Late HDV RNA Relapse After Peginterferon Alpha-Based Therapy of Chronic Hepatitis Delta

Benjamin Heidrich,1,2,11 Cihan Yurdaydın,3 Gökhan Kabaçam,3 Boris A. Ratsch,4 Kalliopi Zachou,1,5 Birgit Bremer,1 George N. Dalekos,5 Andreas Erhardt,6 Fehmi Tabak,7 Kendal Yalcin,8 Selim Gürsel,9 Stefan Zeuzem,10 Markus Cornberg,11 C.-Thomas Bock,4 Michael P. Manns,1,2,11 Heiner Wedemeyer,1,2,11 for the HIDIT-1 Study Group

Interferon alpha is the only treatment option for hepatitis delta virus (HDV). Trials investigating the efficacy of pegylated interferon alpha (PEG-IFNa) showed HDV RNA negativity rates of 25-30% 24 weeks after therapy. However, the clinical and virological long-term outcome of HDV-infected patients treated with PEG-IFNa is unknown. We performed a retrospective-prospective follow-up of 77 patients treated for 48 weeks with either PEG-alfa-2a and adefovir (ADV) or either drug alone in the Hep-Net-International-Delta-Hepatitis-Intervention-Study 1 (HIDIT-1) trial. Long-term follow-up data were available for 58 out of 77 patients (75%) with a median time of follow-up of 4.5 (0.5-5.5) years and a median 3 visits per patient. Patients treated with ADV alone received retreatment with PEG-IFNa (48% versus 19%; \( P < 0.02 \)) more often. Hepatitis B virus surface antigen (HBsAg) became negative in six PEG-IFNa-treated patients until the end of long-term follow-up (10%). Sixteen patients tested HDV RNA-negative 6 months after PEG-IFNa treatment who were entered in the long-term follow-up study. Out of these, nine individuals tested HDV RNA-positive at least once during further long-term follow-up, with seven patients being HDV RNA-positive at the most recent visit. Clinical endpoints (liver-related death, liver transplantation, hepatic decompensation, hepatocellular carcinoma) were observed in three PEG-IFNa-treated (8%) and three ADV-treated (14%) patients during posttreatment long-term follow-up with an overall annual event rate of 2.5% (4.9% in cirrhosis). Sequencing confirmed the reappearance of pretreatment virus strains in all cases. Conclusion: Late HDV RNA relapses may occur after PEG-IFNa therapy of hepatitis delta and thus the term sustained virological response should be avoided in HDV infection. The annual posttreatment rate of clinical events in hepatitis delta patients eligible for PEG-IFNa therapy is about 2.5% and 4.9% in patients with cirrhosis. (HEPATOLOGY 2014;60:87-97)

Hepatitis delta is considered the most severe form of chronic viral hepatitis, frequently leading to advanced liver disease with development of cirrhosis and hepatocellular carcinoma (HCC).1-4 Hepatitis delta is caused by infection with the hepatitis delta virus (HDV), which is a defective...
RNA virus using the hepatitis B virus (HBV) surface antigen (HBsAg) as its envelope. Thus, hepatitis delta occurs only in HBsAg-positive individuals. HDV viremia may fluctuate over time and HBV DNA is frequently low or suppressed in hepatitis delta patients. In contrast to HBV DNA, HBsAg levels are almost as high in HDV-infected individuals as in HBV-monoinfected patients, which can be explained by opposing effects of HDV on HBV replication and HBV transcription.

Interferon alpha is the only current treatment option for hepatitis delta. Recent studies investigating the efficacy of pegylated interferon alpha (PEG-IFNa) showed HDV RNA negativity rates of about 15-40% 24 weeks after therapy. In the largest randomized treatment trial in hepatitis delta so far, the Hep-Net-International-Delta-Hepatitis-Intervention-Study 1 (“HIDIT-1”), 28% of patients treated with PEG-IFN-2a with or without adefovir were HDV RNA-negative 24 weeks after the end of 48 weeks of therapy. However, the clinical and virological long-term outcome of HDV-infected patients treated with PEG-IFNa is unknown. Specifically, it is not clear if an HDV RNA response is maintained in all patients for several years after therapy. Even more important, the course of liver disease after PEG-IFNa-based therapy of hepatitis delta needs to be determined. In hepatitis B, prolonged suppression of HBV replication has been linked to a reduced frequency of hepatic decompensations and HCC. Similarly, sustained virological responses (SVRs) to standard therapy of hepatitis C have been associated with lower liver-associated and overall mortality.

Long-term follow-up after treatment of hepatitis delta with conventional interferon alpha suggested that higher doses of interferon alpha therapy could have favorable effects on the course of liver disease. Still, this beneficial effect could not be linked to on-treatment virological responses concerning HDV RNA, as even patients treated with the highest dose of interferon alpha were HDV RNA-positive at the end of therapy when stored sera were retested with more sensitive assays. Thus, it is not clear yet if HDV infection can be completely cured by interferon alpha in the absence of HBsAg seroconversion or if HBsAg clearance is a prerequisite for true recovery from hepatitis delta.

The aim of this study was therefore to investigate the clinical and virological long-term follow-up of hepatitis delta patients treated in the HIDIT-1 study.

Patients and Methods

Patient Population. We performed a retrospective-prospective long-term follow-up study of patients treated in the HIDIT-1, an investigator-initiated, randomized, controlled trial. Ninety patients with chronic hepatitis delta were randomized into three treatment groups and were treated for 48 weeks with additional 24 weeks of posttreatment follow-up. In the first group, 31 patients received peginterferon alfa-2a (180 µg once weekly) plus adefovir (10 mg daily) (Group I); the second group included 29 patients who received peginterferon alfa-2a (180 µg once weekly) plus placebo (Group II); and in the third group 30 patients were treated with adefovir dipivoxil (10 mg daily) alone (Group III). All patients treated within the HIDIT-1 study were eligible for the long-term follow-up study presented here. In Group I a total of 24 patients completed the HIDIT-1 study per protocol. Long-term follow-up data were available for 18 of the 24 patients (75%). Twenty-five patients completed posttreatment week 24 in Group II with 19 patients entering the long-term follow-up study (76%). Finally, in Group III long-term follow-up data were available for 21 (81%) of the 28 patients who completed the initial study according to protocol (Fig. 1).

Data Collection. Data were collected retrospectively every year after the HIDIT-1 trial for 5 years or until patients reached a clinical endpoint or were again...
treated with an interferon-based regimen. Data were collected during routine visits. No additional visits were scheduled.

Several biochemical and hematological parameters were analyzed. These parameters were locally tested during routine visits and were part of the clinical work-up of each patient. In addition, frozen samples were tested with a quantitative HDV RNA assay centrally at Hannover Medical School as previously described. Other virological parameters were tested locally using the Abbott Architect assay for HBsAg and the Roche Cobas TaqMan or other highly sensitive assays for HBV DNA. Biochemical markers included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) as well as bilirubin, creatinine, and albumin. Hematological parameters included platelet counts and prothrombin time. All of these tests were done locally.

**Definition of Clinical Endpoints.** Clinical endpoints were defined as liver-related death, liver transplantation, development of HCC, and hepatic decompensation defined as development of Child-Pugh scores B or C or an increase in Model for End-stage Liver Disease (MELD) scores of five or more points in relation to baseline values. Additionally, treatment or retreatment with interferon-based treatment regimens was considered as an endpoint in line with European Association for the Study of the Liver (EASL) clinical practice guidelines. The combined endpoint of event- and interferon treatment-free survival was also investigated.

**HDV RNA Sequencing.** Reverse transcription of HDV RNA was performed using the OneStep reverse-transcription polymerase chain reaction (RT-PCR) Kit (Qiagen, Netherlands) and HDV-specific primers HDV-04 (GGATGCCCAGGTGGACCG) and HDV-05 (AAGAAGAGTAGCCGGCCG). The HotStar Taq Master Mix (Qiagen, Netherlands) and primers HDV-06 (ATGCCATGCCGACCAAGA) and HDV-07 (GGGGAGCGCGGCGGCGG) were used to amplify a 235-bp fragment covering the region coding for the carboxy terminus of the delta antigen and the less well-conserved region thereafter. Sanger sequencing with PCR primers was used to obtain genomic information of patient-specific isolates.
Statistical Analysis. For statistical analysis as well as for graphic design we used SPSS, v. 15.0.1 (November 2006, SPSS, Munich, Germany) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Quantitative values are indicated in median and statistical differences were assessed using the Student t test. For analyses of qualitative data we used the chi-square test. Differences between clinical outcomes were determined using Cox regression analysis. Differences were considered significant at \( P \leq 0.05 \).

Ethical Approval. The former HIDIT-1 trial was approved by the Ethics Committees of each participating institution in line with the 1975 Declaration of Helsinki. Each patient signed a written informed consent form. The long-term follow-up study was a retrospective study using data and serum samples already collected during routine clinical visits. Retrospective data collection and testing of stored samples was approved by the central coordinating Ethics Committee of Hannover Medical School which was valid for all participating sites in line with the 1975 Declaration of Helsinki.

Results

Long-term follow-up data were available for 58 out of 77 (75%) patients completing the HIDIT-1 trial. The median follow-up time in Group I (PEG-IFNa–ADV) was 4.3 years (range 0.5 to 5.3 years). In Group II (PEG-IFNa monotherapy) and Group III the median follow-up times were 4.4 and 4.9 years (range 1.0–5.4 and 1.3–5.5 years, respectively) (Fig. 1). Overall, median follow-up of three visits per patient was available. Characteristics of patients included in the long-term follow-up study did not differ from the overall HIDIT-1 population (Table 1).

Clinical Long-Term Outcome of Patients Treated in HIDIT-1. The predefined endpoints of this study were event-free survival as well as combined event-free and interferon treatment-free survival. Clinical events occurred during treatment in the initial study in four patients, two of which experienced hepatic decompensation (Group I), one died in Group II, and one in Group III. One patient died from intraperitoneal bleeding as a result of HCC and one after upper gastrointestinal bleeding.

During further long-term follow-up, six additional clinical events occurred including one hepatic decompensation and one liver-related death in Group I, one liver transplantation in Group II, and two liver transplantations as well as one hepatic decompensation in Group III (Table 2), resulting in an overall annual event rate of 2.5%. No statistically significant differences were observed regarding event-free survival between the treatment groups (\( P = 0.73 \), Fig. 2A). Combining patients treated with PEG-IFNa (Group I+II) also revealed a similar clinical long-term course compared to patients treated with adefovir alone (Group III) (\( P = 0.26 \); Fig. 2B). The annual event rates within the different treatment groups ranged from 1.2% to 3.4% (Group I 2.9%, Group II 1.2%, and Group III 3.4%). Patients with cirrhosis defined as ISHAK score 5 or 6 at baseline of the HIDIT-1 trial had an annual event rate after therapy of 4.9% compared to 1.1% of patients without cirrhosis (\( P = 0.019 \) compared to noncirrhosis patients; Fig. 2C). None of the patients who tested HDV RNA-negative at follow-up week 24 experienced a clinical event (Fig. 2D).

Regarding IFN-treatment-free survival, no overall differences were observed. However, patients treated with adefovir alone frequently experienced the IFN-treatment during follow-up compared to patients treated with an interferon-based regimen (\( P = 0.0397 \); Fig. 2E).

Virological Course and Biochemical Disease Activity During Long-Term Follow-up. An overall reduction of biochemical disease activity was observed until the end of long-term follow-up (Table 1). Patients treated with a PEG-IFNa-based regimen had significantly lower median ALT levels than patients treated with adefovir alone (47.0 ± 35.9 versus 99.5 ± 50.3; \( P = 0.002 \)) as well as lower AST values (44.0 ± 20.4 versus 59.5 ± 27.9; \( P = 0.02 \)) (data not shown). ALP levels showed no differences regarding the different treatment regimens.

Patients receiving combination therapy tested HBV DNA-negative significantly more often at the last available timepoint compared with patients treated with adefovir alone (\( P = 0.037 \); Fig. 3A). Interestingly, patients in Group I (PEG-IFNa+ADV) also had low HBV DNA levels or tested HBV DNA-negative more often than patients in Group II (PEG-IFNa; \( P = 0.0096 \)) and Group III (ADV) (\( P = 0.0001 \); Fig. 3B). Overall, patients treated with an interferon-based regimen tested or had HBV DNA levels below 2000 IU/mL more often than patients treated with adefovir monotherapy (\( P = 0.012 \); Fig. 3C).

An HBsAg loss was observed in six individuals (four in group I and two in group II, all HBeAg-negative before therapy). Out of these, three patients (patients 101, 118, and 210) were HDV RNA-negative at follow-up 24 weeks after end of treatment (FU24) of the HIDIT-1 study and remained HDV RNA-negative throughout long-term follow-up. For patients 108 and 225, two HBsAg-negative patients, no data were available regarding HDV RNA levels at FU24. In patient 108 only qualitative testing was performed. The patient stayed positive throughout the whole study.
period including FU24. Patient 225 had high HDV RNA levels at baseline (BL; 3.442.266 copies/mL), showed a steep decline to W24 (negative), and a slight incline to W48 (1.440 copies/mL), while FU24 is missing. One patient (patient 217) suffered a transient late relapse of HDV RNA at year 3 of long-term follow-up. Complete seroconversion to anti-HBs was observed in two out of five patients with anti-HBs data available.

SVR in the HIDIT-1 trial was defined as HDV RNA negativity 24 weeks after end of treatment (FU24VR). Eight patients in Group I, nine in Group II, and none in Group III achieved a FU24VR. Long-term follow-up data were available for 16 FU24VR patients with eight patients in each group. Notably, nine patients (56%) experienced a late relapse defined as HDV RNA-positivity at least once after FU24 virological response. At the last available timepoint, two subjects tested HDV RNA-negative, while seven patients had persistently detectable HDV RNA. Only seven patients (44% of FU24VR and 12% of patients receiving PEG-IFNα in the HIDIT-1 trial) did not show any positive HDV
RNA test results during the entire long-term follow-up period. One patient showed an ALT increase up to 2.4-fold of ULN at year 5 while being HDV RNA-negative. All other patients with long-term virological response had no ALT elevations. In the group of patients with late relapse, two patients had elevated ALT levels during

![Table 2. Baseline Characteristics of Patients With Clinical Events](image)

*Group I PEG-IFNα+ADV; Group II PEG-IFNα; Group III ADV.

1Decompensation: Child-Pugh Score B or C or Increase in MELD Score of 5 or points in comparison to baseline.

2Transplanted due to HCC.

3Transplanted due to hepatic decompensation.

![Fig. 2. Event-free survival. (A) Event-free survival in the three treatment groups. (B) Event-free survival in patients treated with interferon (Groups I and II combined) versus patients treated with adefovir monotherapy. (C) Event-free survival in patients with and without cirrhosis. (D) Event-free survival in patients with a posttreatment week 24 HDV RNA response versus being HDV RNA-positive 24 weeks after PEG-IFNα therapy.](image)
follow-up despite being HDV RNA-negative. Late relapse occurred in both treatment groups containing PEG-IFNa at a similar frequency (Fig. 4A).

**Late HDV RNA Relapses During the Long-Term Follow-up Study.** We next aimed to investigate if FU24VR patients with subsequent detection of HDV RNA during further follow-up had experienced late relapses or if the patients were reinfected with a different HDV strain. HDV RNA was sequenced in samples collected before treatment and during long-term follow-up in seven out of nine patients. Almost identical viral sequences were obtained in all cases, suggesting late relapses rather than *de novo* HDV infections as the cause of redetection of HDV RNA (Fig. 4B; Supporting Fig. 1).

Patients with late relapse showed a more pronounced HDV RNA decline until week 48 of peginterferon treatment compared to patients with long-term virological response (median 2.5.3 ± 1.6 versus −1.7 ± 1.7 log IU/mL; *P* = 0.016) (Fig. 4C). Additionally, patients with long-term virological response were significantly more often male (86% versus 33%; *P* = 0.0361) and tested HBV DNA-negative before therapy more often (86% versus 22%; *P* = 0.0117) than individuals suffering from late relapse. Regarding HBsAg levels, a decrease of −1.6 log IU/mL until week 48 was observed in patients with long-term virological response, whereas in individuals with late relapse HBsAg levels showed an increase of 0.1 log IU/mL.

Median ALT levels showed no significant differences during HIDIT-1 but seven out of seven (100%) patients with long-term virological response showed a reduced biochemical disease activity indicated by a decline of ALT levels at F24 of at least 30 IU/mL, whereas only four out of nine (44%) individuals with late relapse showed lower ALT levels (*P* = 0.0174). Detailed characteristics of patients with late relapse and long-term virological response are presented in Table 3 and Supporting Tables 1 and 2.

**Discussion**

International treatment guidelines recommend IFNa-based therapies for patients with hepatitis delta, and HDV RNA negativation is considered an important endpoint of treatment. However, the durability of an HDV RNA response after PEG-IFNa therapy was unknown and the clinical long-term outcome after treatment has thus far not been studied. Investigating patients treated in the largest investigator-initiated randomized treatment for hepatitis delta so far, we demonstrate here: 1) that the overall posttreatment clinical-event rate in HDV-infected patients fulfilling inclusion criteria for interferon therapies was relatively low, with only 2.5% per year; that 2) PEG-IFNa therapy was associated with an improved biochemical disease activity in hepatitis delta but not with a reduction of hepatic events until year 5 of follow-up; that 3) an
HBsAg loss occurred in 10% of PEG-IFNa-treated patients; and 4) that a posttreatment week 24 HDV RNA response may not be durable as late relapses occurred in more than 50% of patients. However, a late relapse was not associated with the development of clinical complications during the observation period.

Hepatitis delta is clearly the most severe form of chronic viral hepatitis.\textsuperscript{1,2} Recent long-term cohort studies following patients for up to 28 years showed high cumulative rates of hepatic decompensation and liver-related morbidity and mortality.\textsuperscript{26-28} Even though the annual event rate observed in this posttreatment follow-up study seems to be relatively low, the findings are well in line with the previous cohort studies. It has to be considered that patients included in the HIDIT-1 study had to fulfill standard inclusion and exclusion criteria of PEG-IFNa-based treatment trials. Individuals with comorbidities potentially contributing to disease progression were therefore excluded. Moreover, patients with more advanced cirrhosis were not studied. As expected, clinical events were twice as frequent and also occurred earlier in patients who already had liver cirrhosis before treatment than in patients without liver cirrhosis at baseline.\textsuperscript{22,29}

A crucial question for the clinical management of hepatitis delta is if PEG-IFNa treatment is associated with an improved clinical long-term outcome. Successful therapy of both chronic hepatitis B and chronic hepatitis C has been clearly linked to reductions in frequencies of hepatic decompensation, HCC, and liver-related mortality.\textsuperscript{15-21} These beneficial effects correlated
with virological responses as determined by HBV DNA suppression or HCV RNA eradication. Even though usage of high doses of recombinant IFNa has also been associated with a better long-term survival in a smaller trial in hepatitis delta, this potentially clinical effect could not be linked to virological responses concerning HDV RNA negativation.\textsuperscript{22} There is indirect evidence that clearance of HDV RNA from blood may be associated with an improved outcome as an HDV RNA-negative status was associated with lower mortality in the Milan cohort.\textsuperscript{26} After a median follow-up time of 4.5 years, PEG-IFNa therapy was not associated with an improved overall clinical long-term outcome in our study. However, a significant reduction of biochemical disease activity was observed in PEG-IFNa-treated patients which potentially translates into lower disease progression rates beyond year 5 posttherapy. The clinical benefit of IFNa therapy in the previous study by Farci et al. also became evident only after 10 years of follow-up.\textsuperscript{22} Furthermore, it should be stressed that not a single patient in our study with a posttreatment week 24 HDV RNA response experienced a clinical event during subsequent follow-up, indicating that even transient virological response to PEG-IFNa therapy could be of clinical importance in hepatitis delta. This is also suggested by a recent retrospective investigation from the Hep-Net-Greece cohort which similarly linked IFNa-based therapies with an improved overall outcome in hepatitis delta.\textsuperscript{30} Nevertheless, more comprehensive studies are needed to investigate this important question in more detail.

An unexpected finding of this study was the high rate of late HDV RNA relapses after an initial posttreatment response. Even if the detection of HDV RNA during long-term follow-up was only transient in some patients, almost 50% of patients classified as responder patients in the HIDIT-1 trial were HDV RNA-positive at the last long-term follow-up visit and these relapses were also associated with ALT increases indicating disease activity in at least four subjects. Importantly, reinfections with different viruses were excluded as sequencing revealed almost identical HDV strains in all patients with pre- and posttreatment samples available. HDV reinfection would have been theoretically possible as this has been demonstrated in chimpanzees.\textsuperscript{31} The observation of HDV relapse challenges the conventional posttreatment week 24 investigation as a valid virological endpoint in IFNa-based treatment trials for hepatitis delta. While a SVR-24 can be considered as a virological “cure” in HCV infection,\textsuperscript{32} we would suggest that the term “sustained” virological response should be avoided concerning HDV RNA. The clinical observation of this trial implies that HDV may be much more difficult to eradicate from the body than HCV. Indeed, intrahepatic long-term detection of the hepatitis delta antigen has been reported even in the absence of HBsAg in patients after liver transplantation.\textsuperscript{33,34} In addition, intrahepatic HDV RNA may escape detection by innate immunity due to its intranuclear localization, comparable cccDNA in HBV infections, and thus contributes to long-term persistence despite apparent eradication.\textsuperscript{9} Therefore, ongoing and future trials must pursue long-term follow-up of patients after termination of therapy.

Immune responses are likely to play a key role in the control of HDV infection.\textsuperscript{35} This is supported by the fact that most patients without late HDV RNA relapses became HDV RNA-negative only after PEG-IFNa therapy was stopped, which indicates that host immunity caused suppression of HDV replication. The enhanced “immune status” of patients able to achieve long-term HDV RNA responses may also be reflected by a better ability of these individuals to control HBV since HBV DNA was lower in long-term responders. In contrast, HDV relapses occurred even in patients who became HDV RNA-negative early during antiviral therapy, which is again in contrast to the current treatment dogma in hepatitis C, where early virological responses are considered to be predictive of long-term outcome.\textsuperscript{32} It will therefore be very difficult to use on-treatment HDV RNA response kinetics to develop stopping rules as previously suggested\textsuperscript{12} or even to individualize treatment duration of PEG-IFNa therapy in hepatitis delta.
An obvious consequence of the finding of late HDV RNA relapse is that HBsAg loss should be considered as the primary and ideal virological endpoint in the treatment of hepatitis delta. However, this endpoint is only achieved by a minority of patients. Merely 10% of individuals treated with PEG-IFNa became HBsAg-negative during long-term follow-up in this trial, which is generally in line with previous studies showing HBsAg losses between 0% and 14%.\textsuperscript{36-38} Future strategies to enhance HBsAg reduction and clearance could be combination therapies of PEG-IFNa and certain HBV polymerase inhibitors,\textsuperscript{14,39,40} the combination of PEG-IFNa with novel HBV entry inhibitors which are in clinical development,\textsuperscript{41,42} or simply extension of treatment duration beyond 48 weeks of therapy. Several case reports demonstrated HBsAg loss in hepatitis delta patients after prolonged IFNa-based therapies for up to 12 years.\textsuperscript{43,44} A treatment trial investigating prolonged PEG-IFNa therapy of hepatitis delta for 96 weeks with or without tenofovir has been initiated. The so-called HIDIT-2 trial (www.clinicaltrials.gov) and posttreatment week 24 study results are expected to be available during 2014.

The study has obvious limitations. Even though we report here the largest long-term follow-up after an interferon-based therapy of hepatitis delta so far, the overall number of patients studied is still limited. It is also quite possible that during longer follow-up even more patients would experience a reappearance of HDV RNA. The higher retreatment rate in Group III is certainly biased, as investigators were obviously considering PEG-IFNa in patients who did not receive this therapeutic option during the HIDIT-2 trial. Moreover, it needs to be ascertained through extended observation if late-HDV RNA relapses are in fact clinically benign or if disease progression with the development of clinical events is just delayed for some time.

In conclusion, the annual posttreatment rate of clinical events in hepatitis delta patients eligible for IFNa therapy is about 2-3%. A long-term monitoring after therapy is recommended even in patients who test HDV RNA-negative 6 months after PEG-IFNa-based treatment, as late relapses may occur. However, it remains to be determined if even transient suppression of HDV replication improves the clinical long-term outcome, as not a single patient in our study with a posttreatment week 24 HDV RNA response experienced a clinical event. Future studies should aim to increase HBsAg losses, as this should be the preferred virological endpoint in the treatment of hepatitis delta.

References

HEPATOTOLOGY, Vol. 60, No. 1, 2014


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website.