*Case Reports*

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# Advanced hepatocellular carcinoma treated by radiofrequency ablation combined with oncolytic virus and anti-PD-1 antibody therapy: a case report and literature review

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**Abstract**

The development of an effective therapy for advanced hepatocellular carcinoma (HCC) represents an important global concern. In recent years, the combination of multiple treatment methods with immunotherapy has achieved great progress in patients with advanced HCC. Patient survival has been significantly prolonged, but cases of complete response (CR) remain rare. Here, we report two cases in which CR was achieved by radiofrequency ablation combined with an oncolytic virus (recombinant human adenovirus type 5) and anti-programmed cell death protein 1 antibody. Additionally, a literature review is presented to describe similar advancements in this field and explore viable methods for the treatment of advanced HCC.

**Keywords**

Complete response, advanced hepatocellular carcinoma, radiofrequency ablation, anti-PD-1 antibody, oncolytic virus, recombinant human adenovirus type 5

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## Introduction

Hepatocellular carcinoma (HCC) has emerged as the third leading cause of cancer-related deaths globally in recent years and is the second leading cause of cancer-related deaths in China. Approximately 750,000 HCC-related deaths are reported each year, nearly half of which are in China.[1] HCC accounts for 90% of primary liver cancer cases,[2] and most patients are diagnosed at an advanced stage. Although some patients receive curative surgical therapy, 70% to 80% experience recurrence within 5 years of surgery. Thus, the current 5-year overall survival (OS) rate is only 12.1% in China.[3] As a result, the identification of effective treatments for advanced HCC remains an important global concern. Several routine treatment modalities are used for advanced HCC, such as transarterial chemoembolization, chemotherapy, and molecular targeting therapy. Given the wide variety of treatment modalities, a multidisciplinary therapeutic approach is recommended for advanced-stage HCC. However, the outcomes remain limited to prolonging only the OS of patients with HCC.[4] The use of immune checkpoint inhibitors has recently been reported to prolong the median OS and progression-free survival but only in a subset of patients with HCC. The objective response rate (ORR) for immune checkpoint inhibitors is approximately 15%, according to several recently published clinical trials.[5,6] Furthermore, the combination of immune checkpoint inhibitors with other modalities has been shown to increase the ORR by 2-fold for unresectable HCC.[7] Based on this recent advancement in the treatment of HCC, a clinical trial using radiofrequency ablation (RFA) in combination with recombinant human adenovirus type 5 (rhAd5) and anti-programmed cell death protein 1 (PD-1) antibody therapy for the treatment of HCC was performed in our institute. Here, we report two cases in which complete response (CR) was achieved by RFA combined with rhAd5 and anti-PD-1 antibody treatment. Additionally, a literature review is presented to describe similar advancements in this field and explore viable treatment methods for advanced HCC.

## Case report

The reporting of this study conforms to CARE guidelines.[8]A 49-year-old male patient (weight = 60 kg) was diagnosed with HCC by a routine physical examination. He suffered from chronic hepatitis B virus (HBV) infection (for more than 40 years) without treatment. Laboratory findings showed a platelet count of $178 \times 10^9$/L, alanine aminotransferase concentration of 106.6 IU/L, total bilirubin concentration of 14.45 μmol/L, albumin concentration of 38.05 g/L, prothrombin time and international normalized ratio of 0.93, and HBV DNA levels of $2.724 \times 105$ IU/mL. The serum alpha-fetoprotein (AFP) level was 11.060 ng/mL, and the level of protein induced by vitamin K absence or antagonist-II (PIVKA-II) was approximately 3258.00 mAU/mL. The patient did not present with aberrant symptoms, encephalopathy, or ascites. His liver function was well compensated, with a Child–Pugh score of class A. The patient's Eastern Cooperative Oncology Group performance status was grade 1. Additional assessment of immune checkpoint inhibitor therapy for lymphocyte subset profiling and next-generation sequencing-based genetic testing were performed. The blood lymphocyte subtype test showed that the total number of lymphocytes was 939/μL (range of normal values: 1530–3700), the number of CD8+ T cells was 244/μL (range of normal values: 220–1129), and the number of CD4+ T cells was 388/μL (range of normal values: 404–1612). Genetic testing

showed that the tumor mutation burden was 3.98 mutations/Mb (mean value: 5.28 mutations/Mb), and no abnormalities in the genes encoding mismatch repair proteins, MDM2 proto-oncogene, MDM4 regulator of p53, or DNA methyltransferase 3 alpha were noted.

Contrast computed tomography (CT) scanning and gadolinium ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging (MRI) revealed a diffuse arterial hypervascular/portal washout mass in the right anterior, left, and caudate lobes with invasion of the main trunk, right anterior, left portal, and left hepatic veins and inferior vena cava of the liver. Cirrhosis, portal hypertension, splenomegaly, and retroperitoneal lymph nodes were also observed (Figures 1a and 2a).

Typical radiographic features upon CT and MRI together with a marked elevation in tumor markers for HBV-related liver cirrhosis led to a diagnosis of HCC without performing liver biopsy. The following treatment measures were adopted. RFA of the HCC and intratumoral injection of rhAd5 ($1.0 \times 10^{12}$ viral particles, once) were performed. In addition, pembrolizumab (200 mg each dose) was administered intravenously once every 3 weeks after the operation. Entecavir was used routinely to inhibit HBV replication.

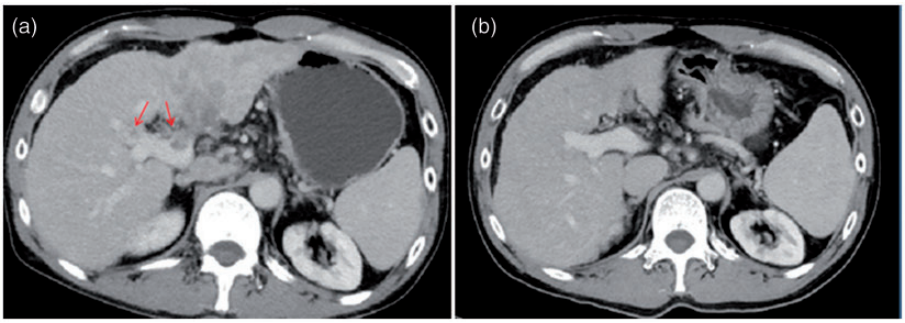


**Figure 1.** Computed tomography imaging of the 49-year-old male patient diagnosed with hepatocellular carcinoma. (a) The tumor invading the portal vein before treatment (indicated by the arrows). (b) The tumor thrombus disappeared after treatment.

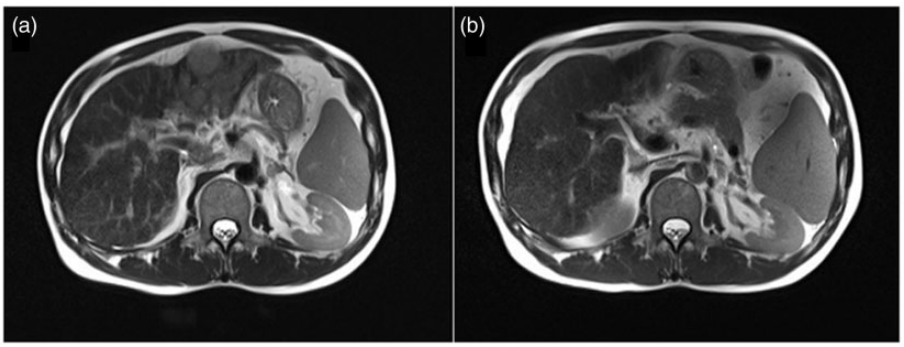


**Figure 2.** Magnetic resonance imaging of case 1. (a) Multiple masses before treatment. (b) The masses disappeared after treatment.

Tumor markers were reexamined 6 weeks after treatment, and both AFP and PIVKA-II had decreased to 10.090 ng/mL and 25.00 mAU/mL, respectively. After 5 months of treatment, reexamination using enhanced CT showed that the tumors and tumor thrombus were considerably smaller after treatment compared with before treatment (Figure 1b). Reexamination using enhanced MRI also revealed that the patchy abnormal signal shadows in the left and caudate lobes of the liver were substantially smaller than those in the previous enhanced MRI, and the tumor thrombus had disappeared or decreased in size (Figure 2b).

After examining the condition of the patient, a multidisciplinary team decided to perform left hemihepatectomy for curative resection of the tumor. Combined with the results of intraoperative color Doppler ultrasonography and liver biopsy, left lateral lobectomy was conducted with consent from the patient and his family. The left branch of the portal vein was opened and showed no tumor thrombosis. Intraoperative frozen-section examination and postoperative pathology of the specimen revealed no tumors; only nodular cirrhosis and chronic inflammation were observed in the portal area (Figure 3). At the follow-up visit 18 months after the operation, no recurrence was found based on imaging and tumor marker measurements.

The second case is a 65-year-old man (weight = 61 kg) who was diagnosed with advanced HCC. CT and MRI showed that the primary tumor of the liver was accompanied by multiple liver metastases and portal vein invasion. A significant increase in both serum AFP and PIVKA-II levels was highly indicative of advanced HCC. He was administered the same treatment regimen and achieved CR according to a positron emission tomography-computed tomography scan 6 months later (Figure 4). However, this patient did not undergo surgery to confirm whether the tumor cells disappeared. At present, this patient has survived for more than 24 months without any symptoms. Adverse reactions were monitored in both patients, and there was no evidence of increased side effects with the combination therapy.

## Discussion

Liver cancer cells tend to invade the portal vein system and form portal vein tumor thrombus (PVTT). The incidence of liver cancer complicated with PVTT is 44.0% to 62.2%.[9] PVTT is associated with the intrahepatic dissemination and extrahepatic

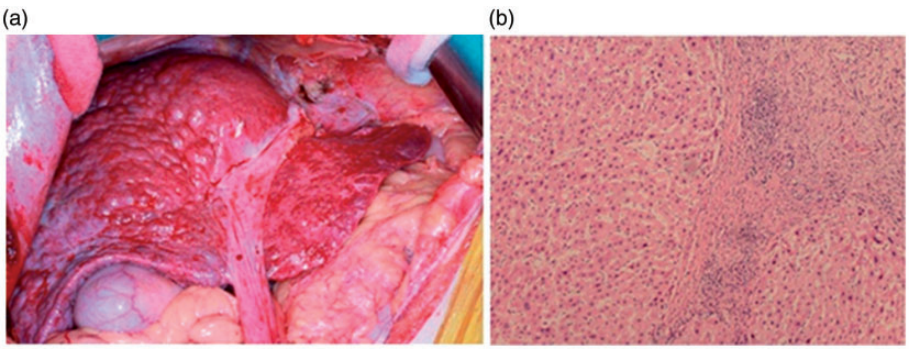


**Figure 3.** Liver biopsy analysis of case 1. (a) Intraoperative condition. (b) Postoperative pathological staining of the specimen.

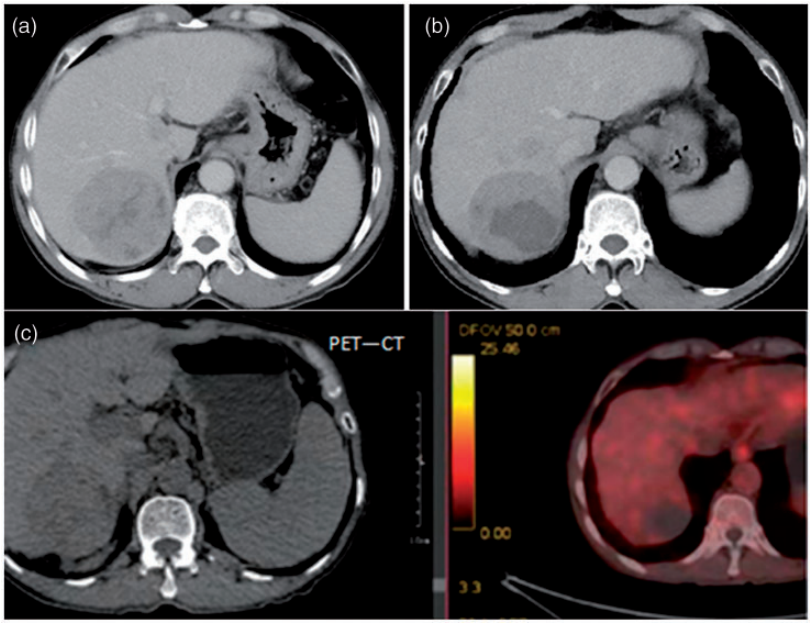


**Figure 4.** Positron emission tomography-computed tomography (PET-CT) imaging of the 65-year-old man diagnosed with advanced hepatocellular carcinoma. (a) CT images before treatment. (b) CT images 5 months after treatment. (c) PET-CT scan 6 months after treatment.

metastasis of tumor cells. The median survival time of patients with HCC and PVTT is only 7.2 months, which is considerably lower than that of patients without PVTT, who show a median survival time of 35.2 months.[10] We used RFA combined with rhAd5 and anti-PD-1 antibody therapy to treat advanced liver cancer and achieved a good therapeutic effect.

RFA not only kills liver cancer cells but also induces and enhances the body's antitumor immune response. Clinical studies have shown that RFA induces specific immunity through a variety of mechanisms.[11–16] The pretreatment of HCC with RFA to induce inflammation or thermocoagulation creates conditions that favor tumor neoantigen generation prior to the initiation of immune checkpoint inhibitor therapy. A clinical trial of this approach was initiated in patients with stage III and IV HCC, and encouraging results have been achieved.[17]

rhAd5 is an oncolytic adenovirus obtained by deleting part of the human adenovirus type 5 E1B-55 kDa protein and E3 regions with genetic engineering technology that can kill tumor cells and induce specific antitumor immunity. This adenovirus undergoes massive replication in specific tumor cells and ultimately leads to cell lysis. The virus progeny released after the lysis of tumor cells continue to infect surrounding tumor cells. This results in tumor destruction and inhibits vascular endothelial growth factor production and tumor neovascularization, leading to an insufficient blood supply and necrosis in tumors.[18] The infection of tumor cells by rhAd5 promotes tumor cell antigen exposure and activates T cells to establish specific antitumor immunity. In addition, rhAd5 itself has antigenicity, inducing the body to produce an immune response against the adenovirus and thus killing adenovirus-infected tumor

cells to establish sustained antitumor immunity.[19] Previous studies have also shown that rhAd5 exhibits a significant antitumor effect on HCC.[20]

Antitumor immunity is reactivated with pembrolizumab, an immune checkpoint inhibitor. Several studies have shown that PD-1 and its ligand (PD-L1) are key factors in limiting the host immune attack in tumors.[21] Specifically, the tumor microenvironment induces high PD-L1 expression in tumor cells. Upon binding with PD-1, PD-L1 negatively regulates the functions of T cells by inhibiting cytotoxic T lymphocyte activities, T cell proliferation, and the production of cytokines, such as interferon-gamma and interleukin-2; thus, tumor immune escape is promoted through this signaling pathway.[22,23] By suppressing the activity of this immune checkpoint, the "brakes" on the local immune system within the tumor microenvironment are released, and the immune response of effector T cells against the tumor is reactivated, thus achieving an antitumor effect. One study found that PD-1 pathway inhibitors not only reversed T-cell suppression but also rescued tumor-related macrophages, prompting macrophage phagocytosis of tumor cells and blocking the spread of cancer cells.[24]

In September 2017 and November 2018, the Food and Drug Administration approved the use of nivolumab and pembrolizumab, respectively, for the second-line treatment of patients with advanced HCC after sorafenib treatment.[5,6] A recent case report from Taiwan, China, showed that pembrolizumab combined with sorafenib was used to treat a 62-year-old patient with metastatic liver cancer, and this patient achieved complete remission.[25] Several clinical studies have shown that the remission rate of patients with PD-L1-positive tumors treated with anti-PD-1/PD-L1 therapy is relatively high. One study indicated that even in the absence of PD-L1 expression, some patients with cancer still benefited from anti-PD-1/PD-L1 therapy for a prolonged period.[26] However, in recent clinical trials of patients with advanced HCC, anti-PD-1 therapy resulted in ORRs of only 10% to 30%,[5,6] but a combination of anti-PD-1 therapy with other treatments improved this efficacy. In a phase-IB clinical trial published by Ribas et al.,[27] the remission rate in patients who received pembrolizumab combined with the oncolytic virus t-vec was 62%, and CD8+ T cells, interferon-gamma gene expression, and PD-L1 protein expression were increased after treatment. This may explain why the oncolytic virus combined with PD-1 antibodies improved treatment efficacy, but more clinical trials are needed for confirmation.

In conclusion, RFA in combination with rhAd5 and anti-PD-1 antibody therapy is an effective method to activate the body's antitumor immunity for the treatment of advanced HCC.

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## Ethics statement

Written informed consent was obtained from the individuals and/or next of kin for the publication of any potentially identifiable images or data included in this article. The studies involving human participants were reviewed and approved by the Department of Hepatobiliary Surgery, The First Affiliated Hospital of The Army Medical University (Southwest Hospital), Chongqing, China.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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## Author contributions

AX collected all references and wrote the draft. FX conceived and designed the experiments, revised the manuscript, and discussed the meaning of the manuscript. JP was responsible for providing patient treatment information. XS and ZS helped collect case data and explore writing ideas.

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