	199	Phase 2	Completed	NCT00636610
	+ Anti-hormone therapy	Prostate Cancer	10	Phase 1|2	Terminated	NCT01163084
	+ Chemotherapy	Pancreatic Cancer	118	Phase 1|2	Completed	NCT01064622
	+ VEGF-A antibody and chemotherapy	Ovarian Cancer|Basal Cell Carcinoma|Metastatic Colorectal Cancer	19	Phase 2	Completed	NCT00959647
	+ Notch inhibitor	Breast Cancer	13	Phase 1	Terminated	NCT01071564
	+ Chemotherapy	Gastric Cancer	124	Phase 2	Completed	NCT00982592
	+ Chemotherapy	Pancreatic Cancer	25	Phase 2	Completed	NCT01195415
	+ Chemotherapy	Myelodysplastic Syndromes,	38	Phase 2	Terminated	NCT01880437
	+ Notch inhibitor	Sarcoma	78 Phase1|2	Completed	NCT01154452	
	+ Photodynamic therapy	Basal Cell Nevus Syndrome	24	Phase 2	Completed	NCT01556009
	+ IGF1R antibody and chemotherapy	Small Cell Lung Carcinoma	168	Phase 2	Completed	NCT00887159
	+ Chemotherapy	Pancreatic Adenocarcinoma	21	Phase 1	Unknown	NCT01713218
	+ Chemotherapy	Medulloblastoma	24	Phase 1|2	Terminated	NCT01601184
	+ Chemotherapy	Metastatic Pancreatic Cancer	98	Phase 2	Completed	NCT01088815
	+ DNMT inhibitor	Acute Myeloid Leukemia	40	Phase 2	Unknown	NCT02073838
	+ mTOR inhibitor	Pancreatic Cancer	31	Phase 1	Completed	NCT01537107
	+ PD1 blockade	Skin Basal Cell Carcinoma	16	Phase 1|2	Completed	NCT02690948
	+ Chemotherapy	Breast Cancer	40	Phase 2	Unknown	NCT02694224
	+ Radiation therapy	Basal Cell Carcinoma	24	Phase 2	Completed	NCT01835626
	+ Radiation therapy	Carcinoma, Basal Cell	14	Phase 2	Terminated	NCT02956889

SMO inhibitor	Combination	Cancer Type	Enrollment	Phase	Status	NCT #
	+ PD1/CTLA4 blockade	Basal Cell Nevus Syndrome	0	Phase 2	Withdrawn	NCT03767439
	+ Tyrosine kinase inhibitor	Basal Cell Carcinoma	84	Phase 2	Recruiting	NCT04416516
	+ Tyrosine kinase inhibitors and PARP inhibitors	Miscellaneous	950	Phase 2	Recruiting	NCT02925234
	+ PDL1 blockade and Tyrosine kinase inhibitors	Miscellaneous	676	Phase 2	Active, not recruiting	NCT02091141
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Lymphoma, Non-Hodgkin	720	Phase 2	Recruiting	NCT03297606
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Cancer of Unknown Primary Site	790	Phase 2	Recruiting	NCT03498521
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Miscellaneous	384	Phase 2	Recruiting	NCT04591431
	+ Targeted therapy and chemotherapy	Glioblastoma, Adult	350	Phase 1|2	Recruiting	NCT03158389
	+ Targeted therapy and chemotherapy	Meningioma	124	Phase 2	Recruiting	NCT02523014
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Miscellaneous	300	Phase 2	Recruiting	NCT04341181
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Miscellaneous	6452	Phase 2	Recruiting	NCT02465060
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Miscellaneous 40	Phase 1	Recruiting	NCT03878524	
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Miscellaneous	131	Phase 2	Not yet recruiting	NCT05238831
	+ PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy	Advanced Cancer|Solid Tumor	250	Phase 2	Recruiting	NCT05159245

SMO inhibitor	Combination	Cancer Type	Enrollment	Phase	Status	NCT #
	+ Radiation therapy and chemotherapy	Medulloblastoma	660	Phase 2	Recruiting	NCT01878617
	+ PDL1 blockade	Cancer Metastatic	1000	Phase 2	Recruiting	NCT04817956
	+ Chemotherapy	Pancreatic Cancer	55	Phase 1	Active, not recruiting	NCT00878163
Sonidegib (Erismodegib, NVP-LDE-225, LDE-225, Odomzo)	+ Radiation therapy and chemotherapy	Medulloblastoma	205	Phase 2	Not yet recruiting NCT04402073	
	+ Chemotherapy	Lung Cancer	19	Phase 1	Completed	NCT01579929
	+ JAK inhibitor	Miscellaneous	50	Phase 1|2	Completed	NCT01787552
	+ Chemotherapy	Pancreatic Ductal Adenocarcinoma	23	Phase 1|2	Terminated	NCT01431794
	+ Chemotherapy	Myelodysplastic Syndrome	63	Phase 1	Completed	NCT02129101
	+ mTOR kinase inhibitor	Esophageal Cancer	25	Phase 1	Completed	NCT02138929
	+ Chemotherapy	Plasma Cell Myeloma	28	Phase 2	Completed	NCT02086552
	+ Chemotherapy	Pancreatic Cancer	78	Phase 1|2	Completed	NCT02358161
	+ Tyrosine kinase inhibitor and chemotherapy	Chronic Myelogenous Leukemia	11	Phase 1	Completed	NCT01456676
	+ Tyrosine kinase inhibitor and chemotherapy	Miscellaneous	108	Phase 1	Recruiting	NCT03434262
	+ Tyrosine kinase inhibitor	Miscellaneous	120	Phase 1	Completed	NCT01576666
	+ Chemotherapy	Advanced Breast Cancer	12	Phase 1	Completed	NCT02027376
	+ Tyrosine kinase inhibitor	Carcinoma, Basal Cell	10	Phase 2	Terminated	NCT02303041
	+ Chemotherapy	Pancreatic Cancer	18	Phase 1	Completed	NCT01487785
	+ PD1 blockade	Miscellaneous	45	Phase 1	Recruiting	NCT04007744
	Neoadjuvant + Surgery	Basal Cell Carcinoma	10	Phase 2	Recruiting	NCT03534947
	+ Chemotherapy	Multiple Myeloma	7	Phase 2	Terminated	NCT02254551

SMO inhibitor	Combination	Cancer Type	Enrollment	Phase	Status	NCT #
	+ Chemotherapy	Solid Tumor|Ovarian Cancer	30	Phase 1	Completed	NCT01954355
	+ Chemotherapy	Pancreatic Cancer	39	Phase 1	Completed	NCT01485744
	+ Chemotherapy	Prostate Cancer	0	Phase 1	Withdrawn	NCT02182622
	+ PD1 blockade	Basal Cell Carcinoma	20	Phase 2	Recruiting	NCT04679480
Glasdegib (PF-04449913, Daurismo)	+ Chemotherapy	Glioblastoma	75	Phase 1|2	Active, not recruiting	NCT03466450
	+ Chemotherapy	Acute Myelogenous Leukemia	30	Phase 2	Recruiting	NCT04231851
	+ Antibody-drug conjugate	Acute Myeloid Leukemia	414	Phase 3	Recruiting	NCT04168502
	+ Chemotherapy	ACUTE MYELOID LEUKEMIA	1	Phase 2	Terminated	NCT04051996
	+ Chemotherapy	Acute Myeloid Leukemia	15	Phase 3	Active, not recruiting	NCT04842604
	+ Chemotherapy	Myelodysplastic Syndrome	73	Phase 1	Completed	NCT02367456
	+ Chemotherapy	Leukemia, Myeloid, Acute	730	Phase 3	Completed	NCT03416179
	+ Chemotherapy	Acute Myeloid Leukemia	0	Phase 1	Withdrawn	NCT04655391
	+ Antibody-drug conjugate	Acute Myeloid Leukemia	28	Phase 3	Terminated	NCT04093505
	+ PD1 blockade, antibody-drug conjugate and chemotherapy	Acute Myeloid Leukemia	138	Phase 1|2	Active, not recruiting	NCT03390296
	+ Chemotherapy	Acute Myeloid Leukemia	48	Phase 1	Active, not recruiting	NCT02038777
	+ Chemotherapy	Leukemia, Myeloid, Acute	0		Withdrawn	NCT04230564
	+ Chemotherapy	Acute Myeloid Leukemia	255	Phase 2	Completed	NCT01546038
	+ Chemotherapy	Adult Acute Myeloid Leukemia	75	Phase 2	Recruiting	NCT03226418
	+ Chemotherapy	Soft Tissue Sarcoma	960	Phase 3	Recruiting	NCT03784014
	+ Chemotherapy and radiation therapy	Glioblastoma	30	Phase 1|2	Not yet recruiting	NCT03529448

SMO inhibitor	Combination	Cancer Type	Enrollment	Phase	Status	NCT #
Saridegib (patidegib, IPI-926)	+ Chemotherapy	Metastatic Pancreatic Cancer	122	Phase 1|2	Completed	NCT01130142
	+ Chemotherapy	Pancreatic Cancer	15	Phase 1	Completed	NCT01383538
	+ Tyrosine kinase inhibitor	Head and Neck Cancer	9	Phase 1	Completed	NCT01255800
Taladegib (LY2940680)	+ Chemotherapy and radiation therapy	Esophageal Adenocarcinoma	7	Phase 1|2	Completed	NCT02530437
	+ Chemotherapy	Small Cell Lung Carcinoma	26	Phase 1|2	Terminated	NCT01722292
	+ Chemotherapy and CDK inhibitors	Breast Cancer|Colon Cancer|Cholangiocarcinoma|Soft Tissue Sarcoma	94	Phase 1	Completed	NCT02784795
BMS-833923 (XL139)	+ Tyrosine kinase inhibitor	Leukemia	33	Phase 1|2	Completed	NCT01218477
	+ Tyrosine kinase inhibitor	Leukemia	70	Phase 2	Terminated	NCT01357655
	+ Chemotherapy	Small Cell Lung Carcinoma	5	Phase 1	Completed	NCT00927875
	+ Chemotherapy	Stomach Neoplasms|Esophageal Neoplasms 39	Phase 1	Completed	NCT00909402	
	+ Proteasome inhibitors	Advanced Cancer, Various, NOS	27	Phase 1	Completed	NCT00884546

Data from clinicaltrials.gov (accessed on 2022/8/22).

Table 1.
Combination therapy of SMO inhibitors under clinical trials.

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