

## Review Article

# Combination of NK-based immunotherapy and sorafenib against hepatocellular carcinoma

Jia Yang<sup>1</sup>, Aydin Eresen<sup>1</sup>, Alessandro Scotti<sup>2,3</sup>, Kejia Cai<sup>2,3</sup>, Zhuoli Zhang<sup>1,4</sup>

<sup>1</sup>Department of Radiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, USA;

<sup>2</sup>Department of Radiology, University of Illinois at Chicago, Chicago, IL, 60612, USA; <sup>3</sup>Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, 60612, USA; <sup>4</sup>Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, 60611, USA

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**Abstract:** Hepatocellular carcinoma (HCC) is the most frequent malignancy of the liver, which is considered the fourth leading cause of cancer-related death in the United States. Liver transplant and surgical resection are curative treatments for HCC, but only 10-15% of HCC patients are eligible candidates. The FDA-approved sorafenib is a multi-kinase inhibitor systemic therapy for advanced HCC that extends the overall survival by over 3 months when compared with placebo. Adoptive transfer of Natural Killer (NK) cells holds great promise for clinical cancer treatment. However, only limited clinical benefit has been achieved in cancer patients. Therefore, there is currently considerable interest in development of the combination of sorafenib and NK cells for the treatment of HCC patients. However, the mechanism of how sorafenib affects the function of NK cells remains to be comprehensively clarified. In this paper, we will discuss NK cell-based immunotherapies that are currently under preclinical and clinical investigation and its potential combination with sorafenib for improving the survival of HCC patients.

**Keywords:** Natural killer cells, sorafenib, hepatocellular carcinoma, combination therapies

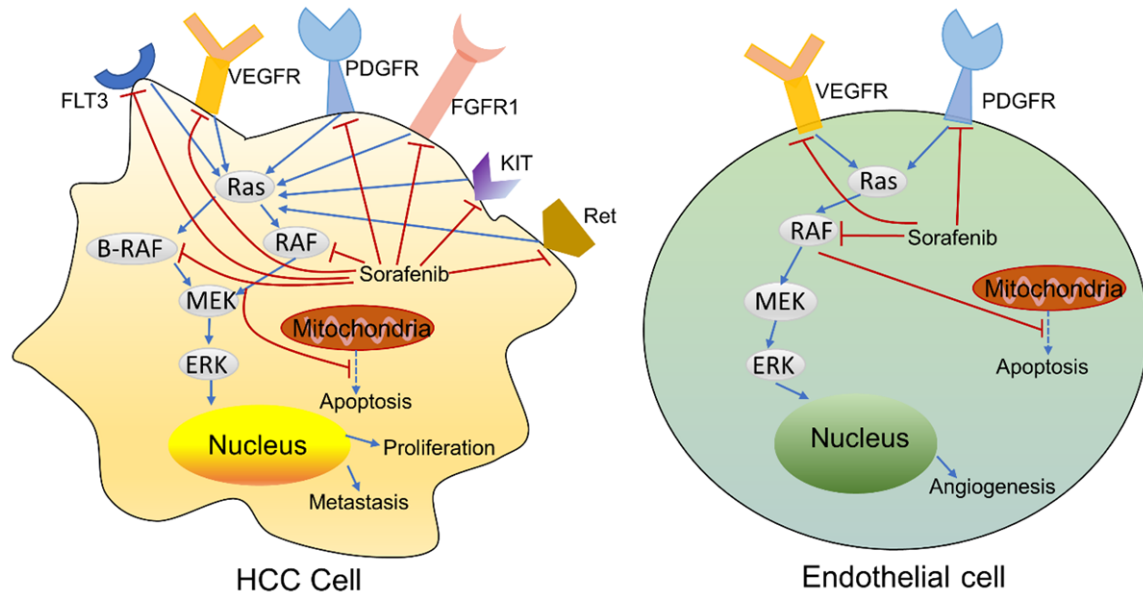
### Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary malignancy in the liver with a high mortality rate worldwide [1]. Chronic infection in the liver with hepatitis B or hepatitis C viruses remains the major risk of HCC. Alcoholic cirrhosis and nonalcoholic steatohepatitis with metabolic syndrome are considered other well-recognized factors that escalate the risk for the development of HCC [2-4]. HCC is a high therapy-resistant tumor that is most frequently diagnosed at advanced stages and thus difficult to treat. Although liver transplant and surgical resection are considered curative treatment options for HCC, it is generally only offered to patients without extrahepatic metastases [5]. Patients who do not qualify for major surgical therapeutic procedures can be treated by other minimally invasive procedures, including radio-frequency ablation, transarterial chemoembolization (TACE), microwave ablation, and irreversible electroporation [6]. However, these therapeutic approaches most often do not provide

a complete cure as a high rate of recurrences has been reported [7]. Recently, sorafenib, an oral multi-target kinase inhibitor that can impair tumor proliferation and angiogenesis, has gained recognition in the clinical treatment development for advanced or metastatic HCC patients who have no viable therapeutic strategies [8, 9]. Despite the fact that sorafenib can bring clinical benefits, the median survival rate for patients who have progressed into the terminal stage is less than 10% [10]. Therefore, novel therapies for HCC remain an urgent medical need.

Immunotherapy, including vaccines, immunomodulatory reagents, and adoptive transfer of immune cells, has been explored in HCC for decades and is considered a promising avenue, in light of the recent progress in the management of other malignancies [11]. The adoptively transferring of Natural killer (NK) cells immunotherapy is a potent and well tolerated treatment for a broad range of malignancies [12]. NK cells have significant advantages for cancer therapy,

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**Figure 1.** The mechanism of action of Sorafenib: tumor proliferation and angiogenesis.

since they do not depend on antigen presentation and spontaneously kill cancer cells, and they are key effectors in cancer immunosurveillance [13, 14]. Recently, adoptive transfer of NK cells has been investigated for tumor immunotherapy in patients and was demonstrated effective anti-tumor effects without any significant adverse effects (AEs) [15]. Furthermore, one of the human NK cell lines, NK-92 cell, has been tested in clinical studies for cancer therapy and is considered safe [16]. However, only limited clinical benefits have been observed thus far using adoptive transfer NK cell immunotherapies for the treatment of cancers including HCC.

The focus of the present article is on the combination of sorafenib with NK cell-based immunotherapy to treat HCC, based on the hypothesis that the combination will augment the therapeutic efficacy. We summarize the preclinical studies and the results of clinical trials, discuss the underlying mechanisms involved in the anti-tumor effects of NK cells and sorafenib as well as their functional interaction, which will provide a theoretic basis for the development of a combined treatment strategy for HCC.

### Sorafenib

Sorafenib (NEXAVAR®), the first FDA approved agent for the systemic treatment of HCC, is a multi-targeted tyrosine kinase inhibitor (TKI)

that impairs angiogenesis, cancer apoptosis, and proliferation by blocking the activity of vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor  $\beta$  (PDGFR $\beta$ ), tyrosine-protein kinase (KIT), fibrosarcoma (Raf) kinases, FLT3, Ret, and fibroblast growth factor receptors (FGFR) (Figure 1) [9, 17]. According to two international randomized controlled trials, sorafenib brings an obvious survival benefit to patients with HCC [8, 18]. However, sorafenib is related to multiple AEs, including hand-foot skin reaction, cardiovascular events, gastrointestinal disturbances, renal toxicity, and fatigue, which can potentially cause treatment discontinuation [8, 18, 19]. Recently, the development of resistance to sorafenib has also raised concerns due to the high heterogeneity of HCC, which can result in different sensitivity to the treatment among patients [20, 21]. Therefore, in recent years it has been suggested that sorafenib combining with other molecular targeted drugs could overcome such limitations by expanding the HCC treatment efficacy.

### Current status of sorafenib based combination therapy

The initiation and progression of HCC is a multi-step and multi-factor process, which suggests that a combination of agents that target multiple critical pathways or key molecules implicated in the hepatocarcinogenesis may achieve

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considerable improvements in the management of this resilient tumor. Sorafenib has been co-administrated with antiangiogenic drugs and inhibitors or agents targeting MEK/ERK pathway, PI3K/AKT/mTOR signal pathway, histone deacetylase, EGF/EGFR pathway, and HGF/c-Met pathway [22]. Combination of sorafenib with other agents, such as doxorubicin [23-25], selumetinib [26] interferon [27], capecitabine [28], tegafur-uracil [29], modified FOLFOLX (5-fluorouracil (5-FU), leucovorin, and oxaliplatin) [30], gemcitabine and oxaliplatin [31, 32], and gemcitabine lonely [33] have also been evaluated. However, until now, no combination treatments involving sorafenib did go through phase III trials.

TACE is currently considered as the standard of care for patients with intermediate-stage HCC according to international guidelines [34]. Over the past decade, numerous studies have tried to combination of TACE and sorafenib for patients with unresectable HCC, while the results of previous trials have been inconclusive [35-57]. Some clinical trials have shown that the combination of sorafenib and TACE brings encouraging efficacy and survival benefits for HCC patients [35, 38, 40-45, 47-50, 52-57]. Contrasting with these findings, other studies showed that sorafenib did not significantly extend overall survival in patients who have responses to TACE [37, 46, 51]. Although selective internal radiation therapy (SIRT) shows efficacy in unresectable HCC, the combination of SIRT and sorafenib did not achieve an improved response in overall survival when compared with sorafenib monotherapy [58].

In recent years, immunotherapy for the treatment of several types of cancer malignancies has advanced rapidly and has shown great promise especially when combined with traditional therapies. The combination of immunotherapy and sorafenib is a very promising therapeutic approach for the HCC. A recent trial reported that the cell-based immune primer ilixadencel in combination with sorafenib can induce antitumor specific immunological responses in patients with advanced HCC [59]. Moreover, in a clinical study, the combination of sorafenib with the treatment of dendritic cells and cytokine-induced killers improved the tumor response rate and prolonged overall survival of advanced HCC patients without increasing the incidence of AEs [60]. Furthermore, the

combination of sorafenib with other immunotherapies, including TLR3 agonists [61], anti-programmed death-ligand 1 monoclonal antibodies [62], dendritic cell therapy [63], chimeric antigen receptor (CAR) T-cell therapy [64], have been shown to induce a considerable reduction in tumor growth in preclinical HCC models.

### *The NK cells in HCC*

NK cells, characterized in humans as CD3<sup>-</sup>CD56<sup>+</sup> lymphocytes and in mice as CD3<sup>-</sup>NKp46<sup>+</sup> or CD3<sup>-</sup>NK1.1<sup>+</sup> cells, are major effectors of innate immunity in defense against pathogens and malignancies. NK cells can recognize and lyse viral infected cells or tumor cells based on the delivery of cytotoxic granules, the secretion of effector cytokines, and their expression of ligands, including inhibitory, activating, adhesion, and cytokine receptors. Additionally, NK cells can effectively kill tumor cells through antibody-dependent cell-mediated cytotoxicity (ADCC).

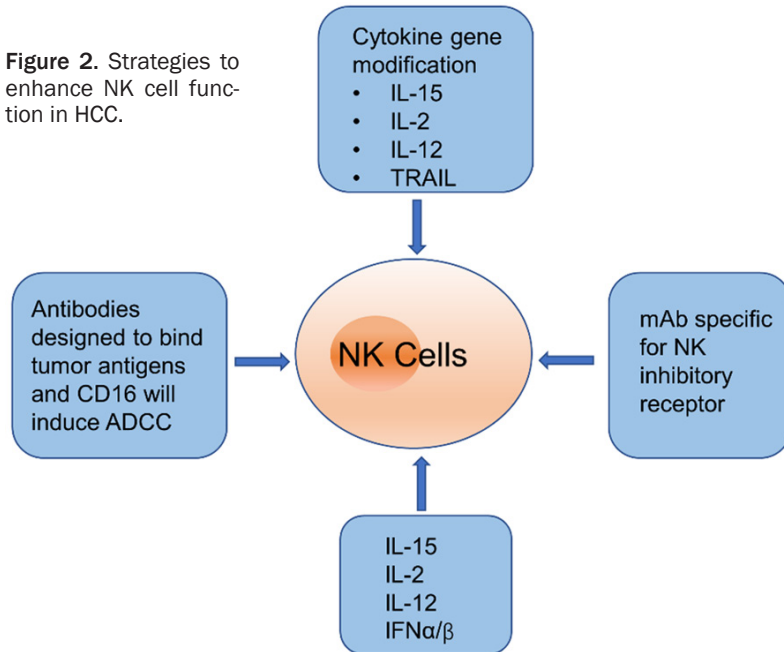
It has been shown that NK cells are enriched in the liver and play key roles in immune surveillance and in controlling the initiation and progression of HCC [65, 66]. Importantly, the ligands of several activating NK receptors, including the major histocompatibility complex class I chain-related protein A and B (MICA/B), CD133, and CD155, are frequently up-regulated on HCC cells [67, 68]. Moreover, a preclinical study observed that the NK cell frequency and function are disrupted during HCC onset and progression in a mouse model [69]. Meanwhile, the liver tumor area showed a lower frequency of NK cells when compared to the nontumor area, and NK cell function with regard to cytotoxic ability, including cytoplasmic granules secretion, TNF- $\alpha$  and IFN- $\gamma$  production, was impaired in HCC patients [70, 71]. Available evidence showed that the low frequency of NK cells in peripheral blood (PB) and liver is associated with the initiation and progression of HCC in humans [70, 72, 73]. Additionally, these patients showed disturbed distributions of NK subtypes, with a dramatic reduction in the CD56<sup>dim</sup>CD16<sup>+</sup> NK cells (more mature and cytotoxic).

### *Targeting NK cell function in the HCC microenvironment*

Among the activating NK receptor pathways, FcR<sub>III</sub> (also known as CD16) is one of the most

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**Figure 2.** Strategies to enhance NK cell function in HCC.



potent receptors that can induce a strong enough activating signal to trigger cytokine production and degranulation via ADCC [74]. It has been reported that an improved outcome after targeting CD16 monoclonal antibody (mAb) treatment can be achieved in HCC patients who show a high affinity to FcγRIII [75], which suggests that the CD16 can serve as a promising treatment target in HCC. A humanized antibody, codrituzumab, targets the glypican-3 (GPC-3) which is up-regulated in HCC cells but generally not in normal hepatocytes, is proven to interact with CD16/FcγRIIIa and trigger ADCC [76]. Importantly, a recent clinical trial showed that elevated CD16 expression on peripheral NK cells and GPC-3 expression on the tumors were correlated with prolonged overall survival and progression-free survival in HCC patients [77]. Therefore, these studies suggest the potential for CD16 and/or GPC-3 as targets to enhance NK cell function in HCC.

As an activating receptor, NKG2D has received increased attention since it mediates the cytotoxicity of NK cells via binding to its ligands such as MICA/MICB which are up-regulated in malignant cells but are generally lacking in the normal cells [78]. It has been reported that the levels of soluble MICA were increased in advanced HCC patients, which was correlated with down-regulated expression of NKG2D and disrupted activation of NK cells [79].

Accordingly, in a recent report, Easom et al. observed that NK cells derived from malignant liver tissue show a down-regulated expression of NKG2D compared with adjacent non-invasive tissue in HCC patients [80]. In contrast, a recent pre-clinical study showed that activating the receptor NKG2D resulted in tumor growth in a model of HCC. These mixed pieces of evidence suggest that the roles of NKG2D in the progression of HCC are highly complicated.

Suppressing inhibitory receptors may provide an alternative method to boost

the function of NK cells. Recently, the monoclonal antibodies that target inhibitory receptors (*i.e.*, immunoglobulin-like receptors (KIR) and NKG2A) have been tested on multiple myeloma patients [81, 82]. However, strategies for blocking inhibitory receptors to activate NK cells' antitumor function need to be further evaluated in patients and mouse models of HCC. To date, several cytokine genes, including IL-12, IL-15, IL-2, IFN- $\alpha$ , and stem cell factors, have been applied to modify NK cell lines to enhance NK cell activity against tumor cells [83, 84]. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is constitutively expressed on hepatic NK cells and plays a key role in the surveillance of tumor initiation, progression, and metastasis [85]. It has also shown that combined gene-based virotherapy involving TRAIL and IL-12 genes have obvious anti-HCC effects through upregulating the production of IFN- $\gamma$  and infiltration of NK cells in the tumor microenvironment [86]. Recently, IL-15 has also been assessed in clinical studies as a potential pharmacological candidate for cancer therapy because of its critical role in the regulation of NK cell proliferation, survival, and cytotoxicity [87]. However, IL-15 can induce severe toxicity which is correlated with consequent IFN- $\gamma$  secreted by NK cells [88]. Additionally, despite advances in NK cell expansion, the administration of IL-15 showed no sustained antitumor responses [89]. **Figure 2**

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describes various strategies to augment the function of NK cells in the HCC microenvironment.

### *NK cell adoptive immunotherapy for HCC*

Adoptive transfer NK cell immunotherapy requires the NK *ex vivo* expansion, maximal *in vivo* activity, *in vivo* long-term persistence, and cytotoxic cells with high specificity. Currently, the source of NK cells can be derived from stem cells, PB NK cells of a healthy donor (allogeneic setting) or from the patient (autologous setting), and the NK cell lines such as the NK-92 cells. Allogeneic NK cells adoptive transfer immunotherapy for HCC have been demonstrated in two recent clinical trials [90, 91]. However, it might cause serious graft-versus-host disease (GVHD) and can limit the clinical benefits due to the inadequate depletion of T cells in grafts. In this regard, autologous NK cell immunotherapy is safer than allogeneic settings with minimal side effects. A recent study observed that co-administration with autologous cytokine-induced killer cells increased overall survival and progression-free survival for patients with HCC [92]. In ClinicalTrials.gov, 6 trials for HCC were found (search with disease and condition: "Hepatocellular Carcinoma"; other term: "NK cell"; recruitment status: "Recruiting" or "Active, not recruiting"; search date 17th Sep 2020). The results are listed in **Table 1**.

The alternative option is NK cell lines that show strong antitumor activities and can be reproducibly and easily expanded and purified. The NK-92 cell, an IL-2 dependent human NK cell line, is comprised of 100% activated NK cells [93]. It is easier to expand and manipulate genetically with transfection efficiency being superior to that of PB NK cells. The NK-92 cells adoptive immunotherapy has been tested in patients with advanced malignancies and showed some antitumor responses in treatment-resistant lung cancer patients [94]. Additionally, genetically engineered NK-92 cells, GPC3-specific CAR-modified NK-92 cells were demonstrated to have significant antitumor effects against HCC both *in vitro* and in xenografts [95-97]. These preclinical studies suggest that CAR-engineered NK cells have the potential for further development as an investigational novel therapeutic approach for HCC patients.

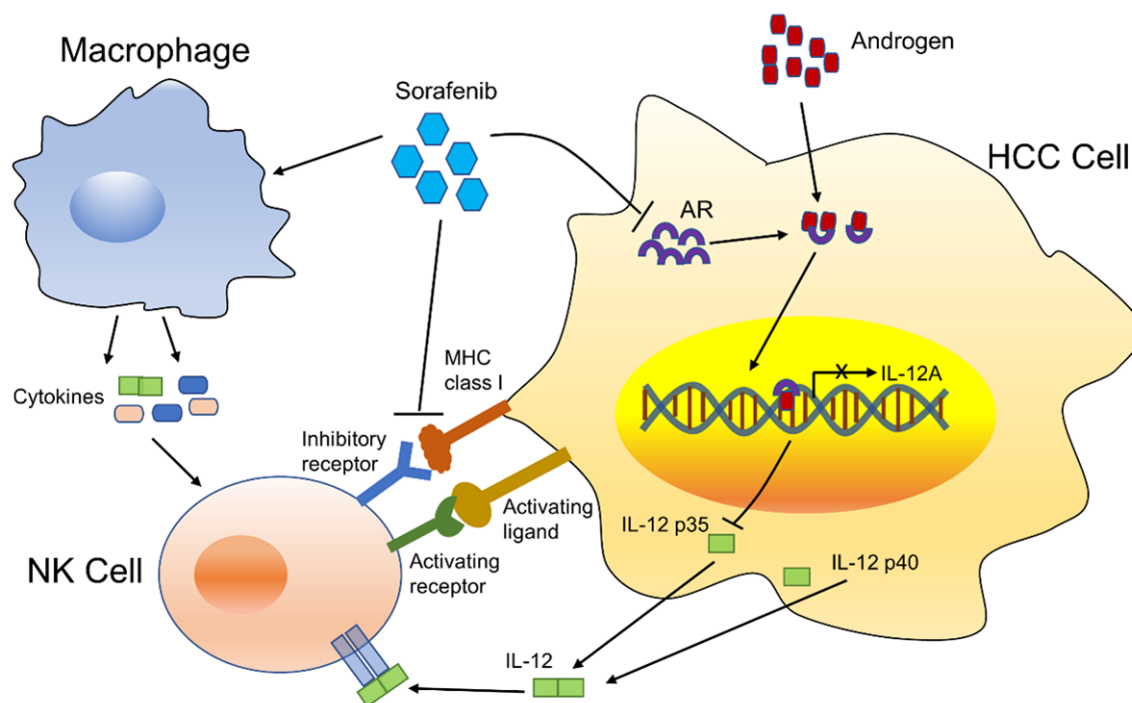
### *The potential of combination sorafenib and NK cell for HCC*

Although NK cells play a key role in the immune surveillance of HCC, the overall efficacy of NK cell-based therapeutic strategies alone is low. Researchers have recently focused on studying the immunological mechanism of sorafenib on NK cells to better inhibit HCC progression (**Figure 3**). It has been reported that the enhanced cytotoxic sensitivity of tumor cells to NK cells is associated with the up-regulated expression of NKG2D ligands after incubation with sorafenib [98]. Meanwhile, a previous study reported that administration of sorafenib stimulates the activation of tumor-associated macrophages and consequently induces the activation of NK cells by a cytokine- and NF- $\kappa$ B-dependent manner in mice liver [99]. This study also showed that NK cells activated by sorafenib-treated macrophages have up-regulated degranulation and IFN- $\gamma$  secretion. On the other hand, it has been reported that expanded NK cells significantly enhanced the antitumor effects of sorafenib and that the cytotoxicity of NK cells has not been affected in the presence of sorafenib [100]. A recent study also reported an immunomodulatory mechanism of sorafenib by unleashing NK cell cytotoxicity against HCC tumors [101]. They indicated that sorafenib can down-regulate MHC class I expression of HCC cells, which may then induce tumor resistance to immune checkpoint therapies and increase sensitivity to NK cell responses. Moreover, Shi et al. demonstrated that androgen receptor (AR) can directly bind to the IL12A promoter region and subsequently down-regulate IL12A expression at the transcriptional level, which resulted in inhibited NK cell cytotoxicity against HCC, whereas sorafenib treatment can boost IL12A signals through suppressing AR signals [102]. Furthermore, our recent study observed that sorafenib can also affect the sub-populations and functions of peripheral CD56<sup>bright</sup>CD16<sup>-</sup> and CD56<sup>dim</sup>CD16<sup>+</sup> NK cells, which were correlated with the treatment outcomes such as the overall survival of HCC patients [103]. Additionally, Lohmeyer et al. reported that sorafenib enhanced the cytotoxicity of NK cells in a time- and dose-dependent fashion via the RAS/RAF/ERK pathway [104], whereas Li et al. suggested that sorafenib decreased the function of NK cells via suppressing ERK1/2 [105]. Therefore, there is a need to further

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**Table 1.** On-going clinical trials on NK cell therapy for HCC

Identifier	Phase	Status	Start Year	Title	Condition	Country	NK Cell Source
NCT04162158	II	Recruiting	2019	Safety and Efficacy of Allogeneic NK Cells Therapy in Patients with Advanced Hepatocellular Carcinoma	Hepatocellular Carcinoma	China	Allogeneic PB
NCT04011033	II	Recruiting	2019	Study of Adoptive Transfer of iNKT Cells Combined with TACE to Treat Advanced HCC	Hepatocellular Carcinoma	China	Invariant Natural Killer T
NCT03319459	I	Active, not recruiting	2017	FATE-NK100 as Monotherapy and in Combination with Monoclonal Antibody in Subjects with Advanced Solid Tumors	Advanced Solid Tumor, including Hepatocellular Carcinoma	United States	Donor-derived NK cell
NCT03841110	I	Recruiting	2019	FT500 as Monotherapy and in Combination with Immune Checkpoint Inhibitors in Subjects with Advanced Solid Tumors	Advanced Solid Tumors, including Hepatocellular Carcinoma	United States	Allogeneic, iPSC-derived NK cell
NCT03592706	II/III	Recruiting	2018	Autologous Immune Killer Cells to Treat Liver Cancer Patients as an Adjunct Therapy	Hepatocellular Carcinoma Liver Cancer	Taiwan	Autologous immune killer cells
NCT04106167	Not applicable	Recruiting	2019	Long-term, Non-interventional, Observational Study Following Treatment with Fate Therapeutics FT500 Cellular Immunotherapy	Advanced Solid Tumors, including Hepatocellular Carcinoma	United States	Allogeneic, iPSC-derived NK cell



**Figure 3.** Hypothetic modes of the effects of sorafenib on NK cell activation against HCC cells.

investigate the comprehensive functional interactions between sorafenib and NK cells.

### Conclusion

There is increasing evidence that sorafenib can regulate the function of immune cells, particularly NK cells. In addition, it has been shown that the combination of NK immunotherapy with sorafenib can be developed as a promising and effective therapeutic approach for the treatment of HCC. However, the key pathways involved in the regulation of NK cell distribution and function in HCC by sorafenib have not been fully illustrated. Therefore, a comprehensive understanding of the mechanism is necessary in order to optimize the combination of sorafenib and NK cell-based immunotherapy for the treatment of HCC.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Zhuoli Zhang, Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine-Northwestern University, 737 N. Michigan Ave, 16th Floor, Chicago, IL 60611, USA. Tel: 224-217-0102; Fax: 312-926-5991; E-mail: zhuoli-zhang@northwestern.edu

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