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Review Article

Trends in the treatment of advanced hepatocellular carcinoma: immune checkpoint blockade immunotherapy and related combination therapies

Huijuan Cheng[1,2*], Guodong Sun[1*], Hao Chen[1,2], Yu Li[1], Zhijian Han[1,2], Yangbing Li[1,2], Peng Zhang[1], Luxi Yang[1,2], Yumin Li[1,2]

[1]Lanzhou University Second Hospital, Lanzhou 730030, Gansu Province, China; [2]The Key Laboratory of The Digestive System Tumors of Gansu Province, Lanzhou 730030, Gansu Province, China. [*]Equal contributors.

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Abstract: Hepatocellular carcinoma (HCC) is the most common liver cancer with high morbidity and mortality worldwide. Systemic treatments with several multi-targeted tyrosine kinase inhibitors (TKIs), including sorafenib, lenvatinib, regorafenib and cabozantinib, have been widely utilized int the treatment of HCC. However, with tolerable adverse events and relative low survival time, neo or optimized therapies for advanced HCC are still urgently needed. New developed immune checkpoint inhibitors therapy have been first demonstrated effective in metastatic melanoma through against CTLA-4 or PD-1/PD-L1 to renew T cell effector function. Preclinical data indicated that interference with immune checkpoint molecules results in HCC growth suppression, suggesting it may bring hope to the HCC treatment. Several clinical trials applying monoclonal antibodies to immune checkpoint molecules demonstrated that immune checkpoint inhibitors are safe and enable durable antitumor activity in advanced HCC patients. Several published immunotherapy trials in HCC using Anti-CTLA-4 agents (tremelimumab) or anit-PD-1 agents (Nivolumab) have showed promising results, in which have similar response rate (15%-30%) and disease control rate with TKIs therapies. This article will review the on-going clinical trials associated with immune checkpoint molecules monotherapy or co, and then discuss the optimal scheme of immune checkpoint therapy for advanced HCC.

Keywords: Hepatocellular carcinoma, targeted chemotherapy, sorafenib, immunotherapy

Introduction

Hepatocellular carcinoma (HCC) is the most common liver cancer worldwide and becomes a leading cause of cancer-related mortality in the last decades [1, 2]. It's a primary liver epithelioid malignancy tumor and often occurs in the patients with underlying hepatic diseases, such as viral hepatitis, alcoholic hepatitis, nonalcoholic fatty liver disease and so on [3]. Orthotopic liver transplantation is the most effective treatment for HCC and cirrhosis so far. However, due to the late appearance of symptoms, less than 20% of the HCC patients is amenable to curative resection or orthotopic liver transplantation and most of the HCC patients at advanced stage only have access to palliative treatments [4]. According to on Barcelona Clinic Liver Cancer staging (BCLC) system, advanced HCC is assigned as unresectable HCC with a liver function defined by a Child Pugh stage not greater than B [3, 5]. Over the decades, few therapies appear to effectively improve the prognosis of advanced HCC. Systemic therapies based on tyrosine protein kinases inhibitors (TKI), sorafenib, regorafenib and lenvatinib are considered to be the most efficient targeted drugs and the only proven treatments for advanced HCC patients [6-9]. However, recently sorafenib is proved to extend median OS by only 3 months with tolerable adverse events according to two large sample clinical trials [10]. Thus, neo-therapies or TKIs combination therapies with less hepatotoxicity are needed for the management of advanced HCC patients to prolong their survival time.

In the recent years, immune checkpoint blockade with anti-cytotoxic T lymphocyte associated antigen 4 (CTLA-4) antibodies and anti-PD-1/

PD-L1 antibodies has been successfully utilized in the treatment of advanced melanoma [11-13], suggesting that immunotherapy with immune checkpoint inhibitors may bring a new hope to advanced HCC treatment and management [14]. On one hand, as a typical inflammation-associated cancer, HCC elicits robust immune response and numerous immune factors in the tumoral microenvironment, which contribute to cancer immune evasion. According to the mechanism of immune checkpoint therapy, it may block the HCC related inflammation and the following immune evasion processes, which elevate the efficiency of HCC treatment [15]. Antibodies to PD-1 have shown durable antitumor responses in advanced HCC patients, and several important clinical trials have been ongoing now [9]. On the other hand, as immunotherapeutic drugs are not metabolized in the liver, no severe hepatoxicity and adverse effect has been observed in the patients accepting antibody-based therapies previously, suggesting that immunotherapeutic drugs may be relatively secure in the treatment of advanced HCC and cirrhosis [16].

Taken together, immune checkpoint inhibitors have emerged as neo-potentially agents for advanced HCC patients. This article will review the on-going clinical trials associated with immune checkpoint molecules monotherapy and combination therapies, and then discuss the optimal scheme of immune checkpoint therapy for advanced HCC.

Development of immunotherapy for HCC

The early strategies of immunotherapy for HCC included non-specific activation of the immune system with cytokines, and antigen-specific immunotherapy with autologous/allogeneic engineered tumor cells, peptides, proteins, DNA vaccines and tumor-specific antibodies [17]. Both strategies were designed to directly manipulate the immune reaction or specifically kill the tumor cells. Among all the early immunotherapies, cytokines, therapeutic vaccines to tumor-specific antibodies and adoptive cell transfer (ACT) have been tested in advanced HCC patients and present various levels of antitumor efficacy [18]. However, tumor microenvironment of HCC is complex and immunogenic, it does not only express tumor antigens, but also orchestrate numerous hepatic antigens presenting cells and thus promote evasion of tumor cells from effective immune response [19]. In this view, immunotherapy for HCC should be developed to overcome the immune evasion of tumor cells and harness the host immune system against the tumor [14]. Immune checkpoints inhibitors, whose deregulation is proved to be involved in the immunotolerance mechanism of various chronic diseases and cancers, may fulfill this purpose [20]. Currently, the most mentioned immune checkpoint molecules in previous studies included CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3 and OX40 (**Table 1**).

Liver immune tolerance and progression of HCC

Liver functions as the systemic filter of the circulation, which has to face multiple harmful agents and antigens. Thus, it develops complex immune tolerance system to avoid immune hyperactivation and autoimmune injury. This self-protective system consists of antigen-presenting cells system, Treg cells, immune checkpoints molecules, and cytorkind profiles. The primary reason of HCC progression is the disorder of these cells, molecules and cellular pathways [19].

Liver immune tolerance

Antigen-presenting cells (APCs) play an important role in the immune tolerance system of liver, which include classic dendritic cells (DCs) and liver-specific APCs, e.g. hepatic stellate cells (HSCs), Kupffer cells (KCs) and liver sinusoidal endothelial cells (LESCs). On one hand, APCs can trigger the pro-inflammatory responses or decrease immune responses by preseting pattern recognitions recepotors [19]; on the other hand, they express histocompatibility complex (MHC) class-2 molecules and bind the T-cell receptor (TCR), leading to CD4 T cells activation. As a result of the above successive responses, CD8+ cytotoxic T lymphocytes (CTLs) exert anticancer effects by producing IFN-γ, granzyme B and perforin [21]. Antigen presentation is a highly complex process which requires multiple signals to complete. Co-stimulatory signals produced by APCs include not only the classical CD28-CD80/86 axis which is necessary for T-cell activation, clonal expansion and survival, but also kinds of immune checkpoint molecules which limit T cell

Table 1. Summary of combinations of dual immune checkpoint inhibitors or with TKIs

PD-1/PD-L1 agent	Combining agents	Mechanism	Status	Patients	Trials identifier
Combinations with other immunotherapies					
Nivolumab	Ipilimumab	Anti-CTLA-4	Active, not recruiting	620	NCT01658878
Nivolumab	Ipilimumab	Anti-CTLA-4	Recruiting	45	NCT03222076
Nivolumab	Ipilimumab	Anti-CTLA-4	Not yet recruiting	32	NCT03682276
Nivolumab	Ipilimumab	Anti-CTLA-4	Recruiting	50	NCT03203304
Durvalumab	Tremelimumab	Anti-CTLA-4	Recruiting	30	NCT03638141
Durvalumab	Tremelimumab	Anti-CTLA-4	Recruiting	1310	NCT03298451
Durvalumab	Tremelimumab	Anti-CTLA-4	Recruiting	545	NCT02519348
Durvalumab	Tremelimumab	Anti–CTLA-4	Recruiting	70	NCT03482102
Durvalumab	Tremelimumab	Anti–CTLA-4	Recruiting	90	NCT02821754
Combinations with TKIs					
Nivolumab	Sorafenib	Multi-TKI	Active, not recruiting	620	NCT01658878
Nivolumab	Sorafenib	Multi-TKI	Recruiting	40	NCT03439891
Nivolumab	Sorafenib	Multi-TKI	Recruiting	27	NCT03211416
PDR001	Sorafenib	Multi-TKI	Active, not recruiting	20	NCT02988440
Nivolumab	Lenvatinib	Multi-TKI	Active, not recruiting	30	NCT03418922
Pembrolizumab	Lenvatinib	Multi-TKI	Active, not recruiting	97	NCT03006926
Pembrolizumab	Regorafenib	Multi-TKI	Recruiting	40	NCT03347292
Nivolumab	Cabozantinib	Multi-TKI	Active, not recruiting	620	NCT01658878
Nivolumab	Cabozantinib	Multi-TKI	Recruiting	15	NCT03299946
Avelumab	Axitinib	Multi-TKI	Active, not recruiting	22	NCT03289533

hyperactivation in physiologic condition. These checkpoint molecules expressed on T cells, including CTLA-4-CD80/86, PD-1-PD-L1, KIR-MHC I/II, LAG3-MHC I/II and TIM-3-GAL9, can interact with the corresponding ligands on APCs and get inloved in the regulation of immune tolerance [22].

By secreting TGF-β, KCs and LSECs can also upregulate the regulatory T cells (Treg), which is another key to HCC immunotolerance. Under the control of transcription factor Foxp3, Tregs express CTLA-4 and participate in the CTLA-4-CD80/86 signals network [23], which could reduce IL-2 in the microenvironment by associating with CD25 [24]. As IL-2 is essential for the differentiation and proliferation of T cells, IL-2 reduction is expected to induce immune suppression. In addition, by expressing CD38 and CD78, Tregs could convert extracellular ATP into adenosine, which binds to the adenosine A2A receptor on effector T cell and suppresses its function [24]. Cytokine profiles in HCC microenvironment also blunt the protective adaptive immune response against malignancy [25], such as TGF-β and IL-35 which suppress T cell proliferation [26].

T cell exhaustion and the progression of HCC

In the physiologic condition, the immune tolerance system comprised of the APCs, Tregs and suppressive cytokines plays a protective role in maintaining immune balance. However, in chronic liver disease, its dysfunction may lead to hyperactivation of immunotolerant signals, and thus induce T cell anergy or exhaustion, which allow emergence and progression of HCC.

Exhausted T cells are characterized by high levels of immune checkpoint molecules, reduced effector cytokines and impaired cytotoxicity [27]. Chronic liver inflammation in HCC pathogenesis creates an environment that favors T cell exhaustion, including increased Tregs and immune checkpoint molecules. For examples, in HCC patients, Elevations of Tregs and MDSCs are observed in both blood circulation and tumor tissue [28, 29]. Besides, Treg accumulation significantly correlates with HCC progression and poor prognosis, while frequency of MDSCs in blood circulation is significantly associated with recurrence-free survival of HCC patients who have undergone RFA [30, 31]. In addition, the expressions of PD-1 on T cells and

PD-L1 on liver APCs are both positively associated with chronic inflammation in HCC. PD-L1 is detected in both immune cells and HCC cells (in 74% of HCC cases), and could be used to predict the recurrence/survival of the HCC patients after resection [32]. Similarly, other immune checkpoint molecules are also observed with high expression in HCC, and most of them are associated with aggressive progress and poor survival [33-36]. Based on these findings, researchers tried to inhibit the immune checkpoint molecules in viral hepatitis and HCC models, and their works have achieved great results that HCC tumor growth was suppressed to a large extent in vivo [37, 38]. Taken together, high expression of immune checkpoint molecules seems to be the key to HCC immunotolerance, which may serve as the potential target in the development of HCC therapy.

Clinical trial landscape for immune checkpoint inhibitors in HCC

Since immune checkpoint molecules are recognized as vital indicators of HCC progress, series of clinical trials with immune checkpoint inhibitors have been implemented to confirm theirs potential function in advanced HCC treatment. Among them, four clinical trials have been published and others are on-going. In most of the trials, the therapeutic effect of the immune checkpoint inhibitors were evaluated in combination with other conventional therapies (locoregional therapy, multi-kinase inhibitors and tumor vaccines), or with other immune checkpoint inhibitors, such as dual inhibition of CTLA-4 and PD-1 which has been successfully trialed in the treatment of metastatic melanoma [39-42] (**Table 1**).

CTLA-4 blockade

CTLA-4 is mainly expressed on activated T cells and Tregs, and act as a "break" for immune responses. Stimulated by T cell receptors, CTLA-4 localizes to the plasma membrane and inhibits T cell activation by competing for B7 ligand with CD28. The first CTLA-4 blocking inhibitor in practical HCC treatment was tremelimumab, which displayed promising antitumor activity and acceptable safety profile. As Sangro et al reported in ClinicalTrials.gov NCT01008358, 21 patients with HCC received tremelimumab 15 mg/kg every 90 days for a maximum of 4 doses, the partial response rate was 18% (3/17), and the disease control rate was 76% (13/17) [36]. Duffy's pilot study evaluated the effect of tremelimumab treatment in 32 advanced, sorafenib-refractory patients who had accepted radiofrequency ablation or trans-arterial therapy [43]. Similar to the earlier study, the partial response rate was 26% (5/19) and the disease control rate was 84% (16/19). Interestingly, the researchers also observed a decrease of > 200-fold in serum HCV viral load in 12 of the 14 patients with hepatitis C, suggested that CTLA-4 blockade could control the viral load in the HCC patients with hepatitis C. An important adverse effect of tremelimumab is transaminitis, as high proportion of reversible grade 3/4 transaminitis was observed in both above menttioned studies.

PD-1 & PD-L1 blockade

Similar to CLTA-4 receptor, PD-1 is also a CD28 superfamily member and function as co-inhibitory signals for TCR receptor. PD-1 is mainly expressed on CD8+ T cells, but it can also be detected on Tregs and MDSCs. By chronic antigen stimulation, IFN-γ induces IRF9 which binds to Pdcd-1 promoter, and prompt PD-1 transcription in T cells [45]. When PD-1 binds to its ligands, PD-L1 or PD-L2, T cell proliferation and cytokine release are inhibited.

Compared to CTLA-4 blockade, PD-1 and PD-L1 blockade showed relatively higher objective response rates (ORR), which could reach 10%-20% in advanced HCC patients. Recent researches also indicate that PD-1/PD-L1 blockade monotherapy is safe and tolerable [44]. Nivolumab is a fully human immunoglobulin (IgG4) monoclonal antibody inhibitor of PD-1 receptor, which has received accelerated US FDA approval in 2017 for advanced HCC patients who previously received sorafenib. Its safety and efficacy have been confirmed in the largest cohort study of HCC patients, CheckMate 040 (NCT01658878) [9]. At first, 48 patients with advanced HCC received nivolumab 0.1-10 mg/kg every 2 weeks in a dose escalation phase. Then, since nivolumab showed adequate safety and feasibility, 214 advanced HCC patients from 39 sites of 11 countries received nivolumab 3 mg/kg every 2 weeks in a dose expansion phase. Since only 25% of patients experienced grade 3/4 hepatotoxicity

in the dose escalation phase, nivolumab are proved to be well tolerated. In all, partial responses were observed in 15% and 20% of patients in the dose escalation and dose expansion phases respectively, which are similar to tremelimumab and superior to sorafenib (2%) and regorafenib (10%). Besides, disease control rates of the two phases were 64% and 58%, respectively. Similar to tremelimumab, no viral reactivation or worsening of viremia were observed in the patients with HBV/HCV. Owing to its safety and efficacy, an open-label, multicenter, randomized Phase III study of nivolumab versus sorafenib in HCC patients was conducted to collect more data to confirm the clinical benefit of nivolumab, and more results are to be revealed (NCT02576509) [4].

Another PD-1 blockade agent, pembrolizumab, is a humanized IgG4 antibody to PD-1. It has been assessed and reported in a Phase II study of advanced HCC patients who were sorafenib-refractory (~80%) (NCT02702414) [46]. Similar to nivolumab, pembrolizumab is safe and tolerable, and results in comparative response rates across subgroups. According to the study, objective response rate of pembrolizumab was 16.3%, while control rate was 44%. Grade 3/4 hepatotoxicity was only reported in 24% of the 104 patients, and no viral reactivation or flare were observed in the virally mediated subtypes. In all, pembrolizumab was effective and tolerable in advanced HCC patients and might be an ideal treatment option. This drug is also undergoing further assessment in two phase III, randomized trials as a second-line treatment in HCC patients (NCT02702401 and NCT03062358).

Furthermore, there are other PD-1 blockade agents undergoing clinical trials. For example, durvalumab, a human IgG1κ mAb to PD-L1, was tested on a Phase I/II trials of the advanced HCC patients who failed prior treatment with sorafenib [47]. The overall response rate of durvalumab was 10.3% in the 39 HCC patients. Besides, other PD-1 blockade agents including avelumab (NCT03389126, NCT03412773), PDR001 (NCT02795429) and BGB-A317 have been tested in HCC-specific studies.

Taken together, these clinical data suggested that both CTLA-4 and PD-1/PD-L1 blockade could provide durable disease control to a proportion of HCC patients. Comparatively, PD-1/PD-L1 blockade agents were more tolerable and less hepatoxicity. Further researches for combined PD-1/PD-L1 and CTLA-4 blockades in HCC treatment are still expected, which may help to mitigate adverse effect of the treatments.

Possible combination therapies with immune checkpoint blockade

As an adjuvant tumor treatment, the most likely strategy for immune checkpoint blockade in advanced HCC should be the combination with other surgical, cytotoxic, immune or targeted therapies. Most ongoing clinical trials have been designed to assess the efficiency of the combination strategies. Combination of immune checkpoint inhibitors with other conventional ablative therapies such as RFA or cryoablation would be the most promising approach for HCC treatment. However, as for advanced HCC which is unresectable, it's more appropriate to search for other combination strategies, such as the combination with multi-kinase inhibitors, vaccines and oncolytic viruses, as well as dual inhibition of two immune checkpoint molecules [40].

Combination immune checkpoint blockade

Combination of two immune checkpoint blockades makes it possible to mediate the function of immune checkpoint molecules at different times in the lifecycle of effector T cells. Preclinical data based on series of solid tumors have indicated that dual immune checkpoint blockade is synergistic, and results in higher response rates and improved outcomes compared to monotherapy [40, 41]. CTLA-4 and PD-1 shared similar mechanism in the tumor tolerance. Thus, most of advanced HCC patients using PD-1/PD-L1 monotherapy could not attain durable control, while combination with CTLA-4 blocking inhibitor will improve the efficacy of treatment.

The first study focusing on combination immune checkpoint blockade in advanced HCC was the fourth cohort of the Check-Mate 040 (NCT01658878), in which CTLA-4 and PD-1/PD-L1 mAbs (nivolumab and ipilmumab) were jointly employed to block the related molecules. In this study, the safety and efficacy of the combination therapy were assessed, and the results are to be published in late 2019. Since

then, there have been a series of phase I/II trails designed to assess the efficacy of combination immune checkpoint blockade in the treatment of advanced HCC (NCT03222076, NCT03682276, NCT03203304). Among them, combination therapy with durvalumab and tremelimumab were also being evaluated on a series of HCC-specific Phase I/II clinical trials (NCT03638141, NCT03298451, NCT02519348, NCT03482102, NCT02821754). In advance, the safety and efficacy of combination therapy with durvalumab and tremelimumab have been assessed preliminarily in 40 patients. Essentially, this combination therapy appears safe and tolerable with a confirmed response rate of 15% (6 of 40 patients) [48]. Given the limited data to date, further testing of this combination is ongoing in a Phase II expansion. The NCT03298451 was a randomized, open-label, multicenter Phase III study of durvalumab with or without tremelimumab versus sorafenib in advanced HCC patients, which will enroll about 1350 patients and explore two dose schedules of durvalumab and tremelimumab therapy.

Presently, anti-PD-1/PD-L1 therapy is being paired with agents targeting TIM-3 (NCT03099109), LAG-3 (NCT03005782 and NCT01968109), KIR (NCT01714739) and several other novel immune checkpoint inhibitors in several umbrella and basket studies. Further clinical trials will continue to reveal the safety and efficacy of these novel immune checkpoint inhibitors in the treatment of advanced HCC.

Combination of TKIs and immune checkpoint blockade

Systemic therapies based on tyrosine protein kinases inhibitors (TKI), sorafenib, regorafenib, and lenvatinib are still the first-line strategies for advanced HCC treatment and management. Mechanically, TKIs affect immune effectors, antigen presentation and tumoral microenvironment, which may dampen or augment the immune response to cancer [49]. Considered the efficacy of TKIs in HCC treatment, the combination of TKIs and immune checkpoint blockade has be highlighted in the recent clinical researches. For example, the second and third cohorts of the Check-Mate 040 (NCT01658878) focused on the combination therapy of nivolumab with sorafenib and cabozantinib respectively, while a series of early phase studies in progress were designed to evaluate the safety and tolerability of combination therapy of anti-PD-1 and TKIs, including sorafenib (NCT03211416, NCT03439891, NCT02988440), lenvatinib (NCT03006926, NCT03418922), regorafenib (NCT03347292), cabozantinib (NCT03299946 and NCT01658878) and axitinib (NCT03289533). The clinical trials indicated that combination therapy may lead to survival advantage in advanced HCC patients compared to TKIs monotherapy. However, some potential pitfalls should be paid attention. For example, in several preclinical models of solid tumors, substantial complexity has been observed in the case of combination therapy of TKI (sorafenib) and PD-L1 blockade. Sorafenib was found to induce PD-L1 upregulation and thus the additive PD-L1 blockade was not able to augment antitumor activity in vivo [50, 51]. Herein, attentions should be paid to the offset effect between the two treatments.

Other combination strategies

In the last twenty years, passive immunotherapy of HCC, such as vaccines, oncolytic viruses and adoptive cellular therapies are under active investigation, some of which present advantages over immune checkpoint inhibitors. AFP-derived vaccines can generate a potent CD8+ T cell response to specific antigenic AFP peptides, and boost the immune system to kill the HCC cells [52, 53]. However, in clinical trails, these vaccines rarely lead to tumoral shrinkage or durable disease control. Besides, vaccines can augment the activity of immune checkpointmolecules, leading to deterioration of HCC [54].

Oncolytic viruses can replicate in cancer cells, activate both the complement cascade and cellular immunity, thereby lead to tumoral cell lysis. Preclinical data indicated that local oncolytic virus injection leads to distant tumor inflammatory immune infiltration, rendering tumors susceptible to checkpoint blockade [51, 55]. Thus, the combination of oncolytic viruses with immune checkpoint blockade have been put on the agenda and look forward for better efficacy and stable tolerance over the monotherapy of immune checkpoint blockade. Indeed, pexastimogene devacirepvec, an oncolytic virus derived from vaccinia with preliminary activity in HCC is currently being tested with nivolumab (NCT03071094) [55].

Finally, adoptive cellular therapy represents a novel but highly challenging modality of drug development in HCC. Several human studies are now ongoing, and more preclinical and clinical data are acquired. The role of pairing these strategies with immune checkpoint blockade will be elucidated in the future.

Conclusion and perspective

Immune checkpoint inhibitors appear to be a promising addition to the treatment armamentarium for advanced HCC. Multiple prospective studies are now attempting to ascertain if PD-1 blockade, alone or in combination with CTLA-4 blockade, will alter the natural history of advanced HCC and improve the overall survival of the patients. Based on current evidence, several first and second-line randomized phase III trials have been initiated on HCC patients, although it will take several years before mature survival data become available.

Combination therapies with other immune checkpoint inhibitors, TKIs, vaccines and oncolytic viruses are now in various stages of clinical development to improve the antitumor efficacy. In the next five years, clinical data from the above studies will inform HCC biology and expand treatment options for patients with this dismal disease. It is anticipated that HCC immune oncology will be the major focus of drug development. But it is also necessary for basic and clinical researchers to continue to uncover, explore, and drug other hallmarks of HCC. Currently, there is great optimism and hope for HCC immune oncology-some research programs may succeed, while others may fail. In either case, it will be critical to learn as much as possible to advance the field and improve patient outcomes.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yumin Li, Lanzhou University Second Hospital, Lanzhou 730030, Gansu Province, China. Tel: +86-931-13893615421; Fax: +86-931-13893615421; E-mail: liym@lzu.edu.cn

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