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# Drivers of adaptive evolution during chronic SARS-CoV-2 infections

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In some immunocompromised patients with chronic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, considerable adaptive evolution occurs. Some substitutions found in chronic infections are lineage-defining mutations in variants of concern (VOCs), which has led to the hypothesis that VOCs emerged from chronic infections. In this study, we searched for drivers of VOC-like emergence by consolidating sequencing results from a set of 27 chronic infections. Most substitutions in this set reflected lineage-defining VOC mutations; however, a subset of mutations associated with successful global transmission was absent from chronic infections. We further tested the ability to associate antibody evasion mutations with patient-specific and virus-specific features and found that viral rebound is strongly correlated with the emergence of antibody evasion. We found evidence for dynamic polymorphic viral populations in most patients, suggesting that a compromised immune system selects for antibody evasion in particular niches in a patient's body. We suggest that a tradeoff exists between antibody evasion and transmissibility and that extensive monitoring of chronic infections is necessary to further understanding of VOC emergence.

**Table 1 | Summary of all 27 patients with chronic SARS-CoV-2 infections**

| Background condition | Anti-B cell background treatment or inferred B cell depletion | High-dosage steroid treatment | Antibody-based COVID-19 treatment[a] | Days of infection (n)[b] | S amino acid replacements[c] | Ref. |
|---|---|---|---|---|---|---|
| Chronic lymphocytic leukemia (CLL) | None | None | CP | 105 | **Δ141-144** | 49 |
| Lymphoma | CD20 bispecific antibodies | Prednisone | CP | 106 | None | 50 |
| B cell lymphoma | CD20 bispecific antibodies | Corticosteroids | None | 171 | V3G, Δ18-30, S50L, N87S, **Δ141-145**, A222V | 6 |
| Kidney transplant | None | Prednisone | CP | 27 | **Δ141-144, E484K** | 51 |
| Severe antiphospholipid syndrome | Rituximab | Prednisone | mAb | 152 | P9L, Δ12-18, Δ141-143, **Y144-**, Y144F, Q183H, **N440D**, T478K, **E484K, E484Q, F486I, Y489H**, Q493K, **S494P, N501Y**, I870V, A1020S | 8 |
| Renal disease | Rituximab | Prednisone | None | 16 | **E484K, E484Q** | 48 |
| AIDS | None | None | mAb, CP | 23 | **E484K**, Q954L | 48 |
| Follicular lymphoma | Obinutuzumab | None | mAb | 89 | **E484K** | 48 |
| Heart transplant | None | Prednisolone | None | 27 | **E484K** | 48 |
| CLL | None | None | mAb | 72 | G1219C, **E484K** | 48 |
| Kidney transplant | None | Prednisolone | mAb, CP | 20 | None | 48 |
| AIDS | None | Dexamethasone | None | 190 | **E484K**, A1078V, R190K, **K417T, F490S**, D427Y, **N501Y**, P9L | 33 |
| Marginal B cell lymphoma | Rituximab | Prednisolone | CP | 101 | **Δ69-70, D796H**, Y200H, T240I, S13I, W64G, P330S, P812S | 7 |

**Table 2 | Recurrent mutations observed along the SARS-CoV-2 phylogeny**

| Protein | Mutation | Clade success | Times observed in chronic infections (n) |
|---------|----------|---------------|------------------------------------------|
| ORF1a | T3255I | + | 1 |
| ORF1a | S3675-,G3677-,F3678- | + | 0 |
| S | L18F | + | 0[a] |
| S | T95I | + | 1 |
| S | L452R | + | 0 |
| S | N501Y | + | 2 |
| S | P681R | + | 0 |
| N | P199L | + | 0 |
| ORF1a | L3606F | - | 1 |
| S | H69-,V70- | - | 1 |
| S | Y144- | - | 7[b] |
| S | E484K | - | 10[c] |
| S | P681H | - | 0 |
| N | S194L | - | 0 |
| N | T205I | - | 0 |
| N | M234I | - | 0 |

High clade success or low clade success is reported based on measurements of clade logistic growth, with + or – representing higher or lower than average growth, respectively[20]. The last column marks the number of patients in the set of chronic infections herein where a substitution was observed. [a]Two deletion events were observed at this locus. [b]An additional two deletion events
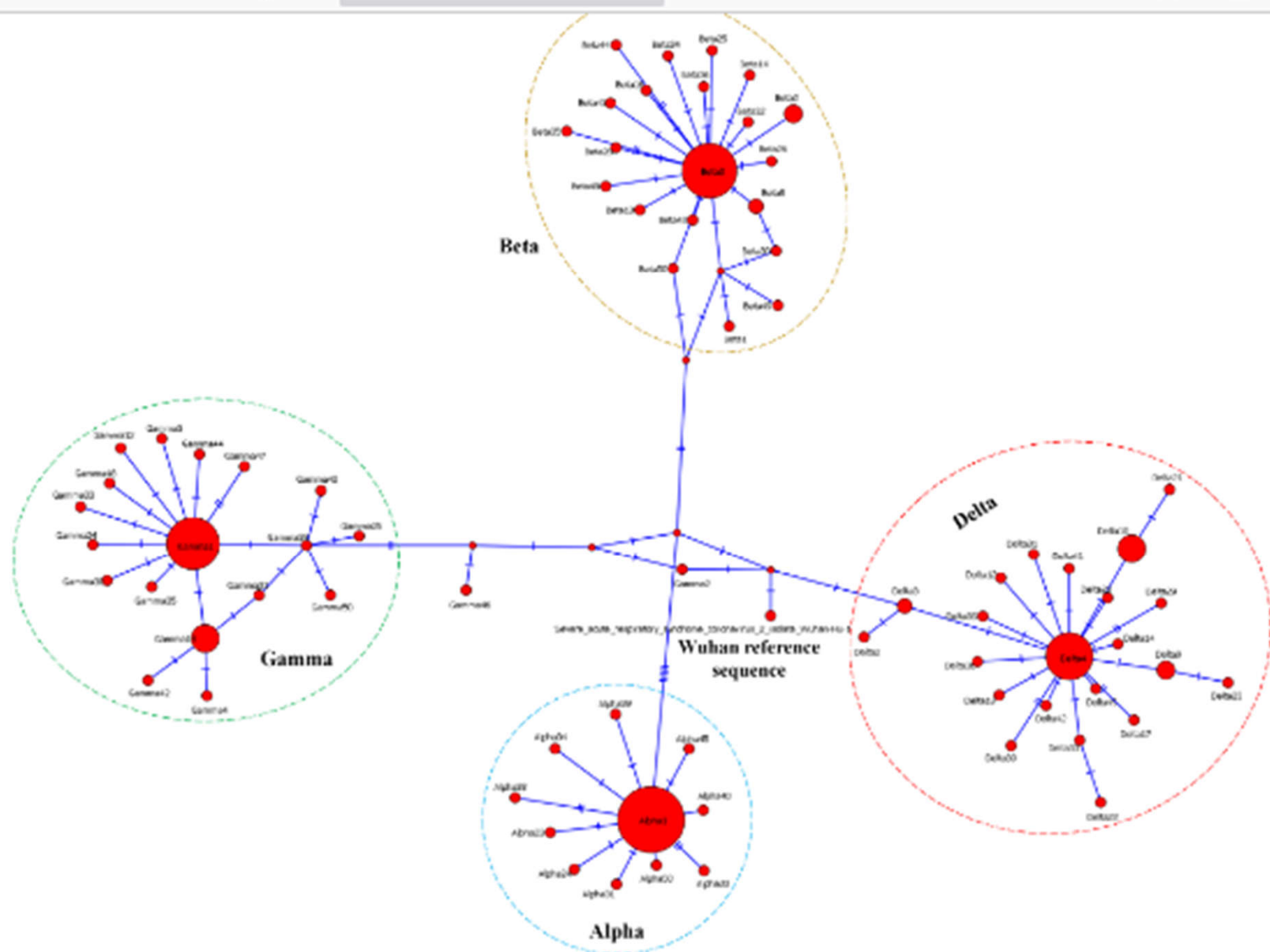
**Fig. 4** Haplotype network of 200 *S* gene sequences of VOCs. The nodes represent the various S gene sequences. Because of space limitations, the samples are abbreviated as alpha1, alpha2, etc., and the corresponding GISAID IDs are presented in Supplementary Table S5. The number of crossbeams on the connecting lines between nodes represents the number of sequence differences between the respective sequences.

and transmission.

To summarize, viral rebound can be viewed as a warning signal that a VOC-like mutation occurred in the patient, and extra caution may be warranted: genetic sequencing, isolation and close monitoring of contacts may be crucial for containment. More extensive monitoring and research of chronic infections is necessary to understand the precise factors determining when and if a variant generated in chronic infection becomes highly transmissible.

Online content

# Primary Small Cell Carcinoma of Liver: A Rare Tumor

Lileswar Kaman[a, c], Javid Iqbal[a], Mahander Pall[a], Amanjit Bal[b]

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## S2691    Primary Small Cell Carcinoma of the Liver. Yes It Exists!

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# Abstract

Primary small cell carcinoma of the liver is very rare tumor. Till date only 12 cases have been reported in the English literature. We are reporting a case of primary small cell carcinoma of the liver in a female patient. She had 13 cm x 7 cm tumor in the right lobe of liver and fine needle aspiration cytology revealed features of small cell carcinoma. After ruling primary from elsewhere, patient underwent central bisectionectomy of the liver and histopathology confirmed the diagnosis of primary small cell carcinoma of the liver. On immunohistochemistry examination, the tumor was positive for Neuron-specific enolase and synaptophysin but negative for Thyroid transcription factor 1 and Hep-Par 1. Here we discuss the clinical course and treatment of primary small cell carcinoma of the liver in our case and review the literature.
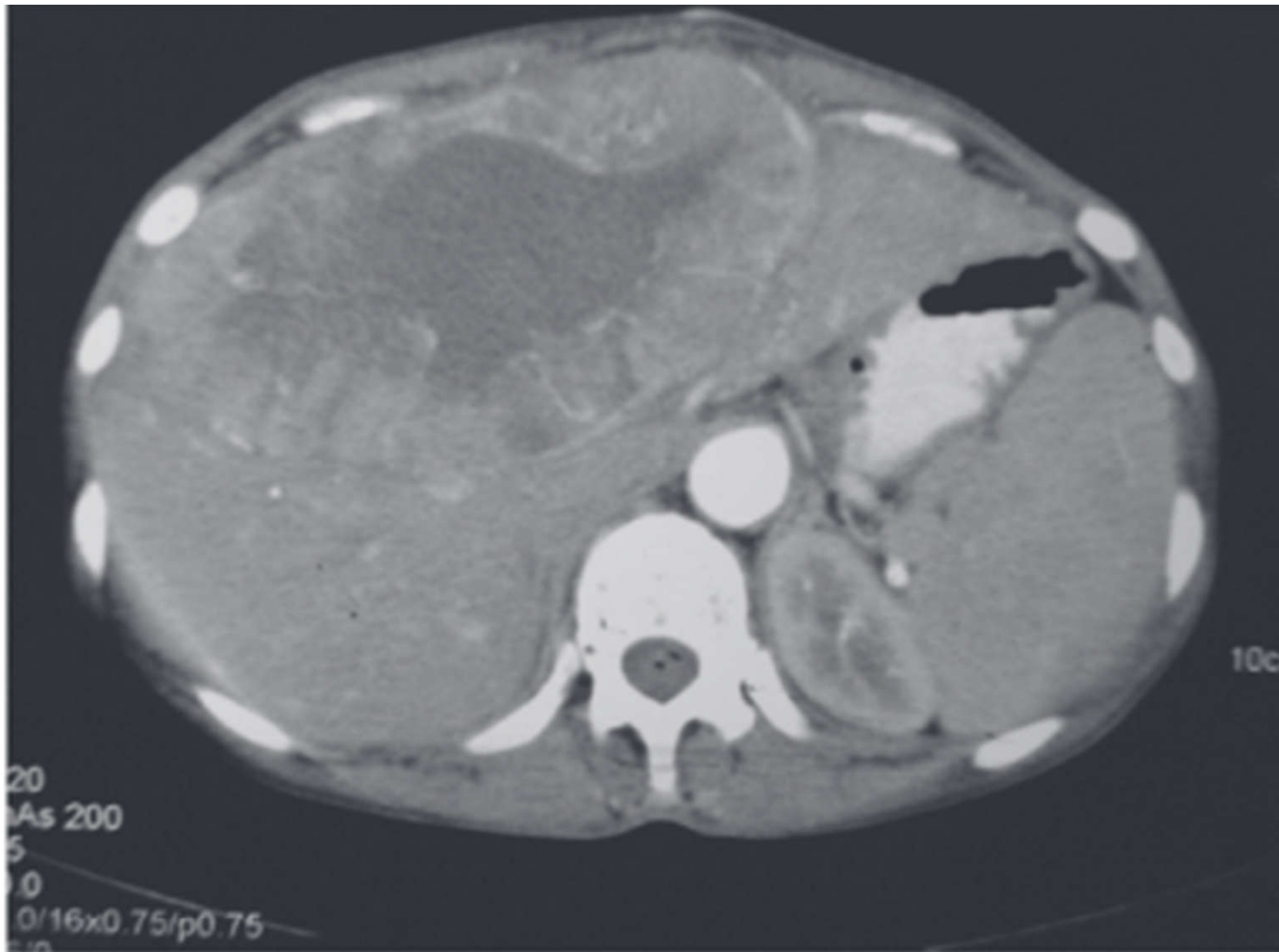
**Figure 1.** Contrast enhanced computed tomography (CECT) abdomen revealed 13.2 x 13.5 x 7.3 cm well defined mass lesion involving segment IV, V and VIII of liver. There was rim enhancement on arterial phase and no contrast retention on venous phase.
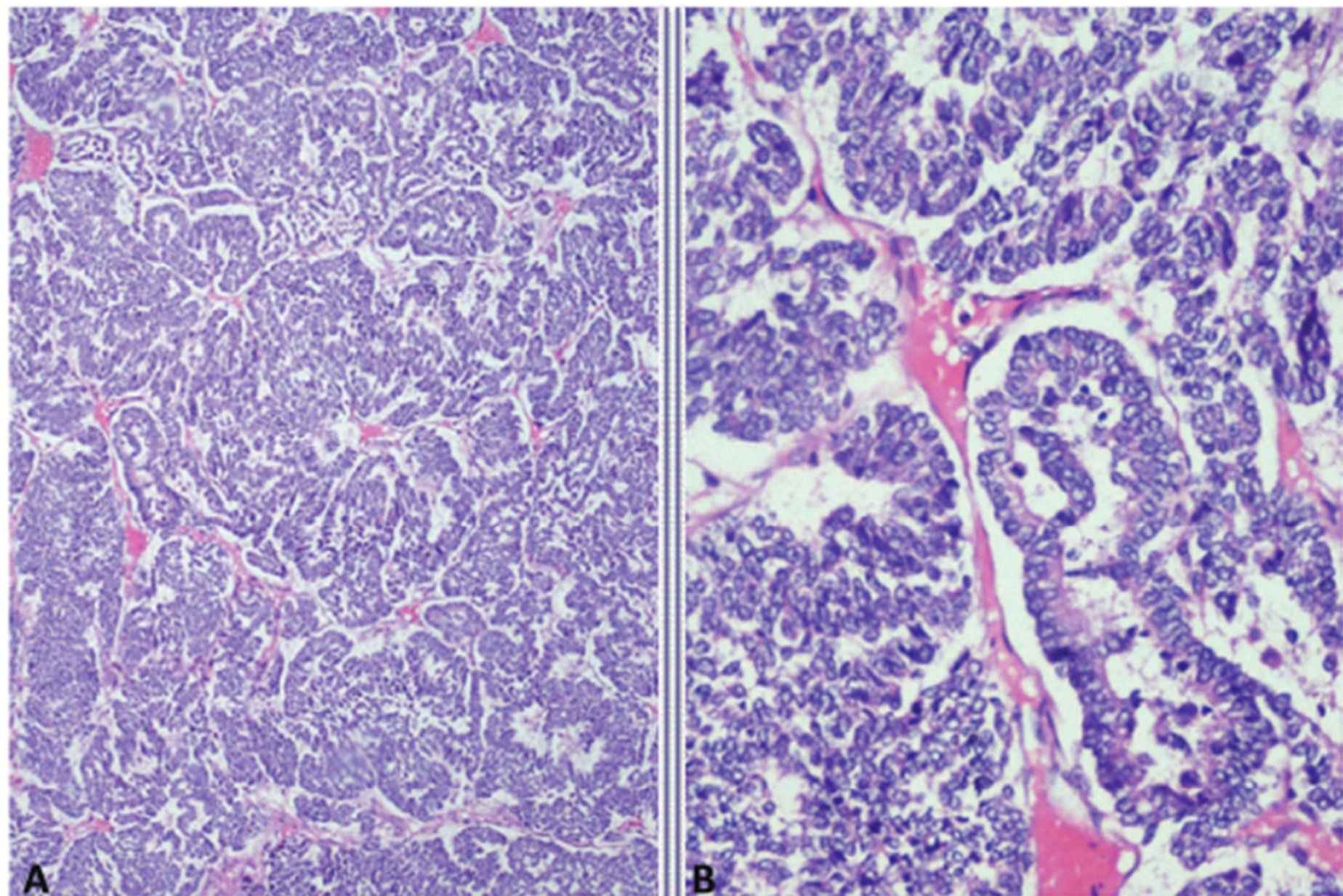
**Figure 2.** Photomicrograph showing A: Organoid and trabecular pattern of tumor cells separated by thin fibrovascular septa; B: Tumor cells with finely granular nuclear chromatin and scanty cytoplasm (Haematoxylin and eosin, x 100, x 400).

**Review**

# Imaging diagnosis of hepatocellular carcinoma: Future directions with special emphasis on hepatobiliary magnetic resonance imaging and contrast-enhanced ultrasound

Junghoan Park[1], Jeong Min Lee[1,2,3], Tae-Hyung Kim[4], and Jeong Hee Yoon[1,2]

Hepatocellular carcinoma (HCC) is a unique cancer entity that can be noninvasively diagnosed using imaging modalities without pathologic confirmation. In 2018, several major guidelines for HCC were updated to include hepatobiliary contrast agent magnetic resonance imaging (HBA-MRI) and contrast-enhanced ultrasound (CEUS) as major imaging modalities for HCC diagnosis. HBA-MRI enables the achievement of high sensitivity in HCC detection using the hepatobiliary phase (HBP). CEUS is another imaging modality with real-time imaging capability, and it is reported to be useful as a second-line modality to increase sensitivity without losing specificity for HCC diagnosis. However, until now, there is an unsolved discrepancy among guidelines on whether to accept "HBP hypointensity" as a definite diagnostic criterion for HCC or include CEUS in the diagnostic algorithm for HCC diagnosis. Furthermore, there is variability in terminology and inconsistencies in the definition of imaging findings among guidelines; therefore, there is an unmet need for the development of a standardized lexicon. In this article, we review the performance and limitations of HBA-MRI and CEUS after guideline updates in 2018 and briefly introduce some future aspects of imaging-based HCC diagnosis. **(Clin Mol Hepatol 2022;28:362-379)**
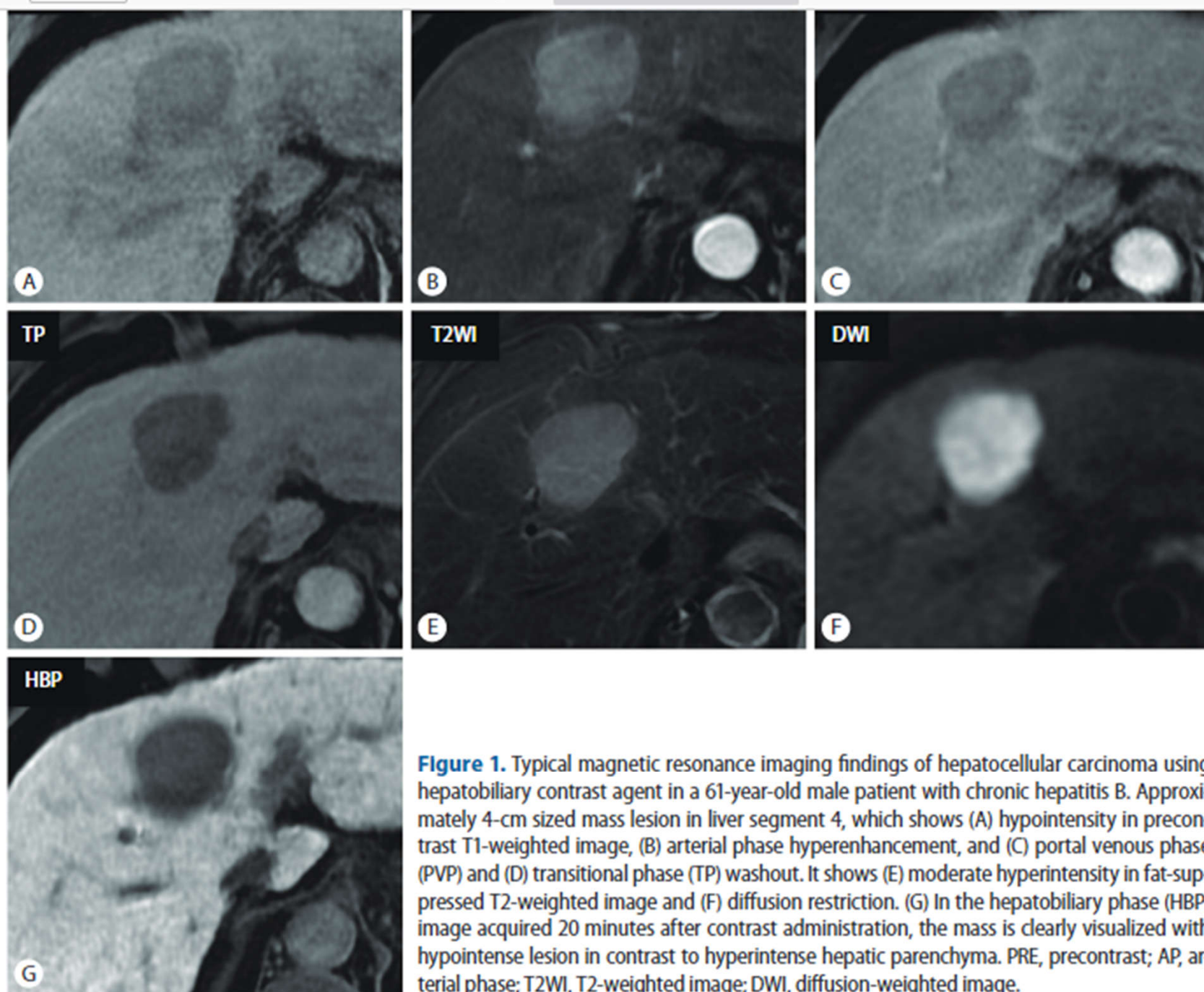
**Figure 1.** Typical magnetic resonance imaging findings of hepatocellular carcinoma using hepatobiliary contrast agent in a 61-year-old male patient with chronic hepatitis B. Approximately 4-cm sized mass lesion in liver segment 4, which shows (A) hypointensity in precontrast T1-weighted image, (B) arterial phase hyperenhancement, and (C) portal venous phase (PVP) and (D) transitional phase (TP) washout. It shows (E) moderate hyperintensity in fat-suppressed T2-weighted image and (F) diffusion restriction. (G) In the hepatobiliary phase (HBP) image acquired 20 minutes after contrast administration, the mass is clearly visualized with hypointense lesion in contrast to hyperintense hepatic parenchyma. PRE, precontrast; AP, arterial phase; T2WI, T2-weighted image; DWI, diffusion-weighted image.
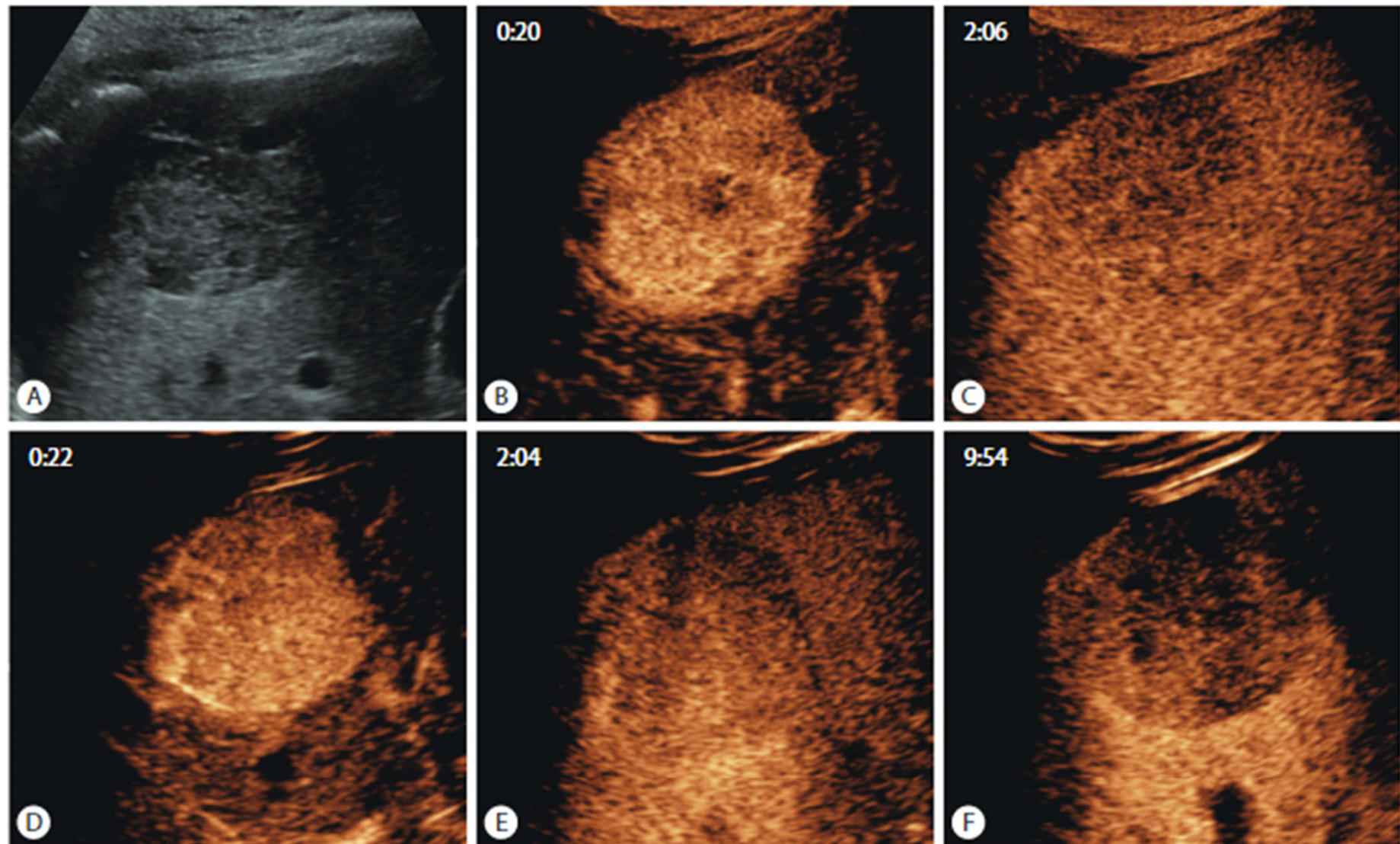
**Figure 2.** Typical contrast-enhanced ultrasound findings of hepatocellular carcinoma using SonoVue and Sonazoid. (A) Approximately 4.5-cm sized hypoechoic mass in liver segment 6 on B-mode ultrasound. After administration of SonoVue, the mass shows (B) hyperenhancement in the arterial phase (20 seconds) and (C) mild washout in the delayed phase, but not devoid of enhancement (126 seconds). The same lesion enhanced with Sonazoid also shows (D) arterial phase hyperenhancement (22 seconds) and (E) mild delayed washout (124 seconds), as well as (F) clear hypointensity in the Kupffer phase (approximately 10 minutes after contrast administration).

**Table 3.** Comparison of diagnostic performance for HCC between CEUS LI-RADS and other diagnostic criteria

| Study | Study type | Contrast agent | LI-RADS | | "APHE and washout" | | On-site diagnosis[†] | |
|---|---|---|---|---|---|---|---|---|
| | | | Sen (%) | Spe (%) | Sen (%) | Spe (%) | Sen (%) | Spe (%) |
| Huang et al.[17,*] (2020) | Retrospective | SonoVue | 73.3 | 97.1 | 88.6 | 87.1 | N/A | N/A |
| Strobel et al.[19] (2021) | Prospective | Unmentioned | 65.2 | 78.6 | 74.3 | 63.0 | 91.5 | 67.4 |
| Schellhaas et al.[20] (2021) | Prospective | Unmentioned | 64.0 | 78.9 | 68.6 | 57.9 | 90.9 | 64.9 |
| Zhou et al.[18] (2022) | Prospective | SonoVue | 38.6–63.6 | 92.7–100.0 | 88.6–100.0 | 28.6–64.3 | N/A | N/A |

HCC, hepatocellular carcinoma; CEUS, contrast-enhanced ultrasound; LI-RADS, Liver Imaging Reporting and Data System; APHE, arterial phase hyperenhancement; Sen, sensitivity; Spe, specificity; N/A, not applicable.
*All lesions are 20 mm or smaller.

## CONCLUSION

In recent years, several major guidelines for HCC have newly included HBA-MRI as the primary diagnostic test or CEUS as a second-line diagnostic test. Several studies have demonstrated that strongly enhanced hepatic parenchyma in HBP allows for better detection of HCC and staging of HCC. In addition, the usefulness of CEUS as a second-line modality has been reported to increase sensitivity without losing specificity. However, to date, there are significant discrepancies in the diagnostic criteria of HBA-MRI and preferred CEUS agents among major guidelines, which could be related to the prevalence of HCC and its treatment patterns in various regions. The application of novel surveillance strategies such as abbreviated MRI or CEUS and deep learning for HCC needs further validation in the near future. To resolve these issues, further large-scale prospective studies are required. Finally, the development and adoption of a universal lexicon for liver imaging would be necessary to decrease gaps between guidelines, enhance communication, and facilitate future scientific research.

**Review**

# RNA interference as a novel treatment strategy for chronic hepatitis B infection

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Chronic hepatitis B (CHB) is a major cause of liver-related morbidity and mortality. Functional cure of CHB, defined as sustainable hepatitis B surface antigen (HBsAg) seroclearance, is associated with improved clinical outcomes. However, functional cure is rarely attainable by current treatment modalities. RNA interference (RNAi) by small-interfering RNA (siRNA) and anti-sense oligonucleotide (ASO) has been studied as a novel treatment strategy for CHB. RNAi targets post-transcriptional messenger RNAs and pregenomic RNAs to reduce hepatitis B virus (HBV) antigen production and viral replication. By reducing viral antigens, host immune reconstitution against HBV may also be attained. Phase I/II trials on siRNAs have demonstrated them to be safe and well-tolerated. siRNA is effective when given in monthly doses with different total number of doses according to different trial design, and can lead to sustainable dose-dependent mean HBsAg reduction by 2–2.5 log. Incidences of HBsAg seroclearance after siRNA therapy have also been reported. ASOs have also been studied in early phase trials, and a phase Ib study using frequent dosing regimen within 4 weeks could achieve similar HBsAg reduction of 2 log from baseline. Given the established efficacy and safety of nucleos(t)ide analogues (NAs), future RNAi regimens will likely include NA backbone. While the current evidence on RNAi appears promising, it remains undetermined whether the potent HBsAg reduction by RNAi can result in a high rate of HBsAg seroclearance with durability. Data on RNAi from phase IIb/III trials are keenly anticipated. **(Clin Mol Hepatol 2022;28:408-424)**

**Keywords:** Hepatitis B virus; Hepatitis B surface antigens; Small-interfering RNA; Anti-sense oligonucleotide; Messenger RNA
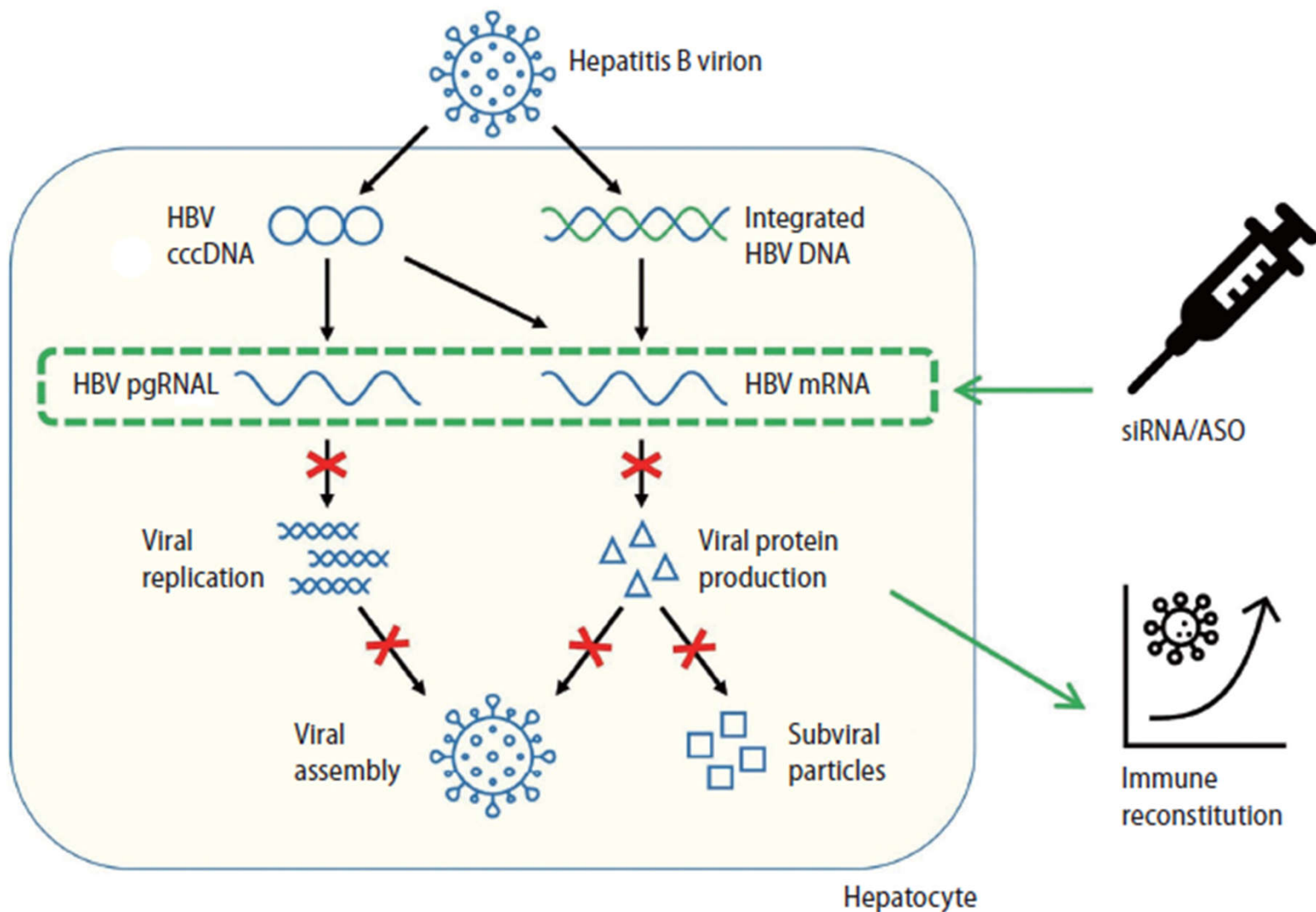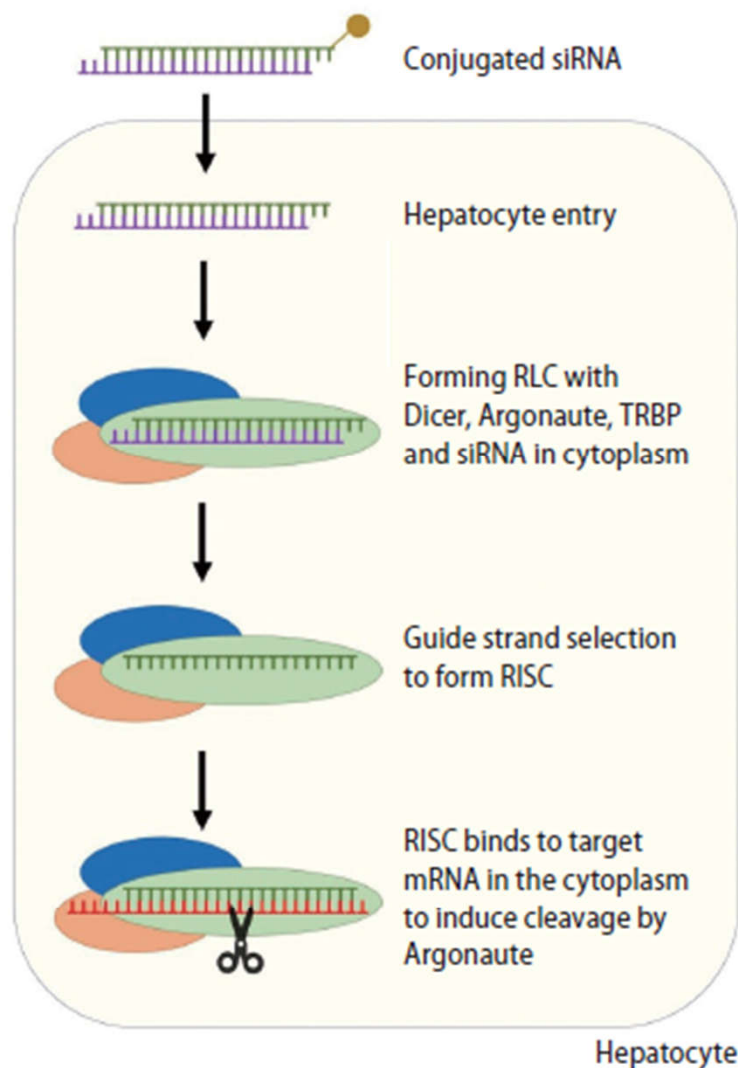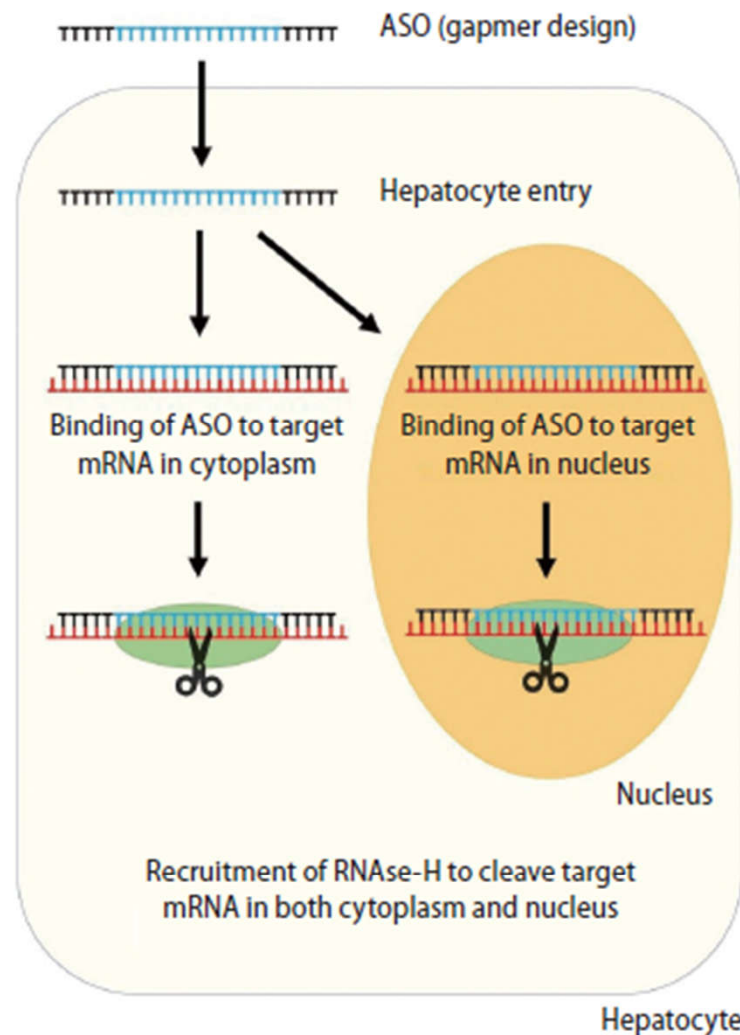
**Figure 2.** Mechanism of RNA interference as a treatment strategy in chronic hepatitis B. HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA; mRNA, messenger RNA; siRNA, small-interfering RNA; ASO, antisense oligonucleotide.

**Figure 1.** Mechanism of small-interfering RNA (A) and antisense oligonucleotides (B). siRNA, small-interfering RNA; RLC, RNA-induced silenc-

## OVERVIEW OF CURRENT EVIDENCE AND CONCLUSION

Clinical trials have consistently demonstrated siRNA to be safe, with most adverse events being mild injection reactions or flu-like symptoms.[76,89,94] ALT flares can occur in siRNA therapy, but are usually transient and associated with HBsAg reduction, suggesting immune reconstitution and elimination of infected hepatocytes.[87-89,94]

siRNA has demonstrated potent HBsAg reduction effects. Among the newer generation siRNA (excluding the older generation ARC-520 and ARB-1467), mean HBsAg suppression by 2–2.5 log is achievable, with over 90% of patients in high dose treatment arms reaching over 1 log IU/mL HBsAg reduction, and 50–97% of patients having HBsAg suppressed to below 100 IU/mL.[75,77,86,89] HBsAg reduction in siRNA was sustainable after end of treatment,[75,86,89] and incidences of HBsAg seroclearance have been documented.[83,95] Nonetheless it remains unclear whether these potent HBsAg reduc-

THE END