Table 2. Baseline viral and host characteristics among genotype-1b ineligible/intolerant patients and their virologic outcome.

Patient	*IL28B* GT	HCV RNA, $\log_{10}$ IU/ml	NS5A polymorphism(s)[a]	NS3 polymorphism(s)[a]	Virologic outcome
P-22	CC	7.1			SVR
P-23	CC	6.9	A92T	Q80L-S122G/S	SVR
P-24	CC	6.6	L28M-R30L-Q54H-A92T	Q80L-S122S/G	D/C at Wk12 due to AE; SVR
P-25	CT	6.8	L31M/L-Y93H/Y		VBT (Wk16)
P-26	CC	5.3			SVR
P-27	CC	6.9	Q54H-Y93H/Y	T54S	SVR
P-28	CC	6.8	Y93H/Y	Q80L	SVR
P-29	CT	6.7	Q54Y-Y93H/Y	Q80L	VBT (Wk16)
P-30	CT	6.7	Q54H		SVR
P-31	CC	6.6	P58S/P-Y93Y/H	S122G	Relapse (FU Wk12)
P-32	CT	6.7	P58L	S122G	Relapse (FU Wk4)
P-33	CT	5.2	Q54H-Q62P/S		D/C at Wk12 due to patient request; SVR
P-34	CC	6.6		Q80L	SVR
P-35	CC	6.4	Q54H-Q62E/A-A92T		SVR
P-36	CC	7.1		S122S/C	Relapse (FU Wk4)
P-37	CC	6.6	Y93H		Relapse (FU Wk4)
P-38	CC	7.5		S122T	SVR
P-39	CC	5.1	R30Q/R		SVR
P-40	CC	6.8	Q54H-A92A/T	Q80L	D/C at Wk8[b]
P-41	CC	6.0		S122G	SVR
P-42	CC	6.5	A92T		SVR
P-43	CT	7.0	Q54Y-Y93H	S122G	VBT (Wk10)

[a]All NS3 and NS5A amino acids were examined with focus on polymorphisms at positions known to be associated with resistance to NS3 protease inhibitors (36, 43, 54, 55, 77, 78, 79, 80, 122, 123, 138, 155, 156, 158, 168, 170, 175) and NS5A inhibitors (21, 23, 24, 28, 30, 31, 32, 54, 58, 62, 92, 93). When a mixture of substitutions is indicated, the most predominant is identified first.

[b]Treatment discontinued at patient request; subsequently lost to follow-up.

AE, adverse event; D/C, discontinued; FU, follow-up; GT, genotype; HCV, hepatitis C virus; SVR, sustained virologic response; VBT, viral breakthrough; Wk, week.

achieving SVR compared with 6.8 (0.3) $\log_{10}$ IU/ml among patients experiencing virologic failure. However, four of six patients with the *IL28B* CT allele subsequently failed treatment (three breakthroughs, one relapse) *vs.* only three of 16 patients with *IL28B* CC (all relapsed).

Genotypic analysis of patients with viral breakthrough. Treatment-emergent RAVs were assessed through post-treatment week 48 in the three patients with virologic breakthrough (Table 3).

Patient P-25: This patient had an *IL28B* CT genotype with a baseline HCV RNA level of 6.8 $\log_{10}$ IU/ml and a linked baseline NS5A-L31M-Y93H/Y polymorphism. Despite undetectable HCV RNA by week 4 (Fig. 4A), viral breakthrough occurred at week 16, associated with high-level resistance to both DCV (NS5A-L31M-P58A-Y93H; 65,000-fold) and ASV (D168A; ~120-fold in GT1b). Other minor variants detected at baseline by clonal analysis (NS5A-Q62R, -A92T) were not present at breakthrough. NS5A variants present at the end of therapy persisted through follow-up week 48, and, although P58A had largely changed to P58G (73% of 33 clones, Fig. 5A) by week 36, a similar ratio of P58G to A was detected at follow-up week 48. By contrast, NS3-D168A had mostly been replaced by wild type at week 48 (83% of 64 clones).

Patient P-29: This patient had an *IL28B* CT genotype, with a baseline HCV RNA level of 6.7 $\log_{10}$ IU/ml and a pre-existing linked NS5A-Q54Y-Y93H/Y and NS3-Q80L (Fig. 5B). Undetectable HCV RNA by week 3 was followed by viral breakthrough at week 16 (Fig. 4A) associated with NS5A-L31M-Q54Y-Y93H (6467-fold DCV resistance) and NS3-Q80L-D168V (~280-fold ASV resistance). These RAVs remained stable through 48 weeks post-treatment.

Patient P-43: This patient had an *IL28B* CT genotype with a baseline HCV RNA level of 7.0 $\log_{10}$ IU/ml, and a pre-existing NS5A-Q54Y-Y93H variant (Fig. 5C). HCV RNA was undetectable at week 2, and breakthrough occurred at week 10 (Fig. 4A), which was associated with a linked NS5A-L31M-Q54Y-Y93H variant (Fig. 5C; 6467-fold DCV resistance) and an NS3-D168V variant (~270-fold ASV resistance). Again, NS5A variants remained stable through week 48 post-treatment, while NS3-D168V was replaced by wild type (100% of 60 clones).

For the three patients experiencing viral breakthrough, DCV and ASV trough exposures were less than drug levels required to achieve a 90% effective concentration (EC_{90}) value against emergent RAVs (Table 3).

Genotypic analysis of patients experiencing post-treatment relapse. Four ineligible patients, with undetectable HCV RNA at the end of treatment, experienced relapse (Fig. 4B). Resistance polymorphisms through week 48 off-treatment are shown in Table 3. Baseline polymorphisms associated with resistance were not detected in two patients (P-32 and P-36), but both displayed post-relapse resistance by follow-up weeks 8 and 4, respectively. Patient P-32 relapsed with NS5A-L31M-P58L-Y93H (8300-fold DCV resistance) and NS3-D168V (270-fold ASV resistance).

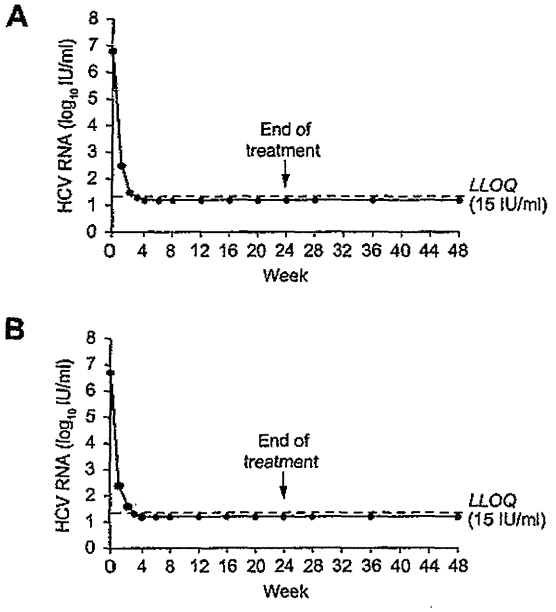

Fig. 1. HCV RNA levels among genotype-1b null responders. Treatment was initiated with (A) asunaprevir 600 mg BID or (B) asunaprevir 200 mg BID, in combination with daclatasvir 60 mg QD. Individual patient HCV RNA levels are shown in grey. Mean HCV RNA levels are shown in black. *BID*, twice daily; *HCV*, hepatitis C virus; *LLOQ*, lower limit of quantitation; *QD*, once daily.

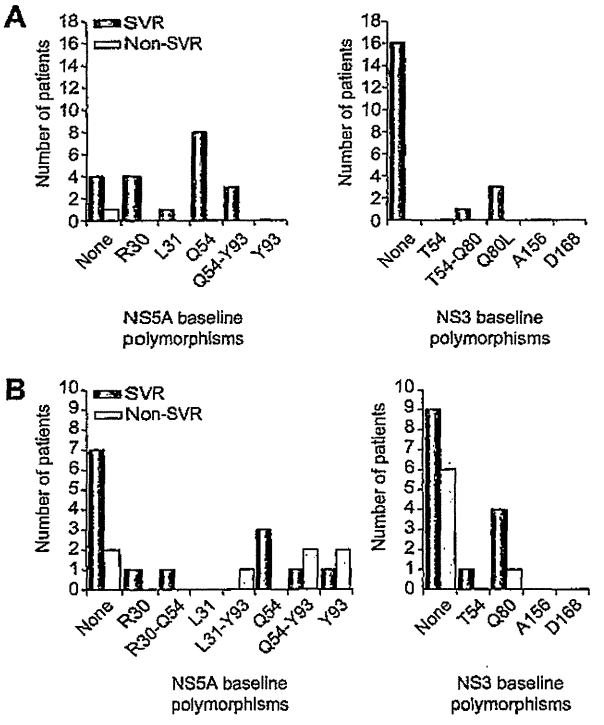

Fig. 2. Impact of baseline polymorphisms associated with resistance on virologic outcome among genotype-1b (A) null responders or (B) ineligible/intolerant patients. The ineligible/intolerant analysis excludes one patient (P-40) who discontinued therapy and was subsequently lost to follow-up. *SVR*, sustained virologic response.

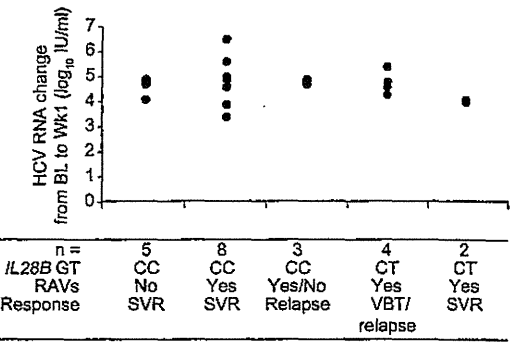

Fig. 3. Early (week 1) declines in HCV RNA were similar among PegIFN-α/RBV ineligible or intolerant patients with and without baseline polymorphisms associated with resistance, virologic failure, and *IL28B* CT genotype. *BL*, baseline; *GT*, genotype; *HCV*, hepatitis C virus; *RAV*, resistance-associated variant; *SVR*, sustained virologic response; *VBT*, viral breakthrough.

Patient P-36 relapsed with an NS5A-L31V/M-Y93H genotype (L31V-Y93H: 14,789-fold DCV resistance *vs.* L31M-Y93H: 7105-fold) [13] and NS3-D168V. The remaining two patients had detectable NS5A-Y93H at baseline (24-fold DCV resistance) and additional substitutions at NS5A-L31 and NS3-D168 were detected after relapse. Patient P-31 displayed NS5A-L31M-Y93H (7105-fold DCV resistance) [13] and NS3-D168A (~120-fold ASV resistance); patient P-37 relapsed with the same NS5A-L31V/M-Y93H and NS3-D168V, as described for patient P-36.

Baseline HCV RNA and *IL28B* genotype did not appear to influence relapse; three of four relapse patients were *IL28B* CC genotype, and baseline HCV RNA was not appreciably higher than for those with SVR (mean HCV RNA [SD]: 6.8 [0.4] *vs.* 6.4 [0.7] log_{10} IU/ml, respectively).

Changes in the DCV resistance pattern present at relapse through follow-up week 48 were seen in three of four relapsers, with Y93H changing to wild type (100% of 68 clones) in patient P-32. Clonal analysis of the baseline sequence revealed the presence of Y93H as a minor species (~2%; 1/61 clones). Genotypic changes resulting in a lower level of phenotypic resistance (L31V-Y93H to L31M-Y93H) were detected in patients P-36 and P-37. NS3 substitutions observed at relapse were not detectable by population sequencing by follow-up week 36. The D168V substitution detected in patient P-37 was replaced by D168E (78-fold ASV resistance [19]) at follow-up weeks 36 and 48. As with the patients who experienced virologic breakthrough, ASV and DCV trough values in the three drug-compliant patients who relapsed were less than the observed EC_{90} values for the respective RAVs.

Discussion

This study assessed resistance and virologic failure in a difficult-to-treat population of null responders and PegIFN-α/RBV ineligible/intolerant patients treated with the dual oral combination of DCV and ASV. Overall, 77% achieved an SVR [11], with all viral breakthroughs and post-treatment relapses occurring in the ineligible/intolerant subpopulation. It is possible that pharmacokinetics may have played a role in these failures, since patients experiencing failure had DCV and/or ASV trough values below median or documented non-compliance [11]. However, since

Table 3. Emergence of resistance-associated variants among genotype-1b ineligible/intolerant patients experiencing viral breakthrough or relapse.

Patient	Time point	DCV/ASV C_{trough} range, nM	NS5A RAVs				DCV EC_{90}, nM	NS3 RAVs			ASV EC_{90}, nM
			L31	Q54	P58	Y93		Q80	S122	D168	
VBT patients											
P-25	BL		M/L	-	-	H/Y	<137	-	-	-	
	Wk16 (VBT)		M	-	A	H	>1000	-	-	A	540
	Wk20		V	-	A	H		-	-	A	
	Wk24	190-261/25-41	M	-	A	H		-	-	A	
	FU Wk4		M	-	A	H		-	-	A	
	FU Wk36		M	-	G	H	>5000	-	-	D/A	
	FU Wk48		M	-	G/A	H		-	-	-	
P-29	BL		-	Y	-	H	0.04	L	-	-	1.6
	Wk16 (VBT)		ND	ND	ND	ND	ND	ND	ND	ND	ND
	Wk20	116-198/18-33	M/V	Y	-	H	750	L	-	V	55
	FU Wk4		M	Y	-	H		L	-	V	
	FU Wk36		M	Y	-	H		L	-	V	
	FU Wk48		M	Y	-	H		L	-	V	
P-43	BL		-	Y	-	H	0.49	-	G	-	2.8
	Wk10 (VBT)		M	Y	-	H	435	-	G	V	279
	FU Wk4	243/69	M	Y	-	H		-	G	V	
	FU Wk36		M	Y	-	H		-	G	-	
	FU Wk48		M	Y	-	H		-	G	-	
Relapse patients											
P-31	BL	573-620/ 153-327	-	-	S/P	Y/H	0.02	-	G	-	
	FU Wk16		ND					-	-	A	
	FU Wk24		M	-	-	H	351	-	G	-	
	FU Wk36		-	-	-	-		-	-	-	
	FU Wk48		-	-	-	-		-	-	-	
P-32	BL	151-306/19-42	-	-	L	-	0.004	-	G	-	
	FU Wk8		M	-	L	H		-	G	V/D	
	FU Wk12		M	-	L	H	543	-	G	-	
	FU Wk36		M	-	L	-	1.5	-	G	-	
	FU Wk48		M	-	L	-		-	G	-	
P-36	BL	138/26	-	-	-	-		-	-	-	
	FU Wk8		V/M	-	-	H		-	-	V	1190
	FU Wk12		V	-	-	H	349	-	-	-	
	FU Wk24		M/V	-	-	H		-	-	V/D	
	FU Wk36		M	-	-	H	137	-	-	-	
	FU Wk48		M	-	-	H		-	-	-	
P-37	BL	75-134/40-93	-	-	-	H	0.49	-	-	-	
	FU Wk8		V	-	-	H		-	-	V	
	FU Wk12		V/I	-	-	H		-	-	V	
	FU Wk24		M	-	-	H		-	-	V	
	FU Wk36		M	-	-	H		-	-	E/D	
	FU Wk48		M	-	-	H		-	-	-	

When a mixture of substitutions is indicated, the most predominant is written first. ASV-resistant variants conferred no cross-resistance to DCV and *vice versa* in a replicon assay. Dashes indicate consensus with control sequence GT1b (Con1).

ASV, asunaprevir; BL, baseline; DCV, daclatasvir; EC_{90}, 90% effective concentration; FU, follow-up; ND, not determined as multiple amplifications failed; RAV, resistance-associated variant; VBT, viral breakthrough; Wk, week.

most patients with troughs below the median achieved SVR, the influence of drug exposure is hard to assess.

NS5A-Y93H was identified as the predominant polymorphism at baseline in all three patients with viral breakthrough and in two of the four patients with relapse. However, three null responders and two ineligible/intolerant patients also had a pre-existing NS5A-Y93H polymorphism and all achieved SVR, making the significance of Y93H alone, for response in the broader patient population, difficult to assess. Furthermore, where Y93H polymorphisms existed at baseline, their effects on

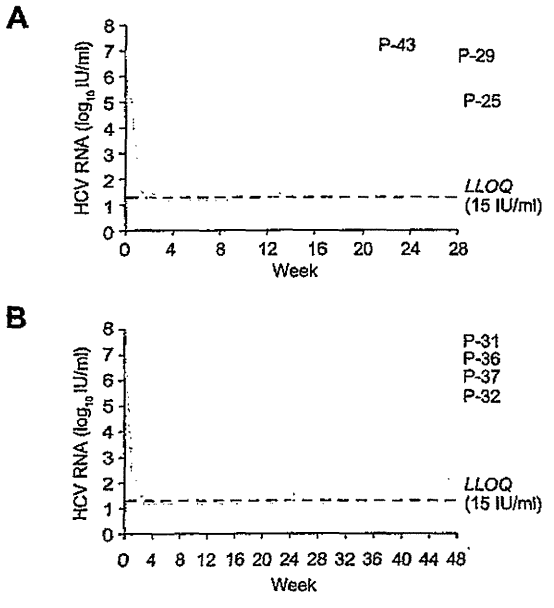

Fig. 4. HCV RNA levels on-treatment and during post-treatment follow-up for genotype-1b ineligible/intolerant patients experiencing (A) viral breakthrough or (B) relapse. Solid lines indicate on-treatment period. Dashed lines indicate post-treatment follow-up. HCV, hepatitis C virus; LLOQ, lower limit of quantitation.

DCV inhibition were minimal (Y93H EC_{50} = 49 pM [6]) compared with C_{trough} values that ranged from 75 to 620 nM. The global prevalence of NS5A-Y93H is approximately 4%, based on data from the Los Alamos database [20] and unpublished data from nine DCV studies, and is approximately 11% in other recent Japanese DCV studies [21], which is considerably lower than the 23% (10/43) prevalence observed in this study. Further analysis of DCV study data indicates that Y93H pre-exists at higher levels in patients infected with GT1b (10%) than GT1a (1%); however, the link with *IL28B* is not so clear given that most failures to date with DCV have been observed in GT1a patients with no baseline Y93H. Other polymorphisms observed at a higher frequency among this GT1b population included NS3-Q80L (~19%, 8/43) *vs.* Q80K, which has been observed more frequently in GT1a populations [18,19].

Baseline HCV RNA did not appear to influence virologic response in either population, and response was too rapid to allow successful genomic sequencing after 1 week of treatment. ASV dose (600 mg or 200 mg twice daily) did not impact the initial decline in HCV RNA in null responders, and the *IL28B* CT allele, present in 86% (18/21) of null responders, did not prevent patients from achieving a very high (90%) SVR. By contrast, although only 27% (6/22) of ineligible/intolerant patients were *IL28B* CT, this genotype was present in all three viral breakthroughs and one of four relapses. While *IL28B* genotype is known to influence response to PegIFN-α/RBV, its apparent impact on virologic suppression in alfa-sparing regimens is unexpected. However, given the small number of patients, any such correlation will require evaluation in a larger dataset.

The emergent RAVs at viral breakthrough or relapse (signature NS5A-L31 and -Y93 substitutions for DCV and NS3-D168 substitutions for ASV) were similar to observations from other

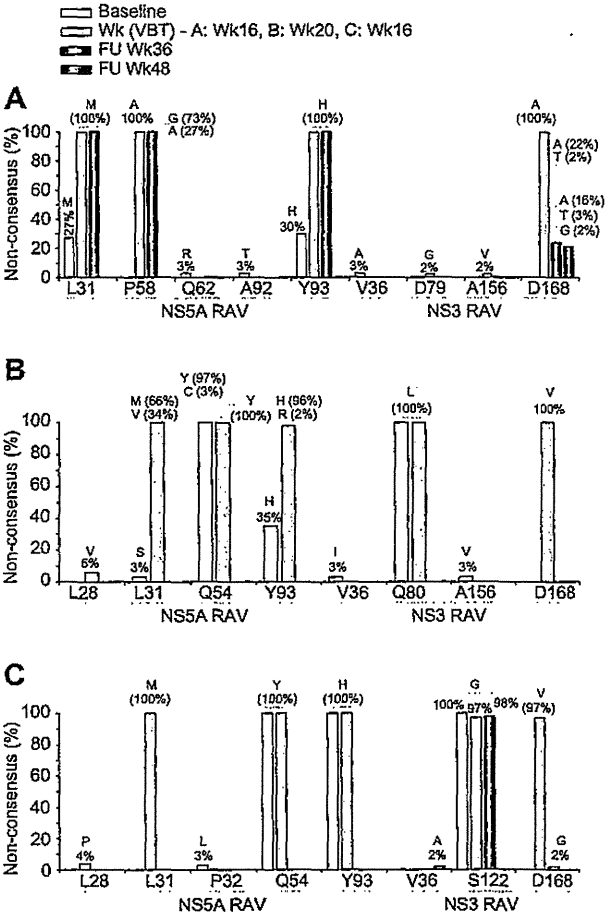

Fig. 5. Clonal analysis of NS3 protease and NS5A resistance-associated variants in patients experiencing virologic breakthrough. (A) Patient P-25. NS5A RAV: baseline 30 clones; Wk16 39 clones; FU Wk36 33 clones; FU Wk48 not performed (no change from FU Wk36 by population sequencing). NS3 RAV: baseline 32 clones; Wk16 41 clones; FU Wk36 56 clones; FU Wk48 63 clones. (B) Patient P-29. NS5A RAV: baseline 37 clones; Wk20 50 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). NS3 RAV: baseline 34 clones; Wk20 47 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). (C) Patient P-43. NS5A RAV: baseline 32 clones; Wk10 47 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). NS3 RAV: baseline 31 clones; Wk10 32 clones; FU Wk36 103 clones; FU Wk48 60 clones. FU, follow-up; VBT, viral breakthrough; RAV, resistance-associated variant.

clinical studies of DCV, and from *in vitro* GT1b replicon resistance studies with ASV [19], although this study represents the first demonstration of emergent clinical ASV resistance. It is possible that signature resistance variants to both DCV and ASV pre-existed as minor species, and subsequently enriched by selective pressure, as predicted by viral kinetic modeling [22]. Although a combination of these NS3 and NS5A variants was not detected by clonal sequencing at baseline, their low-level pre-existence cannot be ruled out. However, assessment of minor NS3 plus NS5A variants from the same RNA sequence is currently not feasible using available deep-sequencing technologies. Nevertheless, additional studies to assess the presence and dynamics of minority baseline variants under drug selection are indicated.

Interestingly, ASV-resistant NS3-D168 substitutions generally decayed during the off-drug follow-up period, implying a lack of replicative fitness relative to wild type, in the absence of selective drug pressure. Indeed, a reduction in replicative fitness has been observed for D168 variants in replicons [19]. Neither of the secondary variants associated with D168V in this study (Q80L or S122G) had an impact on fitness *in vitro* (replication capacity similar or higher than that observed for parental GT1b [Con1] replicon), with both double variants possessing replicative capacities similar to D168V alone [19]. However, clonal analysis indicated that ASV-resistant variants were still detectable in some post-treatment samples as minority species, although not detectable by population sequencing. Deeper sequencing techniques will be required to fully establish the dynamics of decay and whether ASV-resistant strains remain enriched for long periods relative to baseline. Since the re-treatment of patients with prior NS3 protease inhibitor failure has only been assessed in small studies [23], it is not clear whether these NS3 RAVs will form a stable minority capable of rapid overgrowth on re-treatment. By contrast, NS5A variants associated with DCV resistance were observed to be linked and relatively stable through at least 48 weeks post-treatment, although change of DCV-resistance substitutions was noted in four of seven patient samples. As described above, the prevalence of the NS5A variant Y93H, which confers low level resistance to DCV, is approximately 10% in the general HCV GT1b population. Linked NS5A RAVs conferring high level resistance to DCV are less prevalent (<1%). While NS3 RAVs (substitutions at positions V36, T54, R155, or D168) associated with first-generation protease inhibitors have been reported to be present at ⩽2.7% by population sequencing [5,24], emergent NS3 RAVs have been shown to persist for up to 4 years in long-term follow-up studies [25]. Therefore, longer-term studies are indicated to assess what, if any, replicative impairment is conferred by these linked NS5A changes and how long these potentially transmissible drug-resistant strains persist without DCV selection pressure.

In conclusion, high response rates were achieved in this small Japanese study comprising GT1b null responders and PegIFN-α/RBV ineligible/intolerant patients with limited treatment options. Among patients experiencing virologic failure, ASV- and DCV-resistant substitutions emerged together at the time of failure, which were similar to those reported previously. An analysis of persistence demonstrated that DCV-resistant substitutions appeared to have greater fitness over the duration of the study. A loose association with a baseline NS5A polymorphism on virologic outcome was observed; however, further data from larger studies are required. Consequently, a greater understanding of the role and dynamics of pre-existing, emergent, and persistent resistance variants to DCV and ASV will be sought from the planned phase 3 global studies of this combination.

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Conflict of interest

K Chayama has received research grants and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Mitsubishi Tanabe Pharma, Daiichi Sankyo, Toray Industries, Otsuka Pharmaceutical Company, and GlaxoSmithKline KK. Hiroki Ishikawa, Hideaki Watanabe, Wenhua Hu, Dennis Hernandez, Fei Yu, and Fiona McPhee are employees of Bristol-Myers Squibb. All other authors have no conflicts to report.

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Original Article

Randomized controlled trial of a new procedure of radiofrequency ablation using an expandable needle for hepatocellular carcinoma

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Aim: To evaluate the efficacy of a new ablation procedure for the stepwise hook extension technique using a SuperSlim needle for radiofrequency ablation (RFA) treatment of hepatocellular carcinoma (HCC), a randomized controlled trial was performed.

Methods: Thirty patients with HCC measuring 20 mm or less were randomly treated with a conventional four stepwise expansion technique (group 1) and the new stepwise expansion technique (group 2; the electrode was closed in the shaft after the same three steps of the conventional procedure and then fully extended). All patients underwent the RFA procedure using a 10-hook expandable electrode of 17-G diameter (LeVeen SuperSlim 30 mm). We compared the ablation time, required energy and ablated lesions in the two groups.

Results: The long and short diameters of RFA-induced necrosis were significantly larger in group 2 (37 and 28 mm) than group 1 (30 and 26 mm, $P = 0.001$ and $=0.045$, respectively). Irregular and small needle expansion resulting in the parachute-like or irregularly shaped ablated zone was observed in more cases in group 1 than in group 2. The new technique made all tines expand uniformly and largely, which produced a near-oval ablated zone of which the long axis is perpendicular to the needle shaft.

Conclusion: The two kinds of stepwise procedures allow the selection of a more suitable procedure according to the tumor size and shape in each RFA.

Key words: expandable needle, hepatocellular carcinoma, radiofrequency ablation, randomized controlled trial

INTRODUCTION

PERCUTANEOUS TREATMENT INCLUDING radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) is often used for small-size hepatocellular carcinoma (HCC) because it is less invasive than surgical therapy. RFA has become the first-choice local treatment because of the excellent outcome; the efficacy of RFA in HCC tumors measuring less than 2 cm in diameter is similar to that of PEI but it requires fewer treatment sessions, and the efficacy in HCC tumors of more than 2 cm in diameter is better than with PEI.[1] In addition, RFA is also more cost-effective than surgical resection of small HCC.[2] With three commercially-available RFA apparatuses – the radiofrequency tumor coagulation system (RTC system; Boston-Scientific, Natick, MA, USA), radiofrequency interstitial thermal ablation system (RITA; AngioDynamics, Latham, NY, USA) and cool-tip RF system (Valleylab, a division of Tyco Healthcare Group, Boulder, CO, USA) – the volume ablated during one RFA session is of a diameter less than 3.0–4.0 cm, except in ablation with the Starburst XL RFA device (RITA).[3] RFA therapy is currently restricted to tumors measuring less than 3 cm. In this regard, previous studies reported that the necrotic area could be enlarged by saline injection prior to RFA,[4,5] combination of RFA with PEI,[6,7] RFA with ethanol lipiodol injection,[8] RFA with transcatheter arterial embolization[9] and RFA with transient arterial obliteration.[10–12]

Among the above three RFA apparatuses, the RTC system and RITA have adopted the use of expandable needles. We reported previously the efficacy of the

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stepwise hook extension technique for RFA therapy of HCC.[13] The technique allows rapid roll-off at lower power and lower energy and reduces any possible increase in intra-tissue pressure that may cause scattering of intrahepatic metastasis.[14–17]

A more slender expandable needle has been developed (17-G, SuperSlim; Boston Scientific, Natick, MA, USA) for easier and safer insertion into the liver. However, insertion of the slim needle into the liver tissue could result in deformation of the needle and hence possible reduction of the size of the ablated area. To overcome this shortfall, we designed a new technique involving full re-expansion after stepwise extension, to ensure full expansion of the needle. We have already reported the experimental study using healthy pig livers *in vivo* to show that this technique can produce a larger necrotic zone than the conventional stepwise procedure.[18]

The aim of this study was to evaluate the efficacy of the new ablation procedure for the stepwise hook extension technique for RFA therapy of HCC of a patient with cirrhosis or without cirrhosis in a randomized controlled trial.

METHODS

Patients and tumors

FROM NOVEMBER 2006 to March 2010, 30 consecutive patients who met the following criteria were enrolled in this study: (i) HCC confirmed either histopathologically or radiologically; and (ii) diameter of the hepatic tumor of no more than 20 mm. They included 20 men and 10 women, with a median age of 57 years (range, 43–73). Seventeen patients were with cirrhosis and the other 13 were without cirrhosis. Table 1 lists the clinical background of patients of both groups. There were no significant differences between the groups.

A typical hypervascular HCC was diagnosed by typical hypervascular stain on digital subtraction angiography. In addition, one of the following three criteria was used to diagnose a tumor as a well-differentiated HCC: (i) histopathological diagnosis as well-differentiated HCC; (ii) hypo-enhanced lesion on computed tomography (CT) during hepatic arteriography (CTHA) and hypoperfusion on CT during arterial portography (CTAP); and (iii) hypo-enhanced lesion on the equilibrium phase of dynamic CT or hypo-perfused lesion on CTAP and hypointense on the hepatocyte-specific phase of multiple resonance imaging (MRI) using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) (Primovist; Bayer Schering Pharma, Osaka, Japan). A total of 30 patients were treated by the RFA protocol.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital and a signed consent form was obtained from each patient.

RFA protocol

We used the RTC system comprising a slim expandable needle (30 mm, 17-G LeVeen needle, SuperSlim), which consists of 10 expandable monopolar array electrodes, and the RF3000 generator, with a maximum power output of 120 W, and four electrode pads placed on the patient's skin. Instead of using the standard method recommended by the manufacturer, we adopted two types of stepwise hook extension techniques.[18] Patients were randomly divided into two groups based on the RFA protocol used. In group 1, after placing the needle electrode shaft into the tumor with the array retracted, using real-time ultrasound guidance, the electrode tines were expanded to a quarter, a half, three-quarters of the length and full-length in the first, second, third and final steps, respectively. The diameter of the array at each step was 10, 15, 25 and 30 mm, respectively. In group 2, the

Table 1 Background of the patients in groups 1 and 2

	Group 1 (conventional method)	Group 2 (new method)	*P*
Male : female	8:8	12:2	0.042
Age†	69 (45–82)	71 (60–84)	0.270
With cirrhosis: without cirrhosis	11:5	6:8	0.160
Tumor diameter, mm†	12 (9–18)	16 (6–19)	0.179
Hypervascular, yes : no	13:3	11:3	0.520

†Data are median (range).
NS, not significant.

first, second and third steps were similar to those of g1. After the third step, the tines were again closed within the shaft and then fully expanded.[18]

Power was first applied at 30 W and then increased at 10-W increments every minute in each step to a maximum of 120 W. The power was fixed once it reached 120 W. The necessary electric power and tissue impedance were recorded every 15 s. The procedure was applied continuously until a rise in impedance (caused by coagulation necrosis) with a corresponding drop in delivered power (a phenomenon called "roll-off"). The energy requirement for ablation was integration of the electric power (W) over the ablation time (s), which could be calculated approximately by summing a product of 15 (s) and the electric power measured every 15 s.

Image analysis

Tumor size, location and vascularity were evaluated before RFA using contrast-enhanced CT or MRI. Dynamic CT scans were performed using nonionic contrast material unless the patient was allergic to the iodine medium, for whom MRI was performed. Dynamic CT consisted of the arterial phase (30-s delay), hepatic portal phase (60-s delay) and hepatic venous phase (120-s delay) with slice thickness of 5 mm after the start of injection, respectively. Contrast-enhanced MRI was performed with i.v. injection of contrast material Gd-EOB-DTPA (EOB-MRI). Dynamic MRI consisted of the arterial phase (30-s delay), hepatic portal phase (60-s delay) and hepatic venous phase (120- and 180-s delay) with a thickness of 5 mm and hepatocyte-specific phase (>20 min delay) with a thickness of 3 mm. The tumor was appraised as "hypervascular" when it was stained denser on the arterial phase image compared to the surrounding liver parenchyma.

One to three days after the treatment, the size and shape of the RF-induced lesion was evaluated by measuring three perpendicular dimensions of portal phase images of the contrast-enhanced CT or MRI, calculating the hypothetical volume of the ablated zone. In cases in which CT/MRI images were taken along the needle trace and those perpendicular to the needle, we measured the length of the ablated area along the needle tract and the diameter of the area perpendicular to it.

Statistical analysis

The duration of ablation, required energy and the size of the ablated lesions were compared between the two groups using the Mann–Whitney *U*-test. All values were expressed as median. A *P*-value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Ablation time and required energy

ROLL-OFF WAS achieved at each step of ablation in all 30 RFA procedures. Table 2 shows the time to reach roll-off at each step and total ablation time in the two groups. These results indicate that the durations of the first step, second step and third step were similar for groups 1 and 2 ($P = 0.356$, $= 0.457$ and $= 0.590$, respectively), while that of the fourth step and total session were longer for group 2 than group 1 ($P < 0.001$ and <0.001, respectively). The energy required for one procedure was 18.1 kJ (range, 10.7–31.3) and 59.9 kJ (range, 35.1–119.5) for groups 1 and 2, respectively, indicating more energy requirement for group 2 than group 1 ($P < 0.0001$).

Needle expansion

Figure 1 depicts CT images showing the tines in the tumor in the final step; Figure 1(a,b) shows a cross-

Table 2 Comparison of ablation time (in min/s) and radio frequency-induced areas between groups 1 and 2

	Group 1	Group 2	*P*
Duration of the first step	1′ 53″ (0′ 54″–3′ 43″)	2′ 37″ (1′ 00″–4′ 34″)	0.356
Second step	2′ 14″ (0′ 40″–4′ 57″)	2′ 21″ (0′ 16″–3′ 35″)	0.457
Third step	1′ 26″ (0′ 52″–2′ 46″)	1′ 30″ (0′ 57″–4′ 38″)	0.590
Fourth step	1′ 36″ (1′ 02″–3′ 55″)	9′ 20″ (6′ 39″–17′ 13″)	<0.001
Total ablation time	7′ 36″ (5′ 07″–10′ 13″)	15′ 07″ (11′ 22″–25′ 05″)	<0.001
Required energy for ablation, kJ	18.1 (10.7–31.3)	59.9 (35.1–119.5)	<0.001
Long diameter, mm	30 (21–37)	37 (31–60)	0.001
Short diameter, mm	26 (16–32)	28 (25–39)	0.045
Axial diameter, mm	35 (20–45)	40 (30–50)	0.018

Data are median (range).

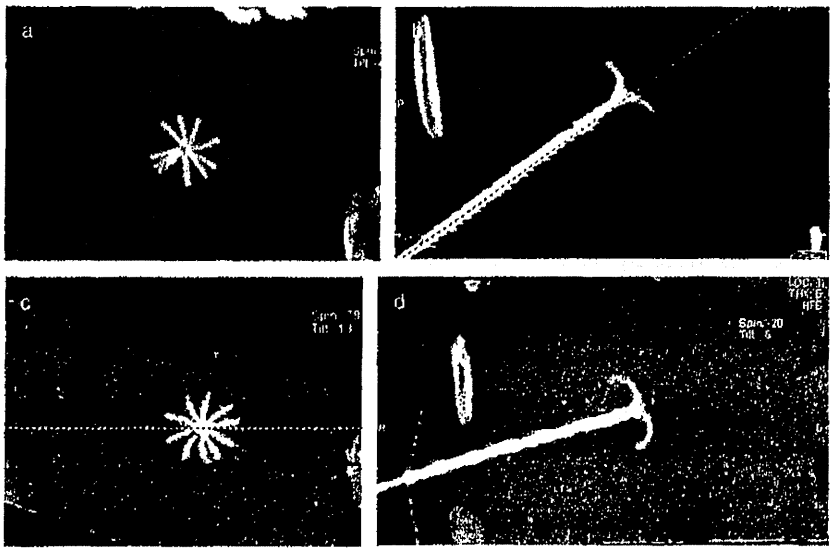

Figure 1 Electrode tines in the final step. (a,b) Tines are uniformly and fully expanded. (c,d) Tines are irregularly and insufficiently expand.

section perpendicular to the needle axis and that along the axis of group 1. Figure 1(c,d) shows those of group 2. All tines are almost uniformly extended as shown in Figure 1(c), while two tines remained attached to each other in Figure 1(a). We checked for needle expansion during RFA in six cases; three cases of group 1 and three cases of group 2. No uniform expansion was detected in any of the cases (0%) of group 1 and 2 cases (67%) of group 2, while irregular expansion was identified in three cases (100%) of group 1 and one case (33%) of group 2. Furthermore, the extent of the expansion at the final step was larger in Figure 1(b) than in (d).

Size and shape of ablated tissue

Table 2 also shows the long and short diameters of the axial cross-section and axial length of the ablated lesions measured on CT images in the two groups. The long diameter was 30 mm (range, 21–37) in group 1 and 37 mm (range, 31–60) in group 2. The short diameter was 26 mm (range, 16–32) in group 1 and 28 mm (range, 25–39) in group 2. The axial length was 35 mm (range, 20–45) in group 1 and 40 mm (range, 30–50) in group 2. All three diameters of group 2 were significantly longer than those of group 1.

In six patients, we reconstructed the post-RFA CT images to show the length of the ablated zone along the shaft and its vertical diameter (Fig. 2). When the tines were uniformly expanded as shown in Figure 1(c), the cross-sectional shape of the ablated zone perpendicular to the axis was nearly circular (Fig. 2c). The zone was more irregular when the tines were non-uniformly separated (see Fig. 1a); the cross-section was also irregular in shape similar to Figure 1(a). In the former case, the ablated zone along the shaft was near-oval in shape with the short axis equivalent to the shaft (Fig. 2d), while the shape was parachute-like or was irregularly shaped sometimes in the latter case (Fig. 2b).

Comparison of the long and short diameters in patients with cirrhosis and without cirrhosis showed that neither the long axis nor the short axis were significantly different; the long diameters in patients with cirrhosis and without cirrhosis were 33 mm (range, 21–53) and 32 mm (range, 25–60), respectively ($P = 0.451$). The short diameter in patients with cirrhosis and without cirrhosis were 27 mm (range, 16–39) and 27 mm (range, 21–36), respectively ($P = 0.983$).

Complications

We did not encounter any episodes of heat injury to adjacent organs, skin burn, symptomatic pleural effusion, intrahepatic abscess, intraperitoneal bleeding or renal failure in either group.

DISCUSSION

RADIOFREQUENCY ABLATION THERAPY is one the curative therapies for HCC measuring less than 30 mm in diameter, whereas surgical resection is the only curative treatment for HCC of more than 30 mm and less than 50 mm in diameter. However, surgical resection cannot be performed in patients with severe liver dysfunction or severe vascular invasion. In Japan,

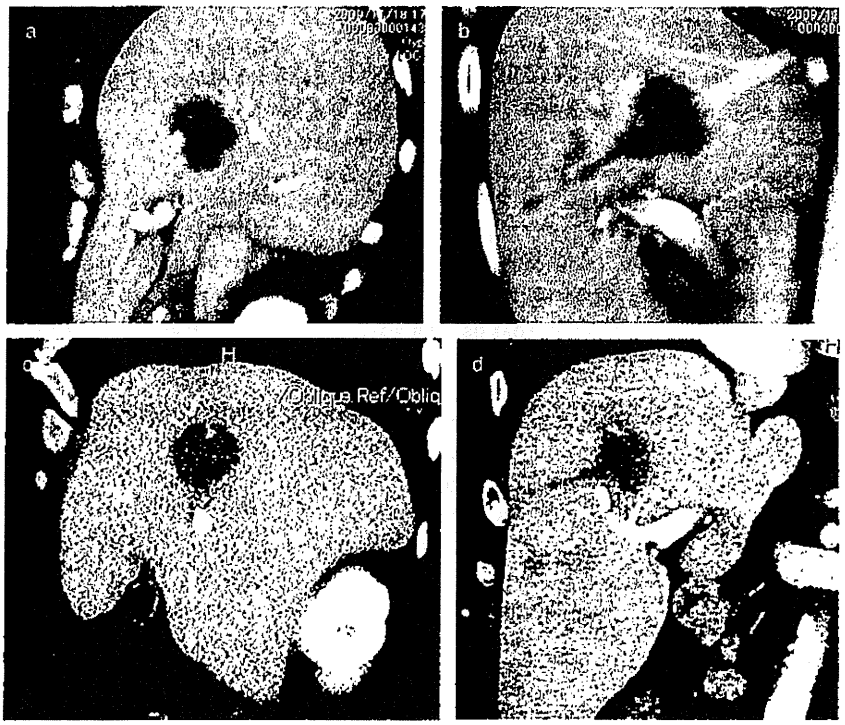

Figure 2 Dynamic computed tomography images of post-radiofrequency ablation lesions produced by the conventional procedure (group 1) and the new procedure (group 2). (a) The shape of the lesion perpendicular to the axis in group 1 is irregular. (b) The shape of the lesion along the axis in group 1 is parachute-like. (c) The shape perpendicular to the axis in group 2 is nearly circular. (d) The shape along the axis in group 2 is ellipsoid.

the Japan Society of Hepatology issued consensus-based HCC treatment guidelines in 2010, which include a HCC treatment algorithm. In this algorithm, resection can be selected with a patient with liver function Child–Pugh class A/B and without vascular invasion or with Vp1 or 2.[19] Thus, a technique that widens the RF-ablated area can improve, at least theoretically, the survival of cirrhotic patients with HCC over 30 mm in diameter.

The shape of the ablated zone depends on the needle type.[3] For example, the path along the shaft is longer than the transverse diameter when using the cool-tip electrode (cool-tip RF system), shorter when using the expandable needle of the RTC system and compatible with each other when using the LeVeen needle (RITA system). The shorter path is less disadvantageous than the shorter perpendicular diameter, because the ablated zone along with the needle trace can be enlarged by repeating the procedure as the needle is extracted while that perpendicular to the tract cannot be enlarged during one insertion. Although it is often difficult to achieve roll-off during a single-step full expansion procedure using the LeVeen needle, our stepwise procedure[13] overcomes this difficulty and produces an oval ablation zone similar to the single-step procedure. The more slender expandable LeVeen Superslim needle is easier and safer to insert into the liver. However, it is easier to deform during insertion and hardly extend as expected; it cannot be fully extended when expanded slowly. This is because the shaft is pushed back as the electrode is inserted toward the liver. To overcome this inconvenience, we designed a new technique, full re-expansion after stepwise extension, which allows a sharper and definite expansion of the slim needle to full length.

We have demonstrated in our previous experimental study,[18] using the pig liver *in vivo*, that the new extension procedure for the expandable needle allows coagulation of a larger and more oval lesion even when using the slim needle. One of the differences between the pig experimental study and the clinical study is that RFA is applied in patients with HCC who have chronically damaged livers. The results showed that the new procedure can also produce a larger ablated zone of which the long axis is perpendicular to the needle shaft compared to that of the conventional procedure in chronically damaged livers; the size of the ablated zone was independent of the liver architecture and liver fibrosis.

The ablation times in this clinical study were similar to those of the experimental studies; the duration of the first, second and third steps were similar in groups 1 and

2, while those of the fourth step and total session were longer in group 2 than group 1. The energy required for one procedure was larger in group 2 than in group 1. The roll-off phenomenon represents marked increase in tissue impedance due to coagulation necrosis. In other words, once the roll-off occurs, the tissue in contact with tines is isolated. Thus, the additional electric current and energy cannot be introduced when the positions of tines are kept in the same position just after the roll-off. After the humors soaks into necrotic tissue from outside normal liver tissue, the additional electric current and electric power can be input. But because the penetrating humor is of small amount, the input electric power shortly enables humors to evaporate and roll-off may occur again soon. Therefore, the second ablation using conventional procedure cannot prominently enlarge the ablated area over 30 mm of diameter which the RTC system exhibits. The shape of ablated area also cannot be clearly changed. A few papers reported the results of double roll-off ablation procedure without change in probe positions,[20–22] showing that this double roll-off procedure cannot ablate the zone bigger than the diameter of the fully expanded needle. The ablation zone was approximately 3 cm with a 14-G LeVeen needle 35 mm in diameter.[20] Even with a 12-tine LeVeen needle 40 mm in diameter, the diameter perpendicular to the axis was 34.4 ± 2.1 mm and the axial diameter was 31.0 ± 6.2 mm.[21] The difference of energy between group 1 and group 2 is due to that of ablated volume because the required energy for ablation per volume is almost identical.[18]

We suggested in our previous study[18] that the smaller ablation zone produced by the conventional stepwise method was due to the facts that the hooks of the Super-Slim needles hardly extended to full extension during the slow insertion because the shaft was pushed back as the electrode was inserted toward the liver and that the tanned tumor or parenchymal tissues were removed from the surface of the multiple tines when they were once enclosed within the shaft in the new method, resulting in a better outcome of RF ablation. Our study identified another reason for the difference in the size of the ablated zone; the tines were extended separately in more cases of group 2, while some tines remained attached to each other in more cases of group 1 than of group 2. It is possible that this is because the tines gathered in one direction in the first step as the tip of the needle shaft was diagonally cut and the direction of the extension of each tine could not be reset in the conventional procedure. When all tines were separately extended, the cross-section was nearly circular and its size was larger due to the better RFA, compared with the irregular shape and smaller size when two or three tines remained attached to each other. In addition, the median of the long axis with the new method is much larger not only than that with the conventional method using a slim needle but also that of the conventional method with an old needle of 15-G diameter.[3,13] It means this method using a slim needle is most appropriate when we want the largest ablated zone among various methods: the conventional method using a slim needle, that using a 15-G needle and the ablation using cool-tip needle.

In conclusion, the new extension procedure using the slim expandable needle allows coagulation of the largest area among various procedures using various types of needles. Additionally, the two kinds of stepwise procedures allow the selection of a more suitable procedure based on the tumor size and shape in each RFA.

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Long-Term Entecavir Treatment Reduces Hepatocellular Carcinoma Incidence in Patients With Hepatitis B Virus Infection

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Chronic hepatitis B virus (HBV) infection leads to cirrhosis and hepatocellular carcinoma (HCC). Antiviral agents are thought to reduce HCC development, but agents such as lamivudine (LAM) have a high rate of drug resistance. We compared the incidence of HCC in 472 entecavir (ETV)-treated patients and 1,143 nontreated HBV patients (control group). Propensity score matching eliminated the baseline differences, resulting in a sample size of 316 patients per cohort. The drug mutation resistance was 0.8% (4/472) in the ETV group. The cumulative HCC incidence rates at 5 years were 3.7% and 13.7% for the ETV and control groups, respectively ($P < 0.001$). Cox proportional hazard regression analysis, adjusted for a number of known HCC risk factors, showed that patients in the ETV group were less likely to develop HCC than those in the control group (hazard ratio: 0.37; 95% confidence interval: 0.15-0.91; $P = 0.030$). Both cohorts were applied in three previously reported risk scales and risk scores were generated based on age, gender, cirrhosis status, levels of alanine aminotransferase, hepatitis B e antigen, baseline HBV DNA, albumin, and bilirubin. The greatest HCC risk reduction occurred in high-risk patients who scored higher on respective risk scales. In sub analyses, we compared treatment effect between nucleos(t)ide analogs, which included matched LAM-treated patients without rescue therapy (n = 182). We found HCC suppression effect greater in ETV-treated ($P < 0.001$) than nonrescued LAM-treated ($P = 0.019$) cirrhosis patients when they were compared with the control group. *Conclusion:* Long-term ETV treatment may reduce the incidence of HCC in HBV-infected patients. The treatment effect was greater in patients at higher risk of HCC. (HEPATOLOGY 2013;58:98-107)

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More than 2 billion people worldwide have been exposed to hepatitis B virus (HBV) and about 350 million people are chronically infected, the majority of whom are in Asia (75%). The prevalence of HBV in Japan is 0.8%, which is lower than other Asian countries such as Taiwan (>10%) and China.[1-3] As chronic HBV infection leads to cirrhosis and hepatocellular carcinoma (HCC), published studies have shown that up to 25% of chronically infected patients eventually die of liver cirrhosis or HCC.[4]

A large-scale longitudinal epidemiologic study has shown that a patient's baseline HBV DNA level is an independent predictor for the development of HCC.[5] Studies have begun to show that treatment to decrease HBV DNA reduces the risk of HCC development in HBV patients with cirrhosis or advanced fibrosis or in chronic HBV patients.[6,7]

Within the past 10 years, new antiviral therapies, including nucleos(t)ide analogs (NAs), have been approved and were successful in suppressing circulating serum viral loads. Studies that have examined the relationship between NA therapy and HCC almost exclusively used older drugs such as lamivudine and/or adefovir. Although results of long-term studies showed the importance of antiviral suppression, HCC risk among patients treated by newer NAs remains inconclusive. Entecavir (ETV) is a relatively new antiviral NA that has proved effective in suppressing HBV

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ETV, entecavir; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HR, hazard ratio; NA, nucleos(t)ide analogs; PS, propensity score; ROC, receiver operating characteristic curve.

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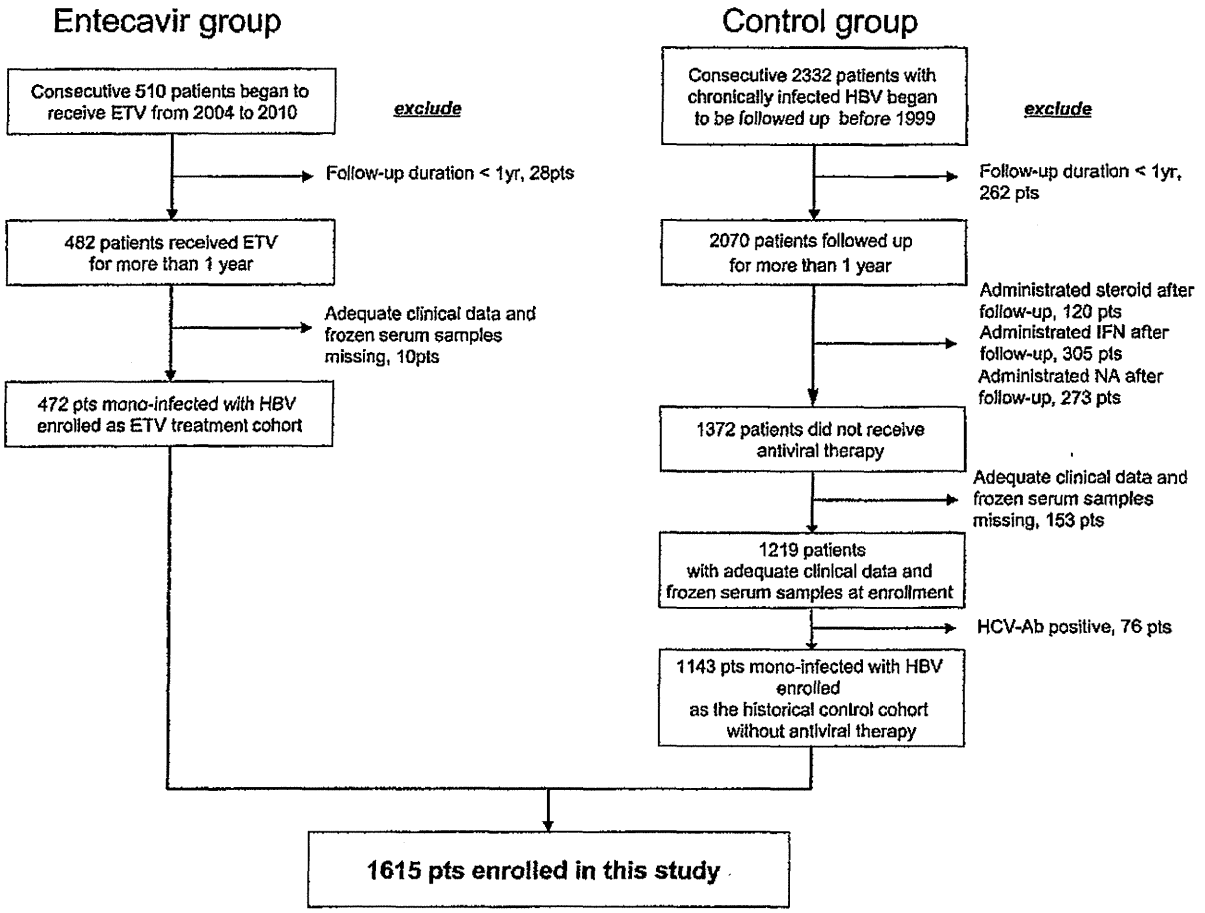

Fig. 1. Entecavir-treated and nontreated cohorts. ETV, entecavir; HBV, hepatitis B virus; IFN, interferon; NA, nucleos(t)ide; HCV-Ab, anti-hepatitis C virus antibody.

DNA replications with minimal drug resistance.[8,9] In this study we examined whether long-term ETV treatment would reduce HCC risk in HBV-infected patients when compared with NA-naïve patients.

Patients and Methods

Patients and Design. From 2004 to 2010, we consecutively recruited 510 patients treated with 0.5 mg ETV (ETV group); the ETV group was compared with a retrospective cohort of 2,332 NA-naïve, HBV-infected patients (control group).

These patients were chronically monoinfected with HBV and were confirmed as hepatitis B s antigen (HBsAg)-positive for at least 6 months. As a general rule, ETV was initiated in a patient who had both abnormal alanine aminotransferase (ALT) levels (defined as ALT $\geq$45) and elevated HBV DNA levels of $\geq$4 log copies/mL. A patient with advanced fibrosis would be treated with ETV if the ALT level was normal; however, a patient without fibrosis or with a normal HBV DNA/ALT level would not be treated with ETV. Among the treated patients, 38 were excluded from the ETV group either because their follow-up period was less than 1 year (n = 28) or because the clinical data or serum samples were incomplete (n = 10). The remaining 472 ETV-treated patients were included in the analysis (Fig. 1). No patient in the ETV group received other NAs before ETV treatment.

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The control group patients were recruited from 1973 to 1999. These patients were NA-naïve at baseline, as no NA therapy had yet been approved. Patients were excluded from the control group if (1) their follow-up duration was less than 1 year (n = 262); (2) corticosteroid withdrawal therapy (n = 120), IFN treatment (n = 305) or NA treatment (n = 273) was initiated during follow-up; (3) clinical data or serum samples were incomplete (n = 153); or (4) patients were found to be positive for anti-hepatitis C virus antibodies (HCV-Ab) (n = 76). The remaining 1,143 patients served as the control population (Fig. 1).

We also made subanalyses to examine the difference of HCC suppression effect between NAs. To make this comparison, we recruited a cohort of 949 consecutive patients from our hospital who were treated with lamivudine (LAM) (September 1995 to September 2007). LAM-treated patients who met the same inclusion criteria as the ETV group, who had no rescue therapy (LAM group, n = 492), were used in the comparison.

We received informed consent from each patient at their entry into the study. Informed consent for the clinical data collection and storage of serum samples were obtained from each patient in the historical control group. The study protocol was in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the Toranomon Hospital Ethics Committee.

Clinical Data Collection and Follow-up. All ETV-treated and untreated patients were followed at 1- to 3-month intervals, during which biochemical and HBV virological markers, blood counts, tumor markers (e.g., alpha-fetoprotein and des-γ-carboxylprothrombin), and cirrhosis and HCC status were monitored. Viral response in the ETV group was defined as a reduction in HBV DNA levels to below 400 copies/mL. Cirrhosis was determined by laparoscopy, liver biopsy, imaging modalities, or portal hypertension. HCC was diagnosed predominantly via imaging, including dynamic computed tomography, magnetic resonance imaging, and/or digital subtraction angiography. When the hepatic nodule did not show typical imaging features, diagnosis was confirmed by fine-needle aspiration biopsy followed by histological examination. Patients were followed until any confirmed HCC diagnosis 1 year after the start of observation (primary outcome) or until the last visit before December 2011. All patients also underwent ultrasonography or helical dynamic computed tomography every 3 to 6 months (cirrhosis patients) or every 6 to 12 months (noncirrhosis patients).

HBV Infection Markers. HBV DNA levels were quantified using the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.6 to 7.6 log copies/mL, or COBAS TaqMan HBV Test v2.0 (Roche Diagnostics) which has a dynamic range of over 2.1 to 9.0 log copies/mL. HBV DNA of the control group was measured from their stored frozen serum (−80°C) using COBAS TaqMan HBV v.2.0 once at the start of observation. Previous measurements were taken using the old DNA polymerase assay in the control group and thus were not used for comparisons. For the ETV group, drug-resistant mutations were determined from a nested polymerase chain reaction, using a primer specific at the polymerase region in patients who had an HBV DNA relapse of $\geq$1 log copies from nadir. Hepatitis B e antigen; (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to serologically determine HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the eight major genotypes (A to H).

HCC Incidence by Risk Scores. To examine HCC incidence by risk scores, we applied published HCC risk scales, which are based on the natural course of HCC among HBV-positive patients, to our cohorts. We first searched Medline/PubMed using "hepatitis B," "cancer," and "risk score" as keywords and found four publications in English that used risk-score estimations.[10-13] One article was rejected because we were unable to compute the risk scores with our variables, and therefore we used only the scales indicated by the remaining three publications to generate the risk scores.[13] The risk scales were based on parameters such as age, gender, cirrhosis, levels of ALT, HBeAg, baseline HBV DNA, albumin, and bilirubin. The original risk score formula and the risk score distributions for our two cohorts derived from these formulas are shown in Supporting Table 1. The risk score cutoff points were determined from the following original articles. In Yang et al.'s article,[10] the risk score was derived from 17-point categories. When we applied the scores to our control group, we found that the 12-point scale was at best in detecting a difference in HCC incidence. With that, we examined the HCC suppression treatment effect by dividing the patients into equal halves with 12 points as the cutoff. Yuen et al.[11] divided their cohort in half and found risk scores of 82 as the optimal cutoff point. We also applied the same cutoff point to our cohorts. Wong et al.[12] used their risk scores to categorize their cohort into low-risk, medium-risk, and high-risk groups with respective cutoff points at <4, 4-19, $\geq$20. We also applied the same cutoff points to our cohorts to examine the treatment effect. Cumulative

HCC incidence rates were compared by these risk scores between the ETV and control groups.

Statistical Analysis. Categorical data were compared using chi-square or Fisher's exact tests. Continuous variables with normal distributions were compared using Student's t test, and those without normal distributions were compared using the Mann-Whitney U test. Cumulative HCC incidence rates were analyzed using the Kaplan-Meier method; patients followed beyond 5 years were censored to better compare the two cohorts because the ETV group had a shorter follow-up period when compared with the historical control group. We compared the cumulative incidence of HCC using the log-rank test, and Cox proportional hazard regression analysis, which was used to assess the variables that were significantly associated with the development of HCC. Deaths before HCC development were censored. Significance was defined as $P < 0.05$ for all two-tailed tests.

We used the propensity score (PS) matching method to reduce significant differences in demographics between the ETV and control groups.[14,15] Using multiple logistic regression analysis, a PS was estimated for all patients treated with ETV.[14] Variables used in the model included age, sex, presence of cirrhosis, HBeAg, HBV DNA< aspartate aminotransferase (AST), ALT, γ-glutamyl transpeptidase; (γ-GTP), bilirubin, albumin, and platelet counts. We performed caliper matching on the PS (nearest available matching). Pairs (ETV and the control group) on the PS logit were matched to within a range of 0.2 standard deviation (SD).[16,17] The PS logit distributions for each cohort showing the overlaps and SD ranges are shown in Supporting Fig. 1. The balance of covariates was measured by their standardized differences. A difference >10% of the absolute value was considered significantly imbalanced.[17] The cohorts were divided into five PS quintiles (Supporting Table 2). We also made subanalyses to examine the difference of HCC suppression effect between NAs by comparing the HCC incidence between propensity score matched ETV- and lamivudine (LAM)-treated patients without a rescue therapy. The LAM-treated patients were derived from consecutive sampling at our institution and were PS matched with ETV group according to the same method described above. Interaction of the subgroups by preexisting cirrhosis or risk scores and ETV treatment were evaluated. $P < 0.10$ was considered statistically significant. Data analysis was performed using IBM SPSS v. 19.0 software (Armonk, NY) and R software v. 2.13 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

Results

Patient Characteristics. The patient characteristics at the baseline, before PS matching are shown in Table 1. The ETV group was followed for an average of 3.2 years (1,561 person-years), whereas the control group was followed for an average of 9.5 years (12,381 person-years). Before matching, patients in the ETV group and the control group differed significantly in age, gender, genotype, baseline HBV DNA level, and other clinical data. In the ETV group, 421 patients (89%) had HBV DNA (<400 copies/mL) at year 1. Not all patients in the control group were tested for HBV DNA level during follow-up. The drug mutation resistance was 0.8% (4/472). The four patients who had drug mutation did not develop HCC. During follow-up, 12 patients (2.54%) in the ETV group and 144 patients (12.60%) in the control group developed HCC. The incidence rates of HCC for the ETV and the control groups were 76/10,000 patient-years and 116/10,000 patient-years, respectively. During this period, 21 patients in the control group developed liver cirrhosis while no patient developed liver cirrhosis in the ETV group. During the same observation period, there were four deaths in the ETV group and 10 deaths in the control group. We took competing risk into account[18,19] and compared incidence of non-HCC deaths between the cohorts and the results were not different. However, because there were only four patients in the non-HCC deaths in the ETV group (two patients in the PS matched cohort) and 10 patients in the control group (six patients in the PS matched cohort), we considered that it was not meaningful to apply competing risk analysis in our cohorts.

Factors Associated with HCC and Effect of ETV Treatment on HCC Development. To allow a common ground for comparison between the two cohorts, we used PS matching with selected key characteristics and compared the two groups within the same time period of 5 years. The PS matching process resulted in a matched sample size that consisted of 316 patients in each group (Table 1). The PS matching reduced the significant variability of the two cohorts. While five (42%) of the 12 covariates varied by >10% before matching, all covariates differed by <10% of the absolute value after matching (Supporting Fig. 2). In the PS score matched cohort, 10 out of the 231 noncirrhosis patients progressed to liver cirrhosis within the 5 years of observation. The cumulative incidence rates of HCC in the matched ETV groups were 0.7% at year 2, 1.2% at year 3, 2.5% at year 4, and 3.7% at year 5. The cumulative incidence rates of HCC in the

System and other systems, we were not able to compare the results 6 months after treatment because of the lack of such data for the Cool-tip RF System, because of the different GCP guidelines in force at the time of the Cool-tip RF System study. However, the 6-month follow-up data of our study were very satisfactory. Furthermore, because there were no such data available in the reports on the Cool-tip RF System, we could not compare the levels of experience of the operators in the two studies.

In conclusion, the present clinical study confirmed that the CelonPOWER System is a very safe and highly effective RFA system for liver cancer in Japanese patients. In addition, because this system is a bipolar device, it operates with high energy efficiency, and because multiple multipolar applicators can be employed simultaneously, coagulation necrosis of an extensive tumor tissue volume can be achieved in a short treatment time. Moreover, throughout the course of this clinical study, most of the patients did not experience hot flushes or perspiration. It is therefore anticipated that the CelonPOWER System will become used as a next-generation RFA system that is not only safer than existing systems, but is highly effective and places less physical burden on the patient.

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Table 3 Frequently observed adverse effects (5 % or more) (adverse reactions at an incidence of >5 % in the overall study period)

Adverse event	No. of patients	%	No. of patients treated (%)	Treatments
Aspartate aminotransferase (AST) increase	72	79.1	0 (0)	–
Alanine aminotransferase (ALT) increase	69	75.8	0 (0)	–
Lactate dehydrogenase (LDH) increase	22	24.2	0 (0)	–
Total bilirubin increase	20	22.0	0 (0)	–
Pleural effusion	12	13.2	2 (2.20)	Human serum albumin, cefmetazole sodium, tazobactam piperacillin hydrate
Vomiting	12	13.2	7 (7.69)	Metoclopramide
Nausea	10	11.0	9 (9.89)	Metoclopramide, domperidone, diazepam
Postoperative pain	9	9.9	3 (3.30)	Pentazocine, loxoprofen sodium hydrate, acetaminophen, diclofenac sodium
White blood cell count increase	8	8.8	1 (1.10)	Sulbactam sodium–cefoperazone sodium
Platelet count decrease	6	6.6	0 (0)	–
Alkaline phosphatase (ALP) increase	5	5.5	0 (0)	–
Fever	5	5.5	5 (5.49)	Loxoprofen sodium hydrate, acetaminophen, cefmetazole sodium

tip RF System, suggesting that this new system yields efficacy that is at least equivalent to that achieved with the Cool-tip RF System, while causing less of a treatment burden on the patient.

We assessed the TE level, and its maintenance in ITT cases after 10 weeks and 24 weeks (6 months) in the follow-up period of this clinical study and found that the overall TE assessment was not inferior to that of the National Follow-up Survey Report on Primary Hepatic Carcinoma (2004–2005) [24] issued by the Liver Cancer Study Group of Japan (Table 2). Considering that the method for overall TE assessment in that report was the same as that employed in the present study, it is reasonable to conclude that the TE maintenance with the CelonPOWER System is not inferior to that of other local therapy.

Nishikawa et al. reported on local recurrence when using monopolar systems clinically. They found that, in 269 patients with solitary hypervascular HCCs who had undergone RFA, the 1- and 2-year cumulative local recurrence rates were 12.8 and 23.6 %, respectively [25]. We believe that our present results for the cumulative local recurrence rate (5.7 % for 6 months) with the CelonPOWER System are comparable to those reported results.

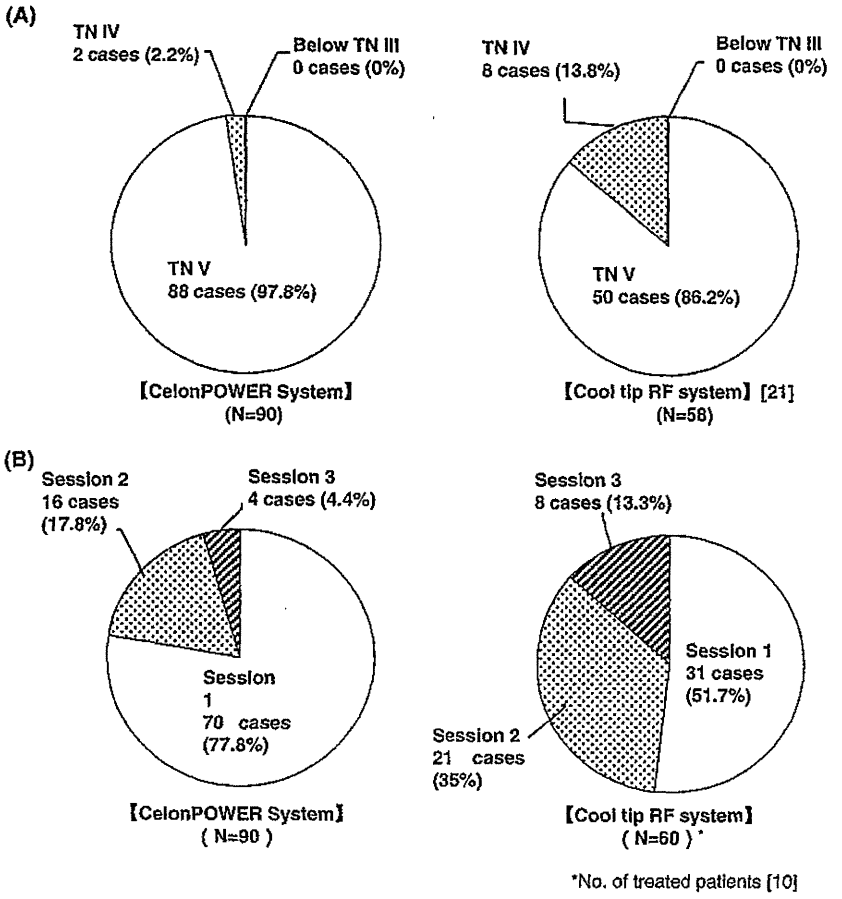
The introduction of a new device inevitably raises the question of its safety. In our series, there were 3 adverse events–one event of abdominal wall burn and one of pleural effusion during the treatment period, and one event of biliary peritonitis during the follow-up period. These adverse events were previously known to be possible adverse events that had been observed with the Cool-tip, RITA, and Boston monopolar RFA systems that have already been approved for clinical use in Japan [21–23]. Therefore, similar caution concerning internal adverse events is necessary when using the CelonPOWER System, although the problem of external burns does not exist with this system.

The high-incidence (≥5 %) device-related adverse event rate during the course of our clinical study was similar to the rates with the Cool-tip, RITA, and Boston monopolar RFA systems [21–23].

Therefore, these events are not unique to the CelonPOWER System, and the safety of the CelonPOWER System is not inferior to that of the existing approved RFA devices.

This study has several limitations. First of all, although it was a prospective study, it was not a randomized controlled clinical study. However, all consecutive patients who satisfied the enrollment criteria were offered the opportunity to participate and the study was performed in all those who provided informed consent and decided to receive the treatment. After providing informed consent, 5 patients decided not to participate and 1 ceased treatment after 1 session, due to the difficulty posed by the proximity of the lesion to the heart and lungs.

Although we were able to compare our own results immediately after treatment with those of the Cool-tip RF



*No. of treated patients [10]

Fig. 6 Comparison of the present results obtained with the CelonPOWER System and the clinical study results reported for the Cool-tip RF System. The percentage of Class V tumor necrosis (*TN*) (TN 100 %) cases (a) and the number of patients in whom each RFA session was completed (b)

simultaneously inserted into an S8 tumor, and treatment was finished in a single ablation.

During the course of the entire clinical study period, serious adverse events (i.e., events for which a causal relationship with the CelonPOWER System could not be ruled out) were seen in 3 patients, consisting of abdominal wall burn, pleural effusion, and biliary peritonitis. Each of those events was judged to be serious because they required prolongation of hospitalization, and each required treatment. In addition, it was judged that each of these serious adverse events was a known adverse event that had been observed with similar, already-approved RFA devices [21–23]. Also, the single fatality, which occurred at home, had occurred in a patient who had been hospitalized for treatment on the suspicion of peritonitis based on the examinations performed after 10 weeks in the follow-up period. The patient's condition had improved and the patient had been discharged, and it was later confirmed that death had occurred at home. Autopsy revealed the cause of death to have been due to the progression of cirrhosis, and it was thus thought that the death was not related to the treatment with the CelonPOWER System. Table 3 shows the most common adverse effects (those observed in 5 % of patients or more) and all of these (pleural effusion, nausea, vomiting, postprocedural pain, and fever) have been known to occur with previously approved local therapeutic devices. Moreover, all the adverse events were easily controllable.

Discussion

We set out to prospectively determine whether a bipolar RFA device (CelonPOWER System) was safe and effective in the treatment of liver cancer and whether it could be demonstrated to be non-inferior to a monopolar RFA system currently approved and employed clinically in Japan (Cool-tip RF System).

Treatment was completed in a fewer number of sessions when using the CelonPOWER System than with the Cool-