Communication

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Sequential Therapy with Ropeginterferon Alfa-2b and Anti-PD1 Antibody for Inhibiting Recurrence of Hepatitis B-related Hepatocellular Carcinoma: From Animal Modeling to Phase I Clinical Results

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Sequential Therapy with Ropeginterferon Alfa-2b and Anti-PD1 Antibody for Inhibiting Recurrence of Hepatitis B-Related Hepatocellular Carcinoma: From Animal Modeling to Phase I Clinical Results

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Abstract: In hepatocellular carcinoma (HCC), recurrence usually occurs after curative surgical resection. Currently, no approved adjuvant therapy has been shown to reduce recurrence rates. In this report, the in vivo mouse effect of sequential combination treatment with recombinant mouse interferon-alpha (rmIFN-α) and an anti-mouse-PD1 antibody on hepatitis B virus (HBV) clearance was evaluated. A phase I clinical trial was then conducted to assess the safety, tolerability, and inhibitory activity of sequential therapy with ropeginterferon alfa-2b and nivolumab in HCC recurrence in patients who have undergone curative surgery for HBV-related HCC. In animal modeling, HBV suppression was significantly greater with rmIFN-α and anti-PD1 sequential combination treatment than with their treatment alone. In the Phase I study, eleven patients completed the sequential therapy with ropeginterferon alfa-2b every two weeks at the dose of 450 μg for six doses, followed by three doses of nivolumab every two weeks up to 0.75 mg/kg. A notable decrease or clearance of HBV surface antigen was observed in two patients. Dose-limiting toxicity of grade 3 alanine transaminase and aspartate aminotransferase increases was observed in one patient. The maximum tolerated dose was determined. Currently no HCC recurrence has been observed. The treatment modality was well tolerated. The data support further clinical development of sequential combination therapy as a post-surgery prophylactic measure against the recurrence of HBV-related HCC.

Keywords: hepatocellular carcinoma; HBV-related; anti-PD1 antibody; ropeginterferon alfa-2b; animal HBV model; clinical trial

1. Introduction

Hepatocellular carcinoma (HCC) is a common and deadly cancer worldwide [1]. It is associated with underlying chronic liver pathological conditions, including chronic viral hepatitis. Chronic hepatitis B (CHB) contributes to more than 50% of global HCC occurrence [2,3]. Surgery is usually the curative treatment modality for early-stage HCC [4]. However, cancer recurrence is frequently observed. Tumor recurrence rate can be as high as 50% to 70% after five years [5–7]. Currently, there are no approved therapies to inhibit recurrence [7]. Effective and well-tolerated adjuvant therapies are urgently needed to reduce recurrence.

Programmed cell death 1 (PD-1) is a negative costimulatory receptor expressed primarily on the surface of activated T cells [8,9]. The binding of PD-1 to its ligand, programmed cell death-1 ligand-

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1/2 (PD-L1/2), inhibits cytotoxic T cell-mediated immunological responses and elicits an immune checkpoint [10]. Tumor cells upregulate PD-L1 and utilize the PD-1 pathway to escape T-cell-mediated immune responses. Anti-PD1 and anti-PD-L1/2 therapeutic antibodies can interfere with the interactions between PD-1 and its ligands, resulting in enhanced anti-tumor immunological response by cytotoxic T cells [10,11]. Anti-PD1 therapy causes a decline or seroclearance of hepatitis B surface antigen (HBsAg) in patients with CHB [12]. HBsAg seroclearance is associated with a lower risk of hepatitis B virus (HBV)-related HCC [13]. Anti-PD1 therapies have been approved to treat advanced HCC, melanoma, metastatic non-small cell lung cancer, and other advanced malignancies [14]. However, anti-PD-1 therapies are also associated with known toxicities [15], which may not be suitable in post-surgical adjuvant settings for patients with HCC. A combination therapy that can potentially minimize toxicity and produce a significant anti-cancer effect may be applicable in this setting.

Ropeginterferon alfa-2b represents a new generation, PEGylated interferon alpha (IFN-α)-based therapy with a favorable pharmacokinetic profile. It can be injected less frequently, e.g., once every two weeks [16–18]. It has been approved for the treatment of polycythemia vera (PV), a myeloproliferative neoplasm (MPN), in the United States and Europe [19,20] and is currently under development for more approvals [21–25]. It appeared to be well-tolerated and showed anti-HBV activities when administered once every two weeks at the dose of 450 μg in a Phase II clinical study [26]. Here, we report our findings in an HBV mouse model sequentially treated with a recombinant mouse-IFN-alpha (rmIFN-α) and an anti-mouse-PD1 antibody and clinical Phase I results of sequential therapy with ropeginterferon alfa-2b and anti-human PD-1 antibody nivolumab in patients with HBV-related HCC after curative surgery.

2. Results

Animal Modeling Data

An HBV mouse model (HBV-HDI) was generated by intravenous injection of an HBV genotype A DNA plasmid into CBA/CaJ mice [27,28]. In this HBV-HDI mouse model, the sequential combination treatment with rmIFN-α and an anti-mouse PD1 antibody (RMP-17) continuously caused a decline in the mean HBsAg values compared to the phosphate-buffered saline (PBS) control. After the sequential combination treatment, the mean HBsAg level was one log lower than that in the PBS control group (Figure 1A). In contrast, the treatment alone with either rmIFN-α or anti-mouse PD1 showed no significant decline in the HBsAg level when compared to the PBS control (Figure 1A). Compared to the rmIFN-α and anti-mouse PD1 treatment alone, the sequential combination of rmIFN-α and anti-mouse PD1 antibody significantly reduced the HBsAg levels and HBV viral titers in the HBV-carrying CBA/CaJ mice (Figure 1A,B). In addition, the HBsAg clearance rate was 44.4% (4/9) in those mice receiving 6-week treatment of rmIFN-α and anti-mouse PD1, which was higher than that in the treatment alone groups (Table 1). The animal data showed a synergistic effect between rmIFN-α and anti-PD1 antibody for HBV suppression or even clearance in the HBV mouse model. We did not observe any adverse effect on animal body weight, liver function and blood cell production (data not shown).

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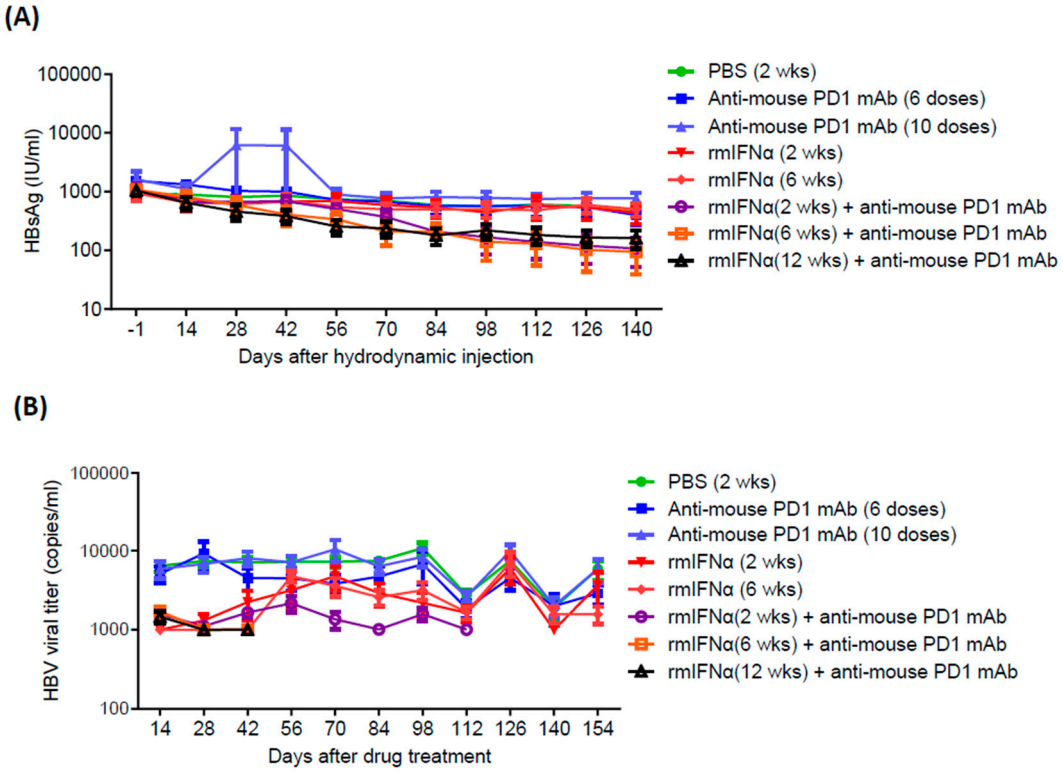

Figure 1. HBsAg (**A**) and HBV viral titer (**B**) in HBV-carried CBA/CaJ mice.

Table 1. Mean HBsAg and HBsAg clearance rate at the end of animal study.

Treatment Groups	Mean HBsAg (IU/ml)	HBsAg clearance rate
PBS	471.9	0% (0/6)
Anti-PD1 (6 doses)	398.6	20% (2/10)
Anti-PD1 (10 doses)	769.1	10% (1/10)
rmIFN-α (2wk)	441.2	0% (0/6)
rmIFN-α (6wk)	504.7	0% (0/6)
rmIFN-α (2wk) +anti-PD1 (6 doses)	107.8 (p value = 0.0012)	20% (2/10)
rmIFN-α (6wk) +anti-PD1 (6 doses)	94.3 (p value = 0.0009)	44.4% (4/9)
rmIFN-α (12wk) +anti-PD1 (6 doses)	162.1 (p value = 0.0040)	0% (0/10)

Phase I Clinical Study

A phase I study was completed to determine the maximum-tolerated dose (MTD). A total of 12 eligible patients were enrolled (Figure 2), including six patients in Cohort 1 and six in Cohort 2. Patients received six doses of ropeginterferon alfa-2b at the dose of 450 μg once every two weeks followed by three doses of nivolumab at 0.3 mg/kg in Cohort 1 or followed by three doses of nivolumab at 0.75 mg/kg in Cohort 2. All patients completed the study treatment except one who withdrew early because of grade 3 anorexia after receiving one dose of ropeginterferon alfa-2b in Cohort 2.

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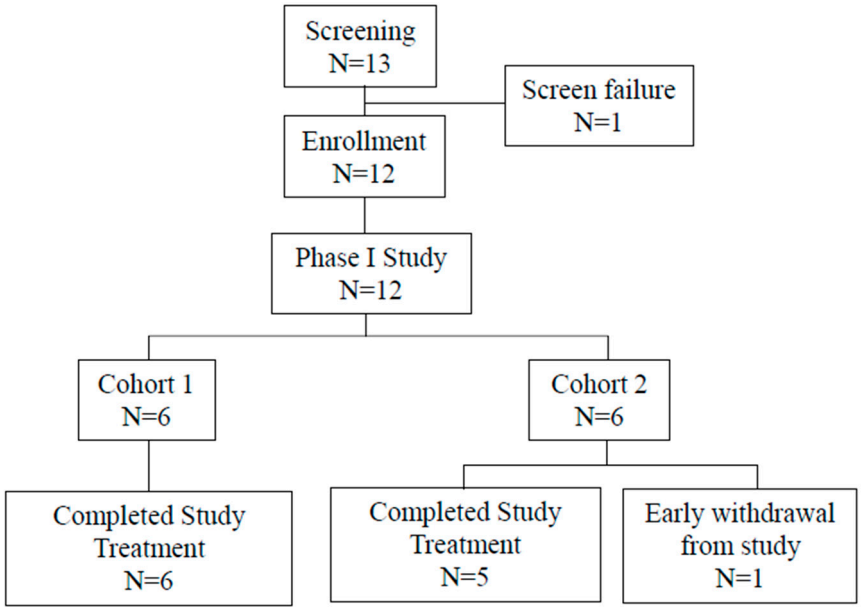

Figure 2. Summary of subject disposition in phase I study.

The mean (standard deviation) age of the eligible patients was 61.8 (10.3) years old (Table 2). Six (50%) patients had liver cirrhosis before participating in this study. All patients experienced at least one adverse event (AE) after treatment. Most AEs were mild or moderate (Table 3). No Grade 4 or 5 AEs were observed. No serious AEs (SAEs) were observed. Four patients (33.3 %) experienced Grade 3 AEs. The most frequent AE was pyrexia (50%), followed by alanine transaminase (ALT) increase (41.7%), aspartate aminotransferase (AST) increase (41.7%), fatigue (33.3%), and neutrophil count decrease (25%).

Table 2. Summary of Demographics and Baseline Characteristics in the Phase I Clinical Study.

Characteristics	P1101 + Anti-PD1 N=12		
	Cohort 1 N=6	**Cohort 2 N=6**	**Total N=12**
Age, years			
Mean (SD)	64.2 (6.1)	59.5 (12.9)	61.8 (10.3)
Range (Min-Max)	53-72	40-75	40-75
Gender			
Male, n (%)	5 (83%)	4 (67%)	9 (75%)
Female, n (%)	1 (17%)	2 (33%)	3 (25%)
Liver Cirrhosis			
Yes	3 (50%)	3 (50%)	6 (50%)
No	3 (50%)	3 (50%)	6 (50%)

Max: maximal; Min: minimal; SD: standard deviation.

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Table 3. Summary of AEs in the Phase 1 Study.

AEs, n (%)	Cohort 1 (N=6)			Cohort 2 (N=6)			Total (N=12)
Any AE	6 (100)			6 (100)			12 (100.0)
Any SAE	0 (0)			0 (0)			0 (0)
AEs occurring in >10% of patients n (%)	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	
Pyrexia	4 (66.7)	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	6 (50.0)
ALT increased	1 (16.7)	1 (16.7)	1 (16.7)	0 (0)	2 (33.3)	0 (0)	5 (41.7)
AST increased	1 (16.7)	1 (16.7)	1 (16.7)	0 (0)	2 (33.3)	0 (0)	5 (41.7)
Fatigue	2 (33.3)	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	4 (33.3)
Neutrophil count decreased	0 (0)	1 (16.7)	1 (16.7)	0 (0)	1 (16.7)	0 (0)	3 (25.0)
Decreased appetite	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	2 (16.7)
Insomnia	1 (16.7)	0	0 (0)	1 (16.7)	0 (0)	0 (0)	2 (16.7)

AE: Adverse Event; SAE: Serious Adverse Event; Note: (1) % = percentage of patients with N as the denominator.

Dose-limiting toxicities (DLTs) were observed in only one patient. Drug-related Grade 3 ALT and AST increases were observed in one patient in Cohort 1. The patient completed ropeginterferon alfa-2b treatment and received one dose of nivolumab. No DLTs were observed in Cohort 2. However, given that the DLT of Grade 3 ALT and AST increases was observed in Cohort 1 and that there were greater levels of Grade 2 ALT and AST increases in Cohort 2, we determined that Cohort 2 reached the MTD of the study based on the overall safety assessment. Therefore, ropeginterferon alfa-2b at 450 ug for six doses followed by nivolumab at 0.75 mg/kg for three doses is the MTD for the adjuvant, sequential combination therapy.

All patients are alive without cancer recurrence as of the date. The mean follow-up period was 716.75 days (minimum: 114 days; maximum: 1416). HBsAg was undetectable in one patient in Cohort 1 at follow-up week 12 and after (Figure 3A). The mean HBsAg levels decreased over time in Cohort 2 during the treatment period (Figure 3B).

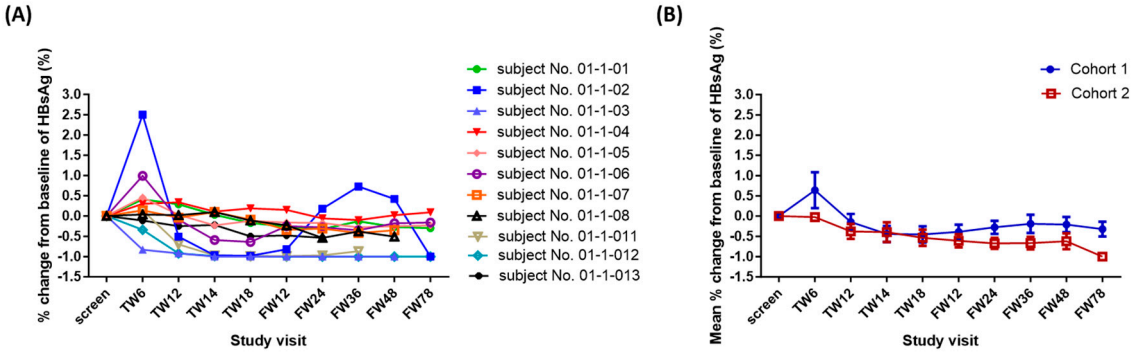

Figure 3. Percentage change of HBsAg in phase I study. (**A**) Percentage change of HBsAg in each subject (**B**) Mean percentage change of HBsAg.

3. Discussion

HCC is a common cause of cancer-related deaths, and most HCC cases are associated with HBV infection. Patients with HCC have a high risk of tumor recurrence after surgical resection. Currently, no approved therapies for recurrence inhibition exist. In this report, we showed our animal modeling data that sequential combination treatment with rmIFN-α and anti-PD1 antibody led to a synergistic effect in HBV suppression and clearance. Our Phase I clinical study further suggested that the sequential combination therapy with ropeginterferon alfa-2b and nivolumab was well-tolerated and might help clear residual HBV infection and inhibit cancer recurrence in patients with HBV-related HCC after curative surgery.

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PD-L1 is often overexpressed during CHB infection [29–31]. In HCC patients with CHB, treatment with nivolumab decreased HBsAg levels [12,32]. Nivolumab is approved for HCC treatment in combination with ipilimumab [33]. It is reasonable to assume that anti-PD-1 antibodies may suppress cancer recurrence in HBV-infected patients with HCC who have undergone surgical resection. However, the toxicities associated with the anti-PD-1 treatment at approved dose levels and HBV reactivation due to the residual viral genome may pose hurdles for its use alone as a prophylactic measure against HCC occurrence [34–36].

Type 1 IFNs, including IFN-α and beta (IFN-β), share the same receptor components and induce similar biological activities [37–39]. They induce anti-proliferative, immune-stimulatory, and anti-angiogenic activities [40–43]. The anti-proliferative effects include cell cycle inhibition and apoptosis [44–46]. In solid tumor cells, they can slow S phase progression by the activation of an intra-S phase checkpoint and induce senescence entry accompanied by a loss of tumorigenicity [47]. In addition, they stimulate the immune system to elicit anti-tumor activities, including inducing natural killer cell-dependent and CD8+ T cell-mediated anti-tumor responses [48,49]. These combined anti-tumor activities can inhibit tumor formation. Pegylated IFN-α treatment has been shown to inhibit HBV and is approved for patients with CHB [50–52]. Its treatment is associated with a lower HCC incidence in HBV infection [53]. Therefore, a PEGylated IFN-α therapy with both anti-cancer and anti-HBV activities may reduce the need of the anti-PD1 treatment at a high dose level for inhibiting the HCC recurrence. Their sequential combination treatment may potentially eradicate residual tumor cells or newly formed tumor cells due to HBV infection in patients with HBV-related HCC after curative surgery. Our results suggest that the sequential combination therapy with ropeginterferon alfa-2b and nivolumab may be a promising regimen for inhibiting cancer recurrence in patients with HBV-related HCC after curative surgery.

4. Methods

HBV-HDI Mouse Model:

Six- to eight-week-old male CBA/Caj mice were bred at the National Taiwan University Laboratory Animal Center. The mice were intravenously injected with 10 μg of HBV genotype A DNA plasmid dissolved in PBS equivalent to approximately 8% of the mouse's body weight as previously described [27,28]. Serum HBsAg and HBV DNA were measured to monitor the HBV persistence. All experiments were performed according to the guidelines established by the Institutional Animal Care and Use Committee at the National Taiwan University College of Medicine.

Preclinical Materials:

Anti-mouse PD1 (RMP-17) is a monoclonal antibody targeting mouse PD-1 generated by hybridoma screening at the PharmaEssentia Corporation Research Laboratory. rmIFN-α was produced using the *Escherichia coli* expression system at PharmaEssentia Corporation.

Treatment of HBV-Carrying CBA/CaJ Mice:

Mice were divided into eight groups to investigate the effect of rmIFN-α in sequential combination with the anti-mouse PD1. The drug dosages and administration routes are described below:

Group 1 (control): Six HBV-carried CBA/CaJ mice were subcutaneously (s.c.) injected with 200 μl PBS at every other day [Q2D] x 8 (Days 0~14).

Group 2: Six HBV-carried CBA/CaJ mice were s.c. injected with 800 IU/g of rmIFN-α (Q2D x 8 , Day 0~14).

Group 3: Six HBV-carried CBA/CaJ mice were s.c. injected with 800 IU/g of rmIFN-α (Q2D x 22, day 0~42)

Group 4: Ten HBV-carried CBA/CaJ mice were intraperitoneally (i.p.) injected with 32 μg/g of the anti-mouse PD1 antibody (Q2D x 6, days 16~26).

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Group 5: Ten HBV-carried CBA/CaJ mice were i.p. injected with 32 μg/g of anti-mouse PD1 antibody (Q2D x 10, days 16~34).

Group 6: Ten HBV-carried CBA/CaJ mice were s.c. injected with 800 IU/g of rmIFN-α (Q2D x 8, days 0~14), and then, i.p. injected with 32 μg/g of the anti-PD1 antibody (Q2D x 6, days 16~26).

Group 7: Ten HBV-carried CBA/CaJ mice were s.c. injected with 800 IU/g of rmIFN-α (Q2D x 22, days 0~42), and then, i.p. injected with 32 μg/g of the anti-PD1 antibody (Q2D x 6, days 44~54).

Group 8: Ten HBV-carried CBA/CaJ mice were s.c. injected with 800 IU/g of rmIFN-α (Q2D x 43, days 0~84), and then, i.p. injected with 32 μg/g of the anti-PD1 antibody (Q2D x 6, days 86~96).

Clinical Materials:

Ropeginterferon alfa-2b was produced by PharmaEssentia Corporation. It was provided as a prefilled syringe of 500 μg/1.0 mL. Nivolumab (OPDIVO®, Bristol-Myers Squibb Company) was obtained via investigator prescription in the dosage form of 20 mg/2 mL or 100 mg/10 mL per vial.

Study Design:

This clinical study was designed as a Phase I/II trial. The Phase I study aimed to evaluate the safety and tolerability and define the MTD of the sequential administration of ropeginterferon alfa-2b and nivolumab in patients who had received curative surgery of hepatitis B-related HCC. The Phase II trial was designed to further evaluate the safety and prophylactic effect of sequential administration of ropeginterferon alfa-2b and nivolumab at the MTD. Phase I was conducted at the National Taiwan University Hospital (NTUH) Taiwan (approval number: 201710061MIPB). The Phase I study was completed, and Phase II has not yet started.

The sequential administration of ropeginterferon alfa-2b and nivolumab was assessed using a 3+3 dose escalation scheme. Eligible patients were planned to be enrolled into four dose cohorts to receive the six doses of ropeginterferon alfa-2b at the dose of 450 μg once every two weeks, followed by three doses of nivolumab every two weeks at a pre-determined dose level by cohort, i.e., 0.3 mg/kg in Cohort 1; 0.75 mg/kg in Cohort 2; 1.5 mg/kg in Cohort 3, and 3 mg/kg in Cohort 4. Patients were followed up by a site visit for an additional 48 weeks after completion of the study treatment. Disease progression and survival status were planned to be continuously monitored.

Patients:

Patients with HBV-related HCC who received the surgical resection within eight weeks were enrolled. Other major inclusion criteria included positive results for HBsAg, undetectable HBV DNA, compensated liver disease, normal fundoscopic examination, and an Eastern Cooperative Oncology Group Performance Status score of 0 to 1. The major exclusion criteria included HCC that was not related to HBV, vascular invasion of HCC on imaging diagnosis, patients who had undergone transcatheter arterial embolization or chemoembolization, transcatheter arterial infusion, or chemolipiodolization in combination with surgery, and a concurrent active malignancy other than HCC.

5. Conclusions

Our animal data demonstrated a synergistic effect between rmIFN-α and anti-PD1 treatment for HBV suppression or even clearance. This effect was observed in patients with HCC who received the sequential combination therapy of ropeginterferon alfa-2b and nivolumab in our Phase 1 clinical study. Most AEs were mild or moderate. Increased liver transaminase increases were common but not associated with increased bilirubin levels or clinical symptoms. No unexpected AEs were observed. The MTD of sequential combination therapy with ropeginterferon alfa-2b and nivolumab was determined. Further exploration and clinical development of the combination therapy are warranted.

Author Contributions: All authors contributed to the work. P-J. C. and M-C. H. enrolled and treated patients. All authors participated in the writing and review of the manuscript and approved it for publication. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Animal experiments were performed by following the guidelines established by the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine. The phase I/II clinical study was approved by the Institutional Review Board of NTUH (201710061MIPB) and conducted according to the principles of the Declaration of Helsinki for all human experimental investigations. Phase I/II clinical studies were registered at ClinicalTrials.gov (NCT04233840).

Informed Consent Statement: Informed consent was obtained from all participating patients.

Acknowledgments: Data will be available to external researchers upon reasonable request from the investigator and PharmaEssentia.

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Conflicts of Interest: Albert Qin and Chanyen Tsai work for PharmaEssentia Corporation. Pei-Jer Chen served as a consultant for PharmaEssentia Corporation. Other authors declare no conflicts of interest.

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