case reports

Spontaneous Immune-Mediated Regression of Hepatocellular Carcinoma With High Tumor Mutational Burden

Joseph W. Franses, PhD, MD, BS[1]; Irun Bhan, MD[2]; Amaya Pankaj, MD[3]; David T. Ting, MD[1]; Vikram Deshpande, MD[3]; and Kenneth Tanabe, MD[4]

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth leading cause of cancer-related death in the United States[1] and the fourth worldwide.[2] Treatment of advanced HCC has been revolutionized in the past few years by combinations of therapies that stimulate the immune system to recognize and attack the cancer.[3,4] The empirical success of this paradigm implies an immune-responsive nature of the HCC tumor microenvironment that can be pharmacologically manipulated. There have been small published series of spontaneous regressions of HCC lesions,[5] and an obvious putative mechanism of such spontaneous HCC tumor regression involves immune rejection. One such recent case report described a series of six patients who experienced varying degrees of HCC tumor regression, one of whom had potentially unique features of the immune microenvironment.[6] Here, we report a patient who experienced a spontaneous regression of an HCC lesion bearing a high tumor mutational burden (TMB) with a brisk-associated lymphocytic infiltrate noted on a preoperative biopsy. We therefore propose a direct mechanism—immune recognition and clearance of a TMB-high tumor, with an unclear stimulus for such antitumor activity—that may underlie rare cases of spontaneous HCC regression.

CASE REPORT

The patient provided permission to use his clinical information for this report. He initially presented in the fall of 2019 as a 64-year-old man with a history of atrial fibrillation (on a stable dose of a direct oral anticoagulant for years) and no known history of chronic liver disease or heavy alcohol use. An abdominal ultrasound obtained for mildly elevated serum aminotransferases revealed a 1.9-cm right-sided liver mass. Magnetic resonance imaging (MRI), which showed no evidence of cirrhotic liver morphology or splenomegaly, confirmed a 2.3-cm segment 4 hepatic lesion exhibiting arterial enhancement, portal venous phase washout, and pseudocapsule; the lesion also exhibited restricted diffusion on diffusion-weighted MRI sequences (Fig 1A). Biopsy of the lesion performed 7 weeks later showed moderately differentiated HCC. He underwent a follow-up MRI approximately 9 weeks after the initial MRI, and this showed a decrease in size to 1.6 cm and a decrease in arterial enhancement of the biopsy-proven HCC (Fig 1B). During this evaluation, the patient's serum alpha fetoprotein level, which was initially elevated to 9.6 ng/mL (reference range: 0-7.8 ng/mL), declined to 5.4 ng/mL, and has since remained within the normal range (Fig 1C). Testing for hepatitis B and C serologies, iron studies, and fasting glucose did not reveal an obvious etiology of cirrhosis.

The patient was taken to the operating room for a planned resection, at which time the surgeon noted no evidence of tumor at the site predicted by the preoperative imaging. Instead, only slight dimpling was noted in segment 4a at the expected site. Ultimately, after re-review of preoperative imaging, the entirety of hepatic segment 4a was resected. Pathologic review of the resected liver segment showed a wedge-shaped subcapsular scar with a marked infiltration by a mature lymphocyte population; adjacent normal tissue was noted to have mild steatosis, without fibrosis.

Spontaneous regression of the HCC was suspected, and the initial biopsy specimen was further analyzed to identify an underlying mechanism. Review of the histology of the biopsy specimen demonstrated HCC with an associated prominent lymphocytic infiltrate resembling a lymphocyte-rich HCC (Fig 2A) composed primarily of CD8+ T cells (1,084 per mm^2) (Fig 2B) and relatively few CD56+ natural killer (NK) cells (Fig 2C). The resected liver segment contained a 4-mm scar that was devoid of cancer cells but which was notable for a substantial density of CD8+ T cells (1,678 per mm^2) (Fig 2D). Programmed death-ligand 1 (Cell Signaling Technology [E1L3N]) was not expressed on either tumor cells or immune cells. There was no evidence of microvascular involvement on the resection specimen.

Nucleic acids extracted from a formalin-fixed paraffin-embedded sample from the patient's biopsy tumor tissue were sequenced using the Massachusetts General Hospital Next-Generation Sequencing Snapshot panel.[7] We identified a total of 34 mutations (reference range for TMB-High on this assay: Absolute

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on May 13, 2021 and published at ascopubs.org/journal/po on June 17, 2021: DOI https://doi.org/10.1200/PO.21.00092

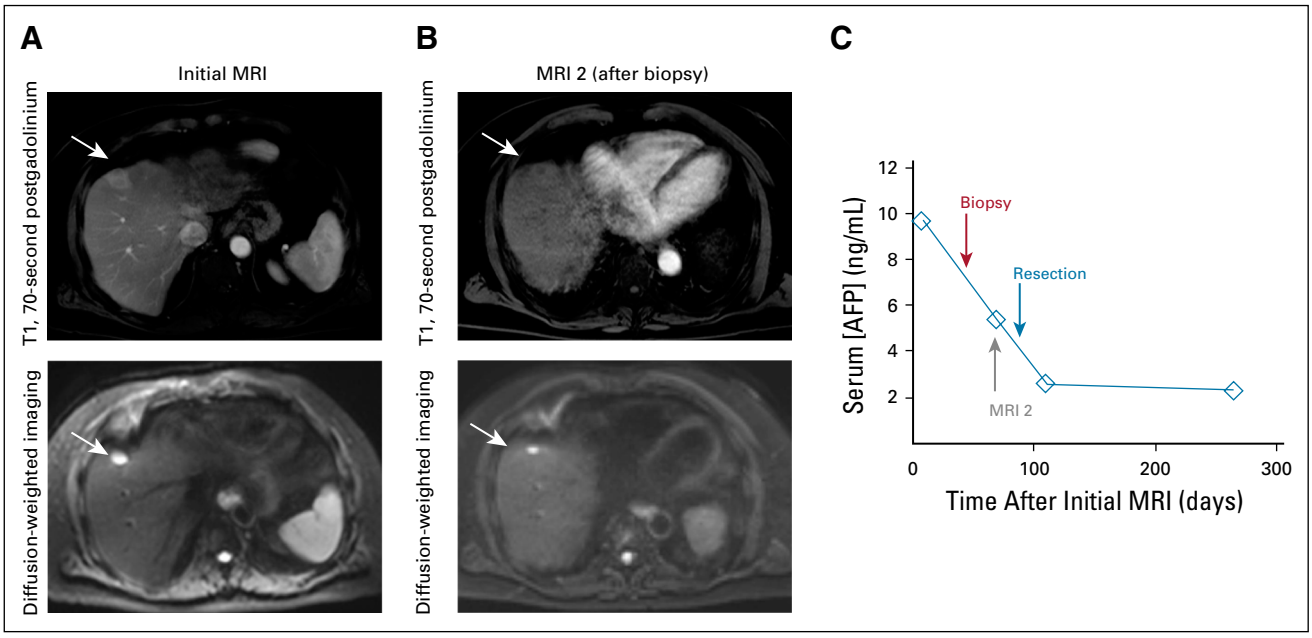

FIG 1. Preoperative clinical parameters implied regression of a biopsy-proven HCC. (A) Initial MRI (top: postcontrast T1 image, bottom: diffusion-weighted image) showed a 2.3-cm tumor bearing the hallmark characteristics of HCC. (B) Postbiopsy MRI confirmed decreasing arterial enhancement (top: postcontrast T1 image) and decreased size (bottom: diffusion-weighted image) of the HCC lesion. (C) Serum AFP declined to within normal limits after the initial scan. AFP, alpha fetoprotein; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging.

count ≥ 15), which is equivalent to 40 mutations/Mb and indicative of a high TMB. This case scores in the 95th percentile across all cancer types (out of 4,800 solid tumor specimens profiled) and in the 86th percentile in the subset of HCC (15 HCC specimens profiled) on our targeted panel assay. Additionally, the sequencing data showed missense substitutions in TP53 and DAXX; there were no genomic fusions identified (Table 1).

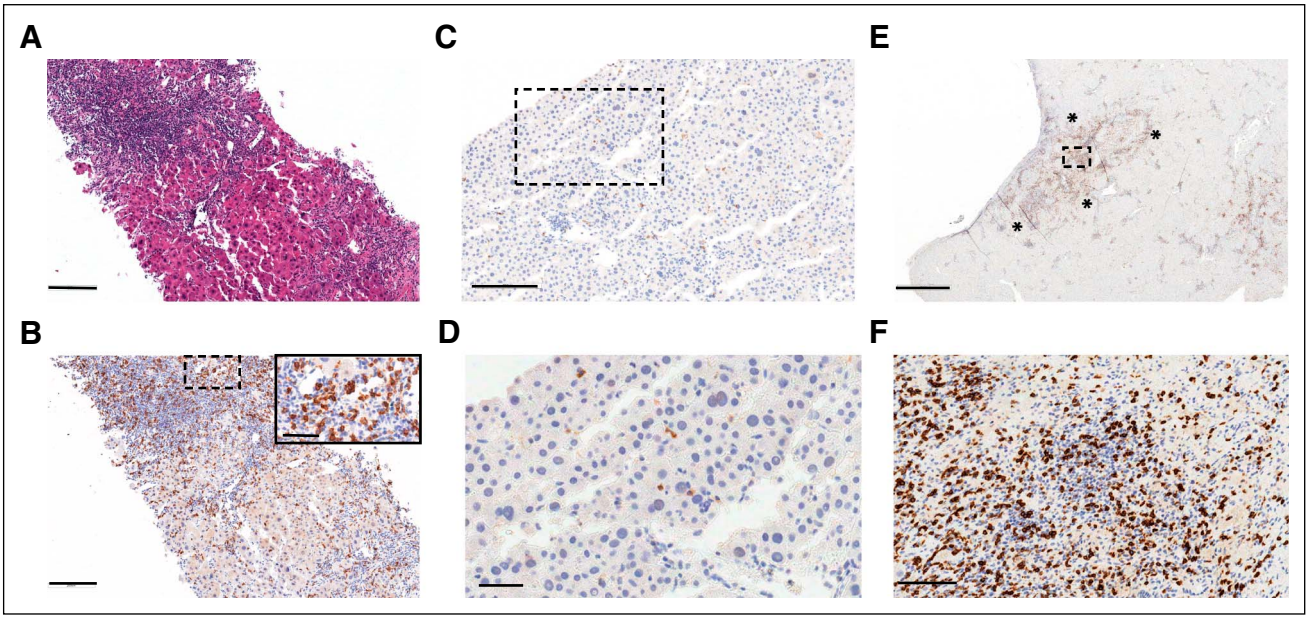

FIG 2. (A) H&E stain of biopsy tissue showing HCC with a prominent lymphocytic infiltrate. Scale bar is 200 μm. (B) IHC staining of biopsy tissue confirmed that the infiltrating lymphocytes were predominantly CD8+ (Leica [4B11]). Scale bars are 200 μm and 20 μm (inset). (C and D) There were also rare CD56+ (Leica [PA0191]) cells present in the biopsy specimen. Scale bars are (C) 200 μm and (D) 50 μm. (E and F) CD8 IHC staining of the resected liver segment showed a residual brisk CD8+ immune cell infiltrate into a 4-mm scar and no residual cancer cells. Asterisks denote the presumed border of the original tumor. Scale bars are (E) 2 mm and (F) 100 μm. HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

TABLE 1. Summary of Genomic Alterations

Molecular Variant	Comment
TP53	Arg110Leu (ENST00000269305.4: c.329G>T)
DAXX	Glu172Gly (ENST00000266000.6: c.515A>G)
Mismatch repair protein (IHC) expression	Intact (MLH1, MSH2, PMS2, and MSH6)
Tumor mutational burden (mut/Mb)	40
Gene fusions	None

Abbreviations: IHC, immunohistochemistry; mut, mutations; Mb, megabase.

The patient remains in complete remission 16 months after the surgical resection. Postoperative imaging has continued to show no evidence of recurrent disease or hepatic vascular compromise or thromboembolism, and the serum alpha fetoprotein has remained within normal limits.

DISCUSSION

To our knowledge, this is the first known case of a spontaneous immune-mediated regression of biopsy-confirmed HCC associated with a documented high TMB. There have been several case reports and case series demonstrating radiographic and/or pathologic evidence of spontaneous HCC regression, even at an advanced stage and sometimes completely.[6,8] In those instances, there was no documentation or report of the TMB status. The assembled case series that document such regression have often invoked inflammatory stimuli or other systemic or physiologic perturbations to explain the antitumor effect.[9] Our patient had no changes to his medications, took no additional conventional or complementary medications, had received no vaccinations within the preceding year, and had no other obvious hemodynamic precipitants to the tumor regression.

Although the biopsy of our patient's tumor may have contributed to the inflammatory local microenvironment, there was evidence of a pre-existing immune infiltrate even in the biopsy specimen. The tumor biopsy specimen demonstrated infiltration of the tumor by large numbers of CD8+ T cells. Tumor-infiltrating lymphocytes have complex roles in hepatocarcinogenesis, including direct cytotoxicity against HCC cells by cytotoxic T cells and NK cells, a phenomenon inhibited by infiltrating regulatory T cells.[10] Multiple studies have documented specific lymphocyte subsets that can contribute to pro- and anti-cancer immunity. Indeed, a recent case report implicated enrichment of CD16+ NK cells in one patient with advanced HCC that spontaneously regressed.[6]

Our patient's tumor exhibited a high TMB (Table 1). TMB is at least partially independent from other immunotherapy biomarkers—such as programmed death-ligand 1[11] and microsatellite instability[12]—and is associated with a primed immune system that is poised to respond to immune checkpoint inhibition in multiple cancer types.[13,14] This immune reactivity is presumably associated with an increased likelihood of peptide neoantigen presence in the setting of a highly mutated genome.[15] High TMB is present in $< 1\%$ of HCC,[16] is more common in TP53-mutated tumors (as was found in our patient), and does not correlate with viral versus nonviral HCC etiologies.[17] Computational deconvolution studies using bulk gene expression analysis of resected HCC specimens has demonstrated a trend toward an enriched CD8+ infiltrate in TMB-high specimens.[18] Our patient's tumor genome also harbored a mutation in the DAXX gene, but its significance remains unclear.

In summary, we report the first case to our knowledge of biopsy-proven completely regressed HCC associated with a brisk lymphocyte infiltrate and a high TMB. Better understanding of the interplay between tumor genomic features and immune microenvironment in similar exceptional cases may lead to deeper insights into the drivers of pharmacologic antitumor immunity and contribute to improved genomic HCC screening methodologies.

AFFILIATIONS

[1]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA
[2]Massachusetts General Hospital Division of Gastroenterology, Harvard Medical School, Boston, MA
[3]Massachusetts General Hospital Department of Pathology, Harvard Medical School, Boston, MA
[4]Massachusetts General Hospital Department of Surgery, Harvard Medical School, Boston, MA

CORRESPONDING AUTHOR

Joseph W. Franses, PhD, MD, BS, Hematology-Oncology, Massachusetts General Hospital Cancer Center, 55 Fruit St, Boston, MA 02114; e-mail: jfranses@mgh.harvard.edu.

AUTHOR CONTRIBUTIONS

Conception and design: Joseph W. Franses, Irun Bhan, Vikram Deshpande, Kenneth Tanabe
Financial support: David T. Ting
Administrative support: David T. Ting
Provision of study materials or patients: Vikram Deshpande, Kenneth Tanabe
Collection and assembly of data: Joseph W. Franses, Amaya Pankaj, Vikram Deshpande, Kenneth Tanabe
Data analysis and interpretation: Joseph W. Franses, Irun Bhan, David T. Ting, Vikram Deshpande, Kenneth Tanabe
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by the authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the

subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Joseph W. Franses

Stock and Other Ownership Interests: Merck, Biogen
Consulting or Advisory Role: Foundation Medicine
Research Funding: Genentech/Roche

David T. Ting

Leadership: PanTher Therapeutics
Stock and Other Ownership Interests: PanTher Therapeutics, ROME Therapeutics, TellBio
Consulting or Advisory Role: ROME Therapeutics, Millipore, Foundation Medicine, Pfizer, NanoString Technologies, Third Rock Ventures
Research Funding: ACD Biotechne, PureTech, Ribon Therapeutics
Patents, Royalties, Other Intellectual Property: Patent licensed to Rome Therapeutics by my Institution, Patent License to PanTher Therapeutics, Patent License to TellBio Inc

Kenneth Tanabe

Stock and Other Ownership Interests: Helix 12 Pharmaceuticals
Consulting or Advisory Role: Best Doctors Inc, Cancer Experts Now, UpToDate, Advance Medical
Research Funding: Enanta, Zafgen Pharmaceuticals
Patents, Royalties, Other Intellectual Property: Patent on use of EGF SNP to identify patients at risk for hepatocellular carcinoma, Patent for use of EGFR inhibitor to inhibit liver fibrogenesis and prevent hepatocellular carcinoma

No other potential conflicts of interest were reported.

REFERENCES

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70:7-30, 2020
2. Bray F, Ferlay J, Soerjomataram I, et al: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394-424, 2018
3. Finn RS, Ikeda M, Zhu AX, et al: Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. J Clin Oncol 38:2960-2970, 2020
4. Finn RS, Qin S, Ikeda M, et al: Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 382:1894-1905, 2020
5. Sakamaki A, Kamimura K, Abe S, et al: Spontaneous regression of hepatocellular carcinoma: A mini-review. World J Gastroenterol 23:3797-3804, 2017
6. Arjunan V, Hansen A, Deutzmann A, et al: Spontaneous regression of HCC—When the immune system stands up to cancer. Hepatology 73:1611-1614, 2021
7. Zheng Z, Liebers M, Zhelyazkova B, et al: Anchored multiplex PCR for targeted next-generation sequencing. Nat Med 20:1479-1484, 2014
8. Koya Y, Suzuki T, Tai M, et al: Spontaneous regression of hepatocellular carcinoma with portal vein tumor thrombus. Case Rep Gastroenterol 12:411-419, 2018
9. Chohan MBY, Taylor N, Coffin C, et al: Spontaneous regression of hepatocellular carcinoma and review of reports in the published English literature. Case Rep Med 2019:9756758, 2019
10. Mossanen JC, Tacke F: Role of lymphocytes in liver cancer. Oncoimmunology 2:e26468, 2013
11. Yarchoan M, Albacker LA, Hopkins AC, et al: PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight 4:e126908, 2019
12. Goodman AM, Sokol ES, Frampton GM, et al: Microsatellite-stable tumors with high mutational burden benefit from immunotherapy. Cancer Immunol Res 7:1570-1573, 2019
13. Marabelle A, Fakih M, Lopez J, et al: Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: Prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol 21:1353-1365, 2020
14. Osipov A, Lim SJ, Popovic A, et al: Tumor mutational burden, toxicity, and response of immune checkpoint inhibitors targeting PD(L)1, CTLA-4, and combination: A meta-regression analysis. Clin Cancer Res 26:4842-4851, 2020
15. Chan TA, Yarchoan M, Jaffee E, et al: Development of tumor mutation burden as an immunotherapy biomarker: Utility for the oncology clinic. Ann Oncol 30:44-56, 2019
16. Ang C, Klempner SJ, Ali SM, et al: Prevalence of established and emerging biomarkers of immune checkpoint inhibitor response in advanced hepatocellular carcinoma. Oncotarget 10:4018-4025, 2019
17. Ho WJ, Danilova L, Lim SJ, et al: Viral status, immune microenvironment and immunological response to checkpoint inhibitors in hepatocellular carcinoma. J Immunother Cancer 8:e000394, 2020
18. Yin L, Zhou L, Xu R: Identification of tumor mutation burden and immune infiltrates in hepatocellular carcinoma based on multi-omics analysis. Front Mol Biosci 7:599142, 2020