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Efficacy of Immunotherapy With TG4040, Peg-Interferon, and Ribavirin in a Phase 2 Study of Patients With Chronic HCV Infection

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BACKGROUND & AIMS: TG4040 is a modified vaccinia Ankara (MVA) virus that expresses the hepatitis C virus (HCV) proteins NS3, NS4, and NS5B. We performed a phase II open-label study to determine the efficacy, safety, and immunotherapeutic properties of TG4040 in combination with pegylated interferon α-2a and ribavirin (PEG-IFNα/RBV) in patients with chronic HCV infection. **METHODS:** Treatment-naive patients with HCV genotype 1 infection were assigned randomly to 1 of the following groups: PEG-IFNα/RBV for 48 weeks (group A, n = 31), PEG-IFNα/RBV for 4 weeks followed by PEG-IFNα/RBV for 44 weeks with 6 injections of TG4040 (group B, n = 63), or TG4040 for 12 weeks (7 injections) followed by PEG-IFNα/RBV for 48 weeks with 6 injections of TG4040 (group C, n = 59). The primary end point was complete early virologic response (cEVR), defined as HCV-RNA level less than 10 IU/mL after 12 weeks of PEG-IFNα/RBV treatment. **RESULTS:** In group C, 64.2% of evaluable patients achieved cEVR, compared with 30.0% in group A and 45.9% in group B ($P = .0003$ for group C vs A). A higher percentage of patients achieved a sustained virologic response 24 weeks after therapy ended in group C (58.2%) than in groups A (48.4%) or B (50.8%). HCV- and MVA-specific T-cell responses were observed predominantly in group C. As expected, most patients given injections of TG4040 developed anti-MVA antibodies. The combination of TG4040 and PEG-IFNα/RBV was reasonably well tolerated. However, PEG-IFNα–associated thrombocytopenia developed in 3 patients who carried the class II HLA allele DRB01*04. **CONCLUSIONS:** A higher percentage of patients with chronic HCV infection who received immunotherapy with TG4040 followed by TG4040 and PEG-IFNα/RBV achieved a cEVR compared with patients who received only PEG-IFNα/RBV therapy. These findings show that immunotherapies that activate T cells are effective in patients with chronic HCV infection. ClinicalTrials.gov number, NCT01055821.

Keywords: Vaccine; ELISpot; Immune Response; SVR24.

Hepatitis C virus (HCV) infection is a global health problem with approximately 180,000 million persons infected worldwide.[1] HCV infection is often a clinically silent disease but its progression can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[2,3] Substantial progress has been made in treating chronic HCV infection with the recent approvals of the NS3 protease inhibitors telaprevir and boceprevir in combination with pegylated interferon alfa-2a (PEG-IFNα) and ribavirin (RBV).[4,5] Although these regimens offer significant benefit in the treatment of HCV genotype 1 infection, the added toxicity profile and potential for resistance mutations can be limiting factors. Clearly, alternate and/or complementary therapies are needed. In addition to the many direct-acting antivirals in development,[4] immunotherapeutics also may offer a unique complementary strategy of combination therapy for the treatment of HCV infection.[6,7]

Targeted, active immunotherapeutics can induce and/or reamplify host immune control of the virus by the combination of various mechanisms of action including the action

Abbreviations used in this paper: **AE, adverse event; cEVR, complete early virologic response; ETR, end of treatment; HCV, hepatitis C virus; ITT, intent-to-treat; LOD, limit of detection; MVA, modified vaccinia Ankara; PBMC, peripheral blood mononuclear cell; PEG-IFNα, pegylated interferon alpha-2a; RBV, ribavirin; SAE, serious adverse event; SVR, sustained virologic response.**

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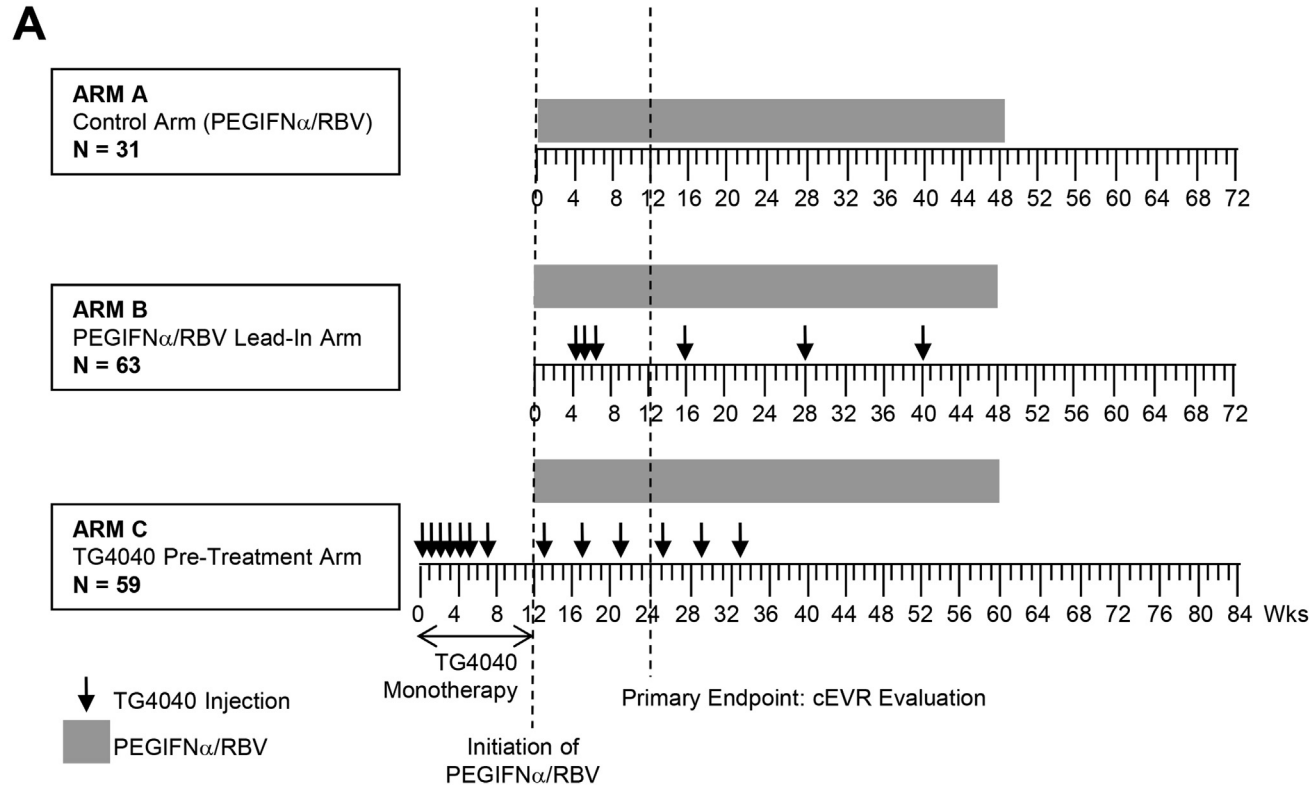
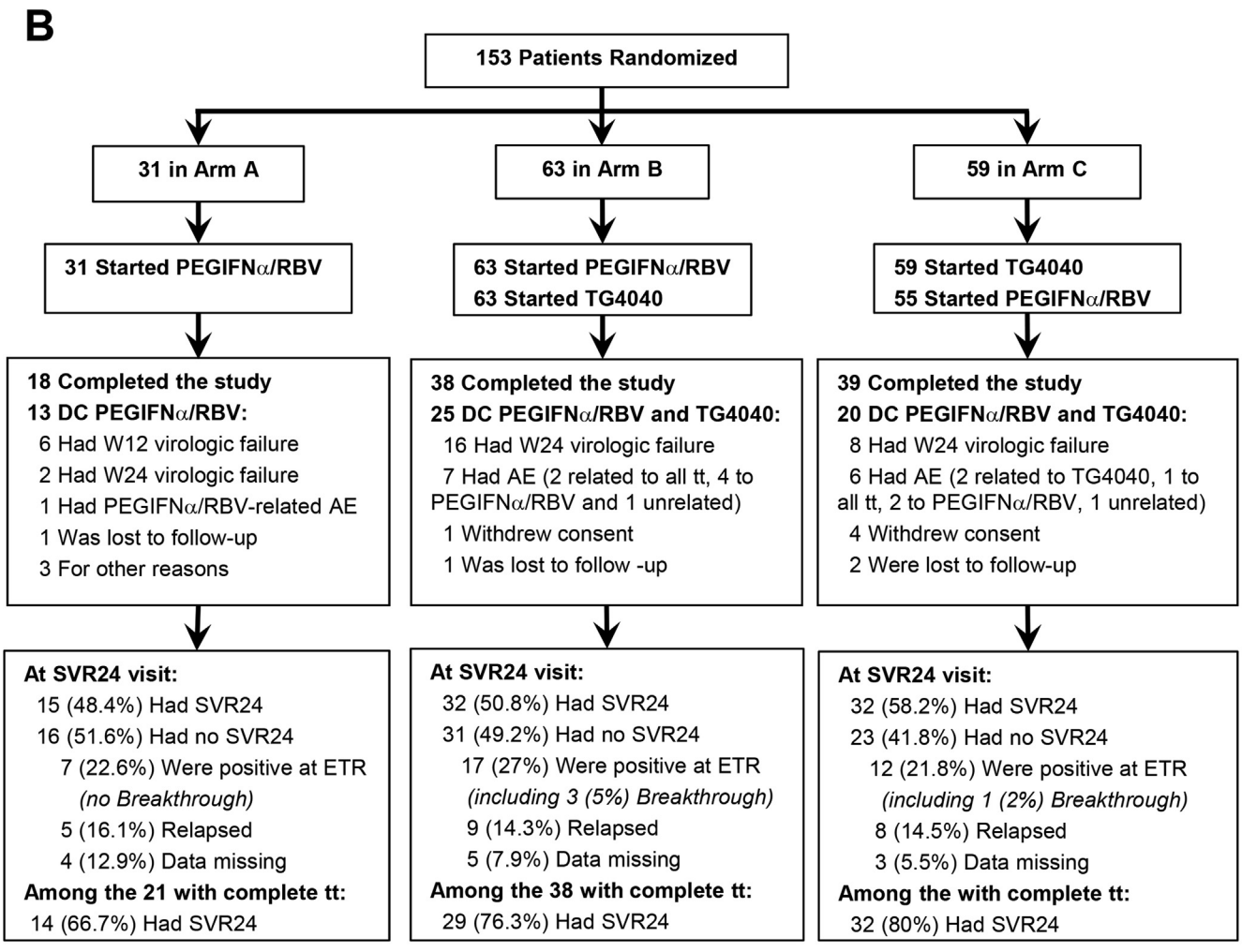

A
ARM A
Control Arm (PEGIFNα/RBV)
N = 31
ARM B
PEGIFNα/RBV Lead-In Arm
N = 63
ARM C
TG4040 Pre-Treatment Arm
N = 59
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 Wks
TG4040 Monotherapy
Primary Endpoint: cEVR Evaluation
TG4040 Injection
PEGIFNα/RBV
Initiation of PEGIFNα/RBV


B
153 Patients Randomized
31 in Arm A
63 in Arm B
59 in Arm C
31 Started PEGIFNα/RBV
63 Started PEGIFNα/RBV
63 Started TG4040
59 Started TG4040
55 Started PEGIFNα/RBV
18 Completed the study
13 DC PEGIFNα/RBV:
6 Had W12 virologic failure
2 Had W24 virologic failure
1 Had PEGIFNα/RBV-related AE
1 Was lost to follow-up
3 For other reasons
38 Completed the study
25 DC PEGIFNα/RBV and TG4040:
16 Had W24 virologic failure
7 Had AE (2 related to all tt, 4 to PEGIFNα/RBV and 1 unrelated)
1 Withdrew consent
1 Was lost to follow -up
39 Completed the study
20 DC PEGIFNα/RBV and TG4040:
8 Had W24 virologic failure
6 Had AE (2 related to TG4040, 1 to all tt, 2 to PEGIFNα/RBV, 1 unrelated)
4 Withdrew consent
2 Were lost to follow-up
At SVR24 visit:
15 (48.4%) Had SVR24
16 (51.6%) Had no SVR24
7 (22.6%) Were positive at ETR (no Breakthrough)
5 (16.1%) Relapsed
4 (12.9%) Data missing
Among the 21 with complete tt:
14 (66.7%) Had SVR24
At SVR24 visit:
32 (50.8%) Had SVR24
31 (49.2%) Had no SVR24
17 (27%) Were positive at ETR (including 3 (5%) Breakthrough)
9 (14.3%) Relapsed
5 (7.9%) Data missing
Among the 38 with complete tt:
29 (76.3%) Had SVR24
At SVR24 visit:
32 (58.2%) Had SVR24
23 (41.8%) Had no SVR24
12 (21.8%) Were positive at ETR (including 1 (2%) Breakthrough)
8 (14.5%) Relapsed
3 (5.5%) Data missing
Among the with complete tt:
32 (80%) Had SVR24

of cellular immune responses, nonspecific responses (innate immunity) resulting in clearance of infected cells, and/or neutralization of circulating viruses via humoral responses. Although various therapeutic vaccine candidates against HCV were evaluated in phase I studies and shown to elicit specific immune responses, to date most were unable to clear infection when administered as monotherapy.[6,7]

TG4040 is derived from the attenuated nonreplicative vaccinia virus of the strain Ankara (MVA), engineered to express HCV nonstructural proteins NS3, NS4, and NS5B. Recently, TG4040 was evaluated in patients with genotype 1 chronic HCV infection in a phase I dose-escalation study.[8] TG4040 was well tolerated, its administration was associated with a transient decrease in serum HCV-RNA level, and elicited HCV-specific immune responses primarily directed against the NS3 protein. Based on these data, this phase 2 study was designed to evaluate the safety and efficacy of TG4040 in combination with PEG-IFNα/RBV for the treatment of genotype 1 HCV infection. Two different TG4040 administration schedules were used, one using lead-in PEG-IFNα/RBV dosing to reduce viral load before treatment with TG4040/PEG-IFNα/RBV, and the other using TG4040 pretreatment first as a stand-alone therapy to activate immunologic responses before the introduction of PEG-IFNα/RBV combined with TG4040.

Patients and Methods

Study Design

This study was a randomized, open-label, phase 2 international study conducted at 28 centers in France, Germany, Israel, Poland, Romania, Spain, and the United States. The first patient was screened in September 2010 and the last observation was made in October 2012. After inclusion/exclusion criteria confirmation by investigators, patients were randomized centrally via an interactive voice response system in a 1:2:2 ratio to arm A (PEG-IFNα/RBV for 48 weeks), arm B (PEG-IFNα/RBV for 4 weeks followed by PEG-IFNα/RBV/TG4040), or arm C (pretreatment with TG4040 for 12 weeks followed by PEG-IFNα/RBV/TG4040) (Figure 1*A*). A permutated block randomization schedule generated by an independent provider was used and patients were stratified by age ($>$50 or $\leq$50 y) and viral load ($>$5.6 or $\leq$5.6 $\log_{10}$ IU/mL). The first 3 patients enrolled in arms B and C were enrolled sequentially with a review of safety data by a data safety monitoring committee before continuing enrollment.

The primary objective of this study was to evaluate the efficacy of TG4040 combined with PEG-IFNα/RBV as measured by the proportion of patients achieving complete early virologic response (cEVR) defined as undetectable HCV-RNA level (ie, $<$10 IU/mL) after 12 weeks of PEG-IFNα/RBV.

Patients were followed up for safety, efficacy, and immune responses every 1–4 weeks until 48 weeks of PEG-IFNα/RBV treatment (week 48 or week 60), and then every 2–12 weeks until post-treatment week 24 (Figure 1*A*). The study conformed to the principles of the 1996 Declaration of Helsinki and Good Clinical Practice as outlined in 21 Code of Federal Regulations, subchapter D, part 312. The protocol and informed consent were reviewed and approved by each ethics review committee or institutional review board. Before the conduct of any study-related procedure, patients provided written informed consent.

Immunotherapeutic TG4040

TG4040 is a viral suspension of nonpropagative, recombinant vaccinia virus from the attenuated MVA strain containing nucleotide sequences encoding HCV nonstructural proteins NS3, NS4, and NS5B derived from a prototypic genotype 1b viral isolate (Transgene, Strasbourg, France) as previously described.[8]

Patients

Eligible patients were between 18 and 70 years, inclusive, had not been treated previously for hepatitis C, and were infected chronically with genotype 1 HCV with detectable HCV-RNA level for more than 6 months. Patients were excluded if they had evidence of cirrhosis (based on a liver biopsy within 24 months or a FibroScan [Echosens, Antony, France] within 6 months), decompensated liver disease, a history of immunodeficiency or autoimmune disorders, significant cardiovascular disease, severe psychiatric disease, malignancy within 5 years, or were co-infected with hepatitis B or human immunodeficiency virus-1.

Treatment Plan

Patients received PEG-IFNα 180 μg/wk subcutaneously and RBV orally according to weight ($<$75 kg received 1000 mg; and $\geq$75 kg received 1200 mg) in a divided daily dose. TG4040 was administered at the hospital in an out-patient setting, subcutaneously, at a dose of 10^7 plaque-forming units as a 6- or 13-injection schedule (arms B and C, respectively). In arm B, patients received TG4040 from week 4 of PEG-IFNα/RBV, 3 weekly injections followed by 3 additional injections, once every 12 weeks. In arm C, patients first received TG4040 weekly 6 times and then monthly for 7 months; PEG-IFNα/RBV was introduced after 12 weeks of TG4040 monotherapy (Figure 1*A*).

Treatment was discontinued for inadequate virologic response as follows: in arm A, if HCV-RNA level failed to decrease by at least 2 $\log_{10}$ IU/mL by week 12 and in all arms if HCV-RNA levels were detectable after 24 weeks of PEG-IFNα/RBV (HCV RNA $\geq$ limit of detection [LOD], with a retest authorized for patients below the limit of quantification).

Antiviral Response

Antiviral response was assessed by measuring serum HCV-RNA levels throughout the study using the Roche AmpliPrep/COBAS TaqMan assay version 2.0 (Mannheim, Germany) with a LOD of 10 IU/mL and a limit of quantification of 15 IU/mL. Laboratory testing was performed by Synlab (Berlin, Germany).

Figure 1. Study design and study disposition. (*A*) Study design. (*B*) Study disposition: randomization, treatment, follow-up evaluation, and SVR among the study patients. In all arms, the main reason for discontinuation (DC) was virologic failure according to stopping rules. Highest SVR rates were seen in arm C. tt, treatment.

IL28B Polymorphism

The IL28B genotype was determined retrospectively from DNA extracted from peripheral blood mononuclear cell (PBMC) aliquots using the allelic discrimination rs12979860 single-nucleotide polymorphism detection assay (Labcorp, Research Triangle Park, NC). Among the 153 randomized patients, 18 samples were not available.

Safety

Safety analyses included all enrolled patients with at least one dose of TG4040 or PEG-IFNα/RBV and included all events up until 24 weeks post-treatment. Adverse events (AEs), serious adverse events (SAEs), adverse drug reactions, laboratory abnormalities, and discontinuations as a result of AEs were recorded, assessed, and graded according to the Common Terminology Criteria for Adverse Events version 4.0. All safety data also were monitored by the data safety monitoring committee approximately every 12 weeks.

MVA- and HCV-Specific Immune Responses

MVA- and HCV-specific cell-mediated immune responses induced by TG4040 were evaluated by ELISpot (CTL-Europe GmbH, Bonn, Germany) interferon (IFN)-γ after in vitro restimulation of PBMCs with TG4040-encoded MVA and HCV (NS3, NS4, and NS5B) antigens. TG4040-unrelated NS5A HCV antigen was used as a negative control (Supplementary Materials and Methods section).

Neutralizing anti-MVA antibody titers were determined by a biological assay and patients were considered seropositive when titers were greater than 66 (Supplementary Materials and Methods).

These assessments were performed by Platine Pharma Services (Lyon, France).

Statistical Analyses

The primary end point of the study was the percentage of evaluable patients who achieved cEVR. The evaluable population included all patients with a viral load measurement available 12 weeks after initiating PEG-IFNα/RBV. Primary and secondary end points also were evaluated based on the intent-to-treat (ITT) population, which included all patients who received at least 1 dose of PEG-IFNα/RBV. Secondary end points included the proportion of patients with undetectable HCV-RNA levels at various time points both on (eg, at week 24 and at end of treatment [ETR]) and off treatment (ie, sustained virologic response 12 or 24 weeks after the last dose [SVR12 and SVR24]). The study used a hypothesis testing framework that allowed 3 possible outcomes: the so-called 3-outcome-1-stage design,[9] for the cEVR response rate in arms B and C separately. The null hypothesis in terms of response rate (r) was chosen to be null hypothesis ($r < 40\%$) and the alternative hypothesis was alternative hypothesis ($r > 60\%$). The 40% response rate was based on expected cEVR rates with PEG-IFNα/RBV.[10] The probability of type I error (α, probability of a false-positive result) was assumed to have a value of 2.5%, adjusted for conducting 2 tests (for arms B and C). The probability of type II error (β, probability of a false-negative result) was assumed to have a value of 10%. Under these assumptions, to reject the null hypothesis for 59 and 63 patients, respectively, 32 and 34 responses had to be observed. Two-sided 95% confidence intervals were computed for the primary response rates according to the Wilson method and the P value was determined using the binomial test. Additional exploratory analyses were performed to evaluate the impact of TG4040/PEG-IFNα/RBV relative to PEG-IFNα/RBV alone. Cochran–Manzel–Haenszel tests, stratified by age and viral load at baseline, were used to compare arm A vs B and arm A vs C. A P value less than 2.5% was considered significant. The impact of TG4040 pretreatment on early viral load decrease was analyzed, comparing the difference between viral load just before administration of PEG-IFNα/RBV (day 1 for arms A and B and week 12 for arm C) and 1 week after PEG-IFNα/RBV introduction; arm C was compared with arms A and B combined (PEG-IFNα/RBV only at week 1) by using a t test on the differences after a logarithm transformation of the viral load.

Viral breakthrough was defined as an increase in serum HCV-RNA level of more than 1 $\log_{10}$ IU/mL above nadir, including an HCV-RNA level greater than 100 IU/mL in patients who had undetectable HCV-RNA levels ($<$10 IU/mL) at a previous time point. Virologic relapse was defined as HCV-RNA level greater than 10 IU/mL during post-treatment follow-up evaluation (12 or 24 weeks after the last dose of treatment) after achieving ETR.

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Patient Characteristics

A total of 153 patients were enrolled across 3 treatment arms (31, 63, and 59 patients in arms A, B, and C, respectively). Patient disposition is shown in Figure 1*B*. Baseline characteristics of the patients were similar across treatment arms with a mean age between 41 and 44 years of age and a mean body mass index of approximately 25 kg/m^2 (Table 1). The majority of patients were Caucasian, had the non-CC IL28B allele, and approximately 55% were male. Most patients were infected with genotype 1b HCV and had mean serum HCV-RNA levels approaching 6 $\log_{10}$ IU/mL.

Antiviral Response

On-treatment. The primary end point of the study was achieved in arm C with TG4040 pretreatment. Thus, 34 of 53 (64.2%) evaluable patients achieved cEVR, which was statistically greater than 40% ($P = .0003$), the predetermined threshold of inactivity. A significant improvement also was observed when considering ITT patients (34 of 55; 61.8%; $P = .001$). In addition, using both evaluable and ITT populations, cEVR rates also were statistically higher than in arm A (9 of 30 evaluable patients [30%] and 9 of 31 ITT patients [29%]), with P values of .0037 and .0040, respectively. Overall, patients treated with TG4040 and PEG-IFNα/RBV experienced greater cEVR rates than patients receiving PEG-IFNα/RBV alone (Table 2), however, the difference between arm A and arm B did not reach statistical significance. Numerically greater cEVR rates also were observed for patients in arm C compared with arm A for all subgroups analyzed (HCV genotype [1a, 1b], IL28B

Table 1. Selected Baseline Characteristics of Enrolled Patients According to Treatment Arm

	Arm A (N = 31), control arm	Arm B (N = 63), PEG/RBV lead in	Arm C (N = 59), TG4040 pretreatment arm	*P* value[a]
Age, *y*	41.0 (12.88)	44.0 (11.11)	43.6 (12.07)	.496
Weight, *kg*	71.9 (13.21)	75.6 (17.06)	75.4 (15.08)	.527
Body mass index	25.0 (4.09)	25.9 (4.45)	25.9 (4.59)	.650
Sex, N				.963
Male	16 (51.6%)	36 (57.1%)	32 (54.2%)	
Female	15 (48.4%)	27 (42.9%)	27 (45.8%)	
Race, N (%)	31	63	59	.601
Caucasian or white	30 (96.8%)	60 (95.2%)	59 (100.0%)	
Hispanic or Latino	0 (0.0%)	1 (1.6%)	0 (0.0%)	
Other	1 (3.2%)	2 (3.2%)	0 (0.0%)	
Genotype, N	31	63	59	.897
1a	6 (19.4%)	12 (19.0%)	15 (25.4%)	
1a/b	0 (0.0%)	1 (1.6%)	0 (0.0%)	
1b	25 (80.6%)	50 (79.4%)	44 (74.6%)	
IL28, N	29	59	53	.834
CC	7 (24.1%)	18 (30.5%)	18 (34.0%)	
CT	15 (51.7%)	34 (57.6%)	26 (49.1%)	
TT	7 (24.1%)	7 (11.9%)	9 (17.0%)	
Fibrosis total,[b] N	31	63	59	.409
F2–F3, F3	1 (3.2%)	7 (11.1%)	9 (15.3%)	
Fibrosis by biopsy, Metavir scale, N	17	37	35	.361
F3	0 (0.0%)	5 (13.5%)	6 (17.1%)	
Fibrosis by FibroScan, N	14	27	25	.935
F2–F3, F3	1 (7.1%)	2 (7.4%)	3 (12.0%)	
Viral load, $\log_{10}$ IU/mL	6.0 (0.68)	5.8 (0.85)	5.7 (0.81)	.370

NOTE. The data are mean (SD) or n (%) unless otherwise stated.
[a]For continuous variables the *P* value was obtained by analysis of variance, and for categoric variables the *P* value was obtained by the Fisher exact test.
[b]When available, biopsy data were prioritized.

genotype [C-C, non C-C], age [≤50 or >50 y], HCV-RNA level [≤5.6 or >5.6 $\log_{10}$ IU/mL], body mass index [<25 or ≥25 kg/m^2], sex, and ALT level [<2 or ≥2 times the upper limit of normal], data not shown). Although numbers were small within some subgroups, cEVR rates did not appear to be influenced by known PEG-IFNα/RBV prognostic factors (not shown). Of note, 60% (21 of 35) of non C-C patients had a cEVR response in arm C vs 27.3% (6 of 22) and 36.6% (15 of 41) in arms A and B, respectively.

TG4040 pretreatment caused a significantly steeper initial decrease in HCV-RNA level 1 week after PEG-IFNα/RBV addition with a mean decrease of 1.39 $\log_{10}$ IU/mL compared with 1.00 $\log_{10}$ IU/mL for PEG-IFNα/RBV alone ($P = .04$) (Figure 2). Furthermore, a greater percentage of patients in arm C achieved HCV-RNA level less than the LOD 5 weeks after initiating treatment with PEG-IFNα/RBV compared with either 4 or 5 weeks of treatment in arms A or B (Table 2).

Administration of TG4040 during the period of monotherapy in arm C was associated with modest antiviral activity in 27 of 59 patients (45.8%). Among those 27 patients, 40.7% (11 of 27) experienced a decrease in HCV-RVA level at 1 time point, 25.9% (7 of 27) experienced a decrease in HCV-RVA level at 2 time points, 22.2% (6 of 27) experienced a decrease in HCV-RVA level at 3 time points, and 11.1% (3 of 27) experienced a decrease in HCV-RVA level at more than 3 time points (of a total of 7 measurements at week (W)1, W2, W3, W4, W5, W9, and W12). This transient antiviral activity was between 0.5 and 5.1 $\log_{10}$ IU/mL and all patients went back to their baseline levels by the time PEG-IFNα/RBV was introduced. Among those who completed this pretreatment period, 72% (18 of 25) achieved cEVR (2 patients discontinued early). Proportionally, fewer patients in arm C vs arm A or arm B met the protocol-defined stopping criteria for virologic failure and discontinued treatment (14.5% [8 of 55] in arm C vs 25.8% [8 of 31] in arm A and 25.4% [16 of 63] in arm B), and few patients in both experimental arms experienced virologic breakthrough (4.8% [3 of 63] and 1.8% [1 of 55] in arms B and C).

Sustained virologic response. Overall, a numerically greater proportion of patients pretreated with TG4040 achieved SVR24; 58.2% (32 of 55) in arm C vs 48.4% (15 of 31) in arm A and 50.8% (32 of 63) in arm B (Figure 1*B* and Table 2). Similarly, a numerically greater proportion of patients who completed 48 weeks of PEG-IFNα/RBV in arm C vs arms A or B achieved SVR24: 80% (32 of 40) vs 66.7% (14 of 21) and 76.3% (29 of 38), respectively. Of note, 51.4% (18 of 35) of non C-C patients had a SVR24 response in arm C vs 36.4% (8 of 22) and 46.3% (19 of 41) in arms A and B, respectively. Compared with arm A, fewer patients relapsed in both experimental arms after achieving HCV-RNA level less than the LOD at the end of treatments

Table 2. Primary and Secondary End Points

Proportion of patients, n (%) [95% CI],[a] *P* value[b]	Arm A, control arm	Arm B, PEG/RBV lead in arm	Arm C, TG4040 pretreatment arm
Evaluable	30	61	53
cEVR (LOD)	9 (30.0%)	28 (45.9%)	34 (64.2%)
	[16.7%–47.9%]	[34.0%–58.3%]	[50.7%–75.7%]
		.1613	.0037
cEVR (LOQ)	16 (53.3%)	34 (55.7%)	37 (69.8%)
	[36.1%–69.8%]	[43.3%–67.5%]	[56.5%–80.5%]
		.8313	.1363
Intent to treat	31	63	55
cEVR (LOD)	9 (29.0%)	28 (44.4%)	34 (61.8%)
	[16.1%–46.6%]	[32.8%–56.7%]	[48.6%–73.5%]
		.1733	.0040
cEVR (LOQ)	16 (51.6%)	34 (54.0%)	37 (67.3%)
	[34.8%–68.0%]	[41.8%–65.7%]	[54.1%–78.2%]
		.8543	.1453
RVR[c] (LOD)	2 (6.5%)	6 (9.5%)	13 (23.6%)
	[1.8%–20.7%]	[4.4%–19.3%]	[14.4%–36.3%]
		.6374	.0669
RVR[c] (LOQ)	4 (12.9%)	9 (14.3%)	21 (38.2%)
	[5.1%–28.9%]	[7.7%–25.0%]	[26.5%–51.4%]
		.9197	.0196
Undetectability after 24 weeks of PEG/RBV	21 (67.7%)	39 (61.9%)	42 (76.4%)
	[50.1%–81.4%]	[49.6%–72.9%]	[63.7%–85.6%]
		.5736	.2805
ETR[d] (LOD)	21 (67.7%)	42 (66.7%)	42 (76.4%)
	[50.1%–81.4%]	[54.4%–77.1%]	[63.7%–85.6%]
		.9244	.2562
ETR48[d] (LOD)	21 (67.7%)	35 (55.6%)	37 (67.3%)
	[50.1%–81.4%]	[43.3%–67.2%]	[54.1%–78.2%]
		.2802	.7780
SVR12[e] (LOD)	15 (48.4%)	33 (52.4%)	33 (60.0%)
	[32.0%–65.2%]	[40.3%–64.2%]	[46.8%–71.9%]
		.7414	.2647
SVR24 (LOD)	15 (48.4%)	32 (50.8%)	32 (58.2%)
	[32.0%–65.2%]	[38.8%–62.7%]	[45.0%–70.3%]
		.8646	.3716
SVR24 (LOQ)	15 (48.4%)	32 (50.8%)	33 (60.0%)
	[32.0%–65.2%]	[38.8%–62.7%]	[46.8%–71.9%]
		.8646	.3050

95% CI, 95% confidence interval; LOQ, limit of quantification; RVR, rapid virologic response.
[a]Two-sided Wilson confidence interval for the binomial proportion.
[b]Cochran–Manzel–Haenszel tests, stratified by age and viral load at baseline to compare arm B vs arm A and arm C vs arm A.
[c]Defined as HCV-RNA level < LOD after 4 weeks of PEG-IFNα/RBV treatment in arms A and B, and after 5 weeks of treatment in arm C (no available visit 4 weeks after PEG-IFNα/RBV initiation).
[d]ETR was defined as undetectability at the end of treatments and ETR48 was defined as undetectability after completion of 48 weeks of PEG-IFNα/RBV.
[e]Patients with ETR and SVR24 responses, but no available SVR12 visit, were considered SVR12 responders.

(28.6% [6 of 21] in arm A, 21.4% [9 of 42] in arm B, and 19% [8 of 42] in arm C). This also is true when considering patients who received a full 48-week schedule of PEG-IFNα/RBV treatment (28.6% [6 of 21], 17.1% [6 of 35], and 17.9% [7 of 39]). Of the 17 patients with virologic relapse in arms B and C, all had detectable HCV-RNA levels after 4 or 5 weeks of PEG-IFNα/RBV and 7 of 9 and 4 of 8, respectively, had detectable HCV-RNA levels after 12 weeks of PEG-IFNα/RBV. Similarly to cEVR, SVR24 rates did not appear to be influenced by known PEG-IFNα/RBV prognostic factors in experimental arms as illustrated by Forest plot representations (Supplementary Figure 1).

HCV- and MVA-Specific Cellular Immune Responses

HCV-specific responses were detected against all TG4040-specific HCV antigens in experimental arms (Figure 3*A*). Pre-existing, mostly weak, HCV-specific immune responses were detected sporadically at baseline against NS3 or NS5B in all arms (Figure 3*B*). HCV-specific

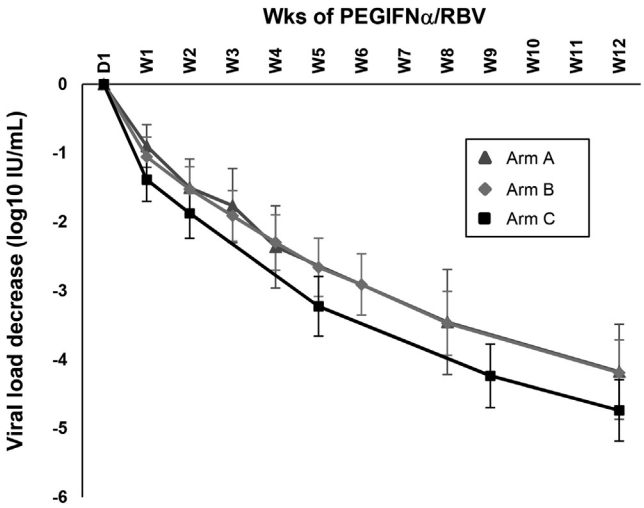


Figure 2. Early decrease in HCV-RNA level. Mean decrease from baseline (day 1 of PEG-IFNα/RBV) $\log_{10}$ HCV-RNA levels until 12 weeks of PEG-IFNα/RBV treatment. HCV-RNA levels measured at week 1 (or week 13 in arm C) reflect a mean decrease from baseline of 0.90, 1.05, and 1.39 $\log_{10}$ IU/mL in arms A, B, and C, showing the benefit of TG4040 pretreatment.

responses induced during therapy were considered mainly TG4040-specific owing to the absence of responses against the TG4040-unrelated NS5A antigen. The majority of TG4040-induced T-cell responses were detected in arm C predominantly against NS3 (46%; 22 of 48 patients) at W9 and/or W12 during the period of TG4040 monotherapy before introduction of PEG-IFNα/RBV (Figure 3*B* and *C*). Specifically, T-cell responses were detected against NS3.1 (20%; 9 of 46 patients), NS3.2 (38%; 18 of 47 patients), NS5B (23%; 8 of 35 patients), and NS4 (18%; 6 of 33 patients) HCV antigens. In arm C, in comparison with baseline, a statistically significant increase in NS3.2-specific T-cell responses was observed after TG4040 monotherapy ($P = .003$), and 6 months after cessation of PEG-IFNα, a significant increase in NS3.1 ($P = .031$), NS3.2 ($P = .041$), and NS5B-specific ($P = .040$) T-cell responses were detected. Of note, polyantigenic T-cell responses were observed in 8 patients, although this probably was underestimated owing to nonanalyzable samples. No significant association was observed between TG4040-specific T-cell responses for any given HCV antigen and cEVR or SVR24: among the 22 of 48 patients with T-cell responses specific to NS3, 59% and 55% achieved cEVR and SVR; similar numbers were observed in the 26 of 48 patients without T-cell responses (62% cEVR and 54% SVR). In contrast, in arms A and B, mostly weak sporadic T-cell responses were observed; mainly against NS3 in arm B (16%; 7 of 43 patients) and only 3 arm A patients developed responses against NS5B (21%; 3 of 14 patients) after treatment with PEG-IFNα/RBV, and therefore were not specific to TG4040 (Figure 3*A* and *B*).

MVA-specific T-cell responses were observed in arms B and C only (Figure 3*A* and *B*). In arms B and C, 53% (8 of 15) and 87% (20 of 23) of evaluable patients had detectable MVA-induced IFN-γ producing T cells after treatment with PEG-IFNα/RBV and TG4040, respectively.

Overall, the level of response was variable (approximately 26–1600 spots), with peak responses during TG4040 monotherapy, which were either maintained or slightly deceased for up to 6 months after cessation of treatment (Figure 3*C*).

MVA-Specific Humoral Responses

Neutralizing anti-MVA antibodies. As expected, at baseline (day 1), 100% (arm A), 97% (arm B), and 95% (arm C) were seronegative for neutralizing anti-MVA antibodies. After TG4040 injections in arms B and C, a significant induction in neutralizing anti-MVA antibody titers was observed (Figure 4*C*, $P < .001$ for both arms). Median titers were low in general and decreased 6 months after cessation of treatment but remained significantly increased. No increase in titers was observed in arm A. In arm B, after TG4040 injections, 49%, 52%, and 60% of patients at weeks 8, 12, and 24 developed detectable neutralizing anti-MVA antibodies (median titers, 188, 224, and 181, respectively), which subsequently significantly decreased at week 72 (31% of patients; median titer, 129; $P < .001$ compared with week 24; Figure 4*A*). In arm C, after TG4040 administration, 66%, 58%, 60%, 66%, and 84% of patients had detectable anti-MVA neutralizing antibodies, respectively, at weeks 9, 12, 14, 24, or 36 (median titers, 222, 261, 230, 203, and 188, respectively), which subsequently decreased at week 84 (42% of patients; median titer, 144; $P < .001$ compared with week 36; Figure 4*B*). In arms B and C, no significant difference in neutralizing anti-MVA antibodies was observed in virologic responders and nonresponders (ie, between patients who achieved cEVR and/or SVR24 and those who did not).

Safety

Adverse events. Overall, more than 90% of patients experienced an AE and a similar proportion of patients across treatment arms experienced AEs, grade 3 or 4 AEs, and SAEs (Table 3). The most common AEs (>20%) in experimental arms included fatigue, pyrexia, neutropenia, anemia, headache, injection site erythema, myalgia, alopecia, and decreased appetite. These AEs are generally consistent with the known AE profile for PEG-IFNα/RBV and with the exception of injection site reactions (which were more frequent in the TG4040 experimental arms), most of these AEs occurred at a similar frequency between arms.

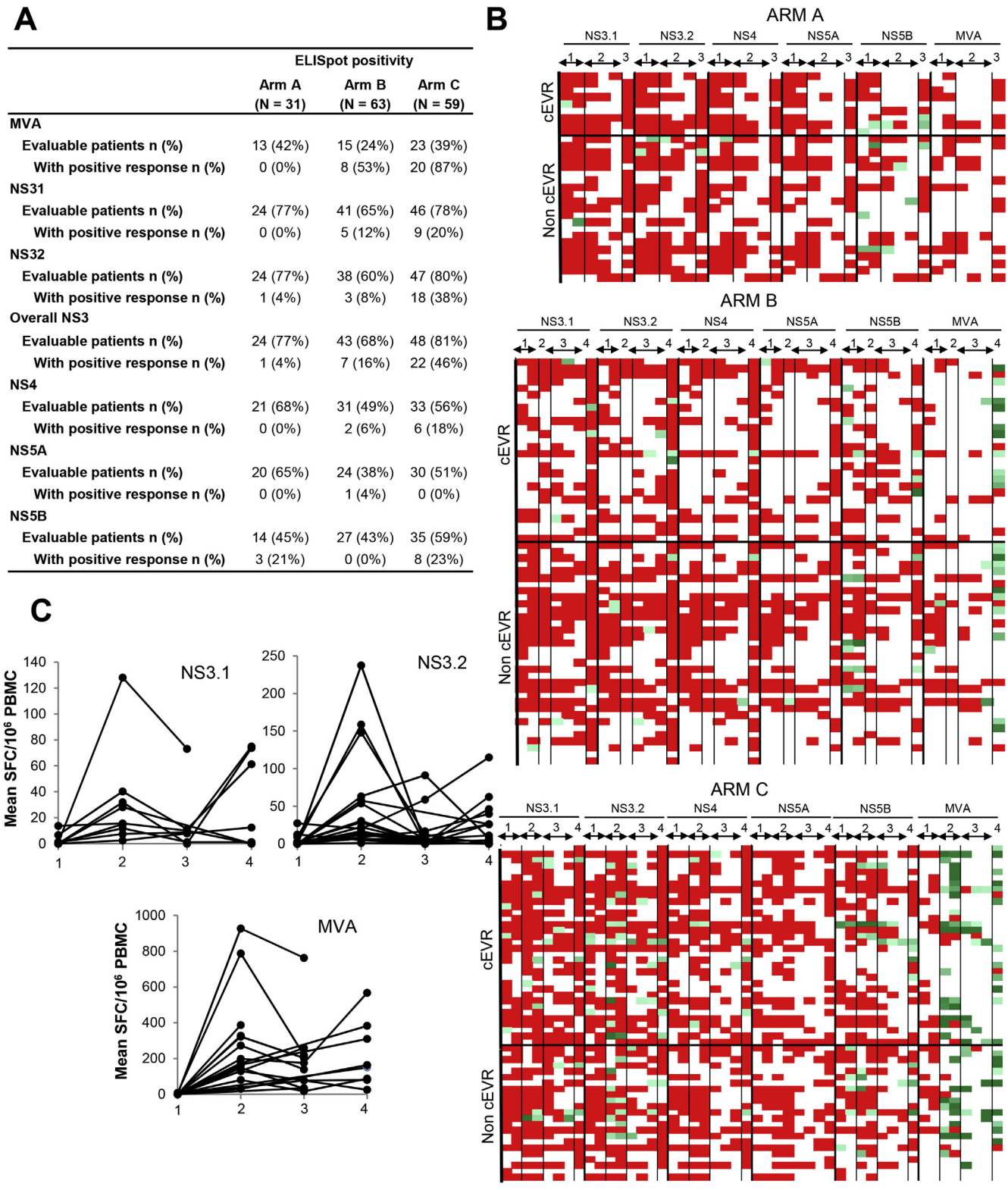
More patients in TG4040 experimental arms discontinued because of AEs, however, few discontinued because of AEs considered related to TG4040 only. Two patients discontinued TG4040 monotherapy, 1 patient because of the development of arthralgia, myalgia, and leukocytoclastic vasculitis, and another patient because of cellulitis, whereas 4 patients discontinued TG4040 and PEG-IFNα/RBV as a result of SAEs considered related to both TG4040 and PEG-IFNα/RBV as discussed later.

SAEs considered related to TG4040 and PEG-IFNα/RBV included severe thrombocytopenia in 3 patients and aplastic anemia in 1 patient. Aplastic anemia occurred after 10.5 weeks of PEG-IFNα/RBV and 10 injections of TG4040

(arm C). Bone marrow analysis showed marked hypocellularity in all hematopoietic cell lines, indicating aplastic anemia of central origin. Also, the presence of lymphoplasmocytes and reactive eosinophils in the bone marrow concomitant to the global depletion of blood cell lines favored immune-allergic mechanisms of toxic or drug-induced origin, which could be explained by the intake of dypirone by this patient in the weeks and days preceding the event (started 8 weeks before the event for dental extraction). This drug is known to induce similar hematologic toxicity.

A

	ELISpot positivity		
	Arm A (N = 31)	Arm B (N = 63)	Arm C (N = 59)
MVA			
Evaluable patients n (%)	13 (42%)	15 (24%)	23 (39%)
With positive response n (%)	0 (0%)	8 (53%)	20 (87%)
NS31			
Evaluable patients n (%)	24 (77%)	41 (65%)	46 (78%)
With positive response n (%)	0 (0%)	5 (12%)	9 (20%)
NS32			
Evaluable patients n (%)	24 (77%)	38 (60%)	47 (80%)
With positive response n (%)	1 (4%)	3 (8%)	18 (38%)
Overall NS3			
Evaluable patients n (%)	24 (77%)	43 (68%)	48 (81%)
With positive response n (%)	1 (4%)	7 (16%)	22 (46%)
NS4			
Evaluable patients n (%)	21 (68%)	31 (49%)	33 (56%)
With positive response n (%)	0 (0%)	2 (6%)	6 (18%)
NS5A			
Evaluable patients n (%)	20 (65%)	24 (38%)	30 (51%)
With positive response n (%)	0 (0%)	1 (4%)	0 (0%)
NS5B			
Evaluable patients n (%)	14 (45%)	27 (43%)	35 (59%)
With positive response n (%)	3 (21%)	0 (0%)	8 (23%)



A

	ARM B (N = 63)					
	D1	W2	W8	W12	W24	W72
Number of samples analyzed	62	60	61	61	57	58
Anti-MVA neutralizing abs n (%)	2 (3%)	2 (3%)	30 (49%)	32 (52%)	34 (60%)	18 (31%)
Median titer of positive samples	95	121	188	224	181	129

B

	ARM C (N = 59)						
	D1	W9	W12	W14	W24	W36	W84
Number of samples analyzed	59	56	55	55	53	50	50
Anti-MVA neutralizing abs n (%)	3 (5%)	37 (66%)	32 (58%)	33 (60%)	35 (66%)	42 (84%)	21 (42%)
Median titer of positive samples	264	222	261	230	203	188	144

C

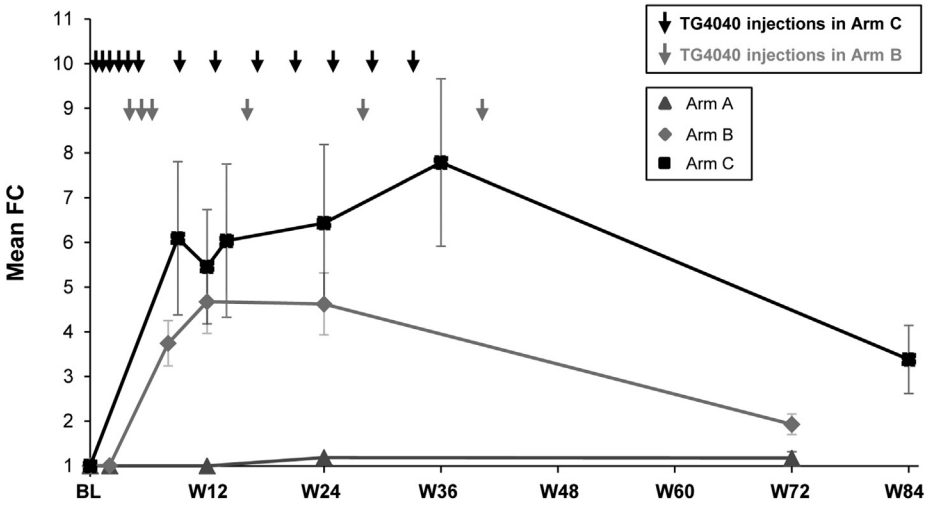


Figure 4. Neutralizing anti-MVA antibodies. (*A* and *B*) Percentage of patients with a positive humoral response and median titer values of positive samples in arms B and C. (*C*) The mean fold change (FC) ± standard error from baseline (day [D]1) of neutralizing anti-MVA antibody titers are shown for each arm. *Overall response evolution over time is statistically significant ($P < .001$).

The severe thrombocytopenia observed in the other 3 patients occurred after 26–48 weeks of PEG-IFNα/RBV and after 4–13 injections of TG4040, with the most recent injection between 2.5 and 6 months before onset. Although platelet transfusions were ineffective in all 3 patients, corticosteroid therapy with or without immunoglobulin infusions led to rapid recovery with improvements in platelet and other blood parameters. These cases of severe thrombocytopenia most likely resulted from peripheral autoimmune mechanisms induced by the combination of known interferon immune side effects potentiated by TG4040 treatment, based on the following evidence: (1) response to corticosteroids, (2) presence of petechial purpura in 2 of the 3 patients, (3) presence of megacaryocytes in bone marrow of 2 of the 3 patients (1 sample could not be analyzed), (4) nonspecific evolution of hematology parameters over time indistinguishable from the other patients in the study, and (5) undetectable HCV

Figure 3. HCV- and MVA-specific T-cell responses. (*A*) HCV- and MVA-specific ELISpot positivity are represented as the percentage of patients with a positive response in arms A, B, and C. Because of PBMC viability after overnight resting, MVA- and HCV-specific T-cell responses were measured in 78%, 56%, and 76% of PBMC samples in arms A, B, and C, respectively. The percentage of evaluable patients was defined as patients with at least one analyzed time point before and after treatment. (*B*) HCV- and MVA-specific ELISpot responses are shown using heat map representation. Each row corresponds to an individual randomized patient and each square corresponds to a study visit as detailed on the bottom of the legend of Figure 3. Green squares represent a positive ELISpot response, shown in light to dark green, with 5 intensities corresponding to fewer than 50, 50–100, 100–150, 150–200, and more than 200 spot forming cells/10^6 PBMCs. Red squares represent a negative ELISpot response (no spot forming cells/10^6 PBMCs). White squares represent nonevaluable or missing samples. (*C*) The mean spot forming cells/10^6 PBMCs is represented for each arm C patient with positive T-cell responses specific to MVA, NS3.1, and NS3.2. (*B* and *C*) Study periods and visits are as follows. Arm A: 1, baseline (M-1, D1); 2, PEG-INFα/RBV (W2, W12, W24); 3, end of study (W72). Arm B: 1, baseline (M-1 [corresponds to the screening visit that occurred 4 weeks before first treatment administration], D1 [corresponds to the day of the first treatment administration]); 2, PEG-INFα/RBV (W2); 3, PEG-INFα/RBV+TG4040 (W8, W12, W24); 4, end of study (W72). Arm C: 1, baseline (M-1 [corresponds to the screening visit that occurred 4 weeks before first treatment administration], D1 [corresponds to the day of the first treatment administration]); 2, TG4040 (W9, W12); 3, PEG-INFα/RBV+TG4040 (W14, W24); 4, end of study (W84).

Table 3. Summary of Adverse Events

Treatment-emergent adverse events, n (%)	Arm A (N = 31), control arm	Arm B (N = 63), PEG/RBV lead-in arm	Arm C (N = 59), TG4040 pretreatment arm
Any AE	28 (90.3%)	58 (92.1%)	58 (98.3%)
Most frequent AE[a]			
Alopecia	4 (12.9%)	8 (12.7%)	13 (22.0%)
Anemia	10 (32.3%)	13 (20.6%)	20 (33.9%)
Cough	5 (16.1%)	7 (11.1%)	7 (11.9%)
Decreased appetite	5 (16.1%)	5 (7.9%)	12 (20.3%)
Dry skin	6 (19.4%)	4 (6.3%)	3 (5.1%)
Fatigue	19 (61.3%)	33 (52.4%)	31 (52.5%)
Headache	7 (22.6%)	14 (22.2%)	18 (30.5%)
Influenza-like illness	8 (25.8%)	11 (17.5%)	9 (15.3%)
Injection site erythema	1 (3.2%)	12 (19.0%)	18 (30.5%)
Injection site induration	0 (0.0%)	3 (4.8%)	9 (15.3%)
Injection site pruritus	0 (0.0%)	4 (6.3%)	9 (15.3%)
Insomnia	7 (22.6%)	9 (14.3%)	8 (13.6%)
Leukopenia	6 (19.4%)	7 (11.1%)	11 (18.6%)
Lymphopenia	5 (16.1%)	3 (4.8%)	9 (15.3%)
Myalgia	4 (12.9%)	14 (22.2%)	8 (13.6%)
Nausea	5 (16.1%)	6 (9.5%)	7 (11.9%)
Neutropenia	11 (35.5%)	23 (36.5%)	20 (33.9%)
Pruritus	6 (19.4%)	11 (17.5%)	9 (15.3%)
Pyrexia	7 (22.6%)	15 (23.8%)	21 (35.6%)
Vitamin D deficiency	2 (6.5%)	9 (14.3%)	9 (15.3%)
Grade 3 or 4 AE	12 (38.7%)	22 (34.9%)	20 (33.9%)
SAE	3 (9.7%)	7 (11.1%)	4 (6.8%)
Related to any study drug	1 (3.2%)	5 (7.9%)	3 (5.1%)
Related to PEG/RBV	1 (3.2%)	3 (4.8%)	1 (1.7%)
Related to TG4040 and PEG/RBV	NA	2 (3.2%)	2 (3.4%)
Unrelated to study drugs	2 (6.5%)	4 (6.3%)	1 (1.7%)

[a]Individual adverse events occurring in more than 15% of patients in any treatment arm.

RNA at the time of the events, minimizing an HCV-specific pathogenic mechanism. All 3 patients who developed thrombocytopenia (but not the patient with aplastic anemia) shared the same class II HLA allele (HLA-DRB01*04), which is known to be associated with a predisposition to autoimmune disease.

Laboratory abnormalities. With the exception of grade 3 or 4 hematology laboratory parameters as described earlier, few other patients experienced a grade 3 or 4 laboratory abnormality (Supplementary Table 1). Consistent with the known hematologic effects of PEG-IFN, the majority of patients experienced neutropenia with a grade 3 or 4 decrease in neutrophil counts. PEG-IFN–induced thrombocytopenia was generally of lesser severity (except in the cases highlighted earlier) with treatment-emergent grade 1 platelet counts (67.7%, 44.4%, and 49.2% in arms A, B, and C, respectively). Anemia also was observed, but with the majority of patients experiencing grade 2 hemoglobin values (22.6%, 17.5%, and 22.9% in arms A, B, and C, respectively).

Discussion

In this phase 2, randomized study, both the safety and efficacy of the immunotherapeutic TG4040 were evaluated alone and in combination with PEG-IFNα/RBV for the treatment of genotype 1 HCV infection. Because it is well established that failure to achieve EVR accurately predicts a subsequent lack of SVR,[11–13] the study was designed to evaluate the proportion of patients with a cEVR to determine the possible added benefit of combining TG4040 with PEG-IFNα/RBV. Treatment with the combination of TG4040 and PEG-IFNα/RBV in arm C met the study primary objective and showed 30% more cEVR responses than with PEG-IFNα/RBV alone, suggesting an early complementary role for TG4040 in the treatment of chronic HCV infection. The group with the PEG-IFNα/RBV lead-in dosing and the less frequent TG4040 injections (arm B) had an intermediate response. TG4040-pretreated arm C patients also had a statistically significantly steeper initial slope of decline in the viral load, a greater proportion of patients achieving undetectable HCV RNA 5 weeks after PEG-IFNα/RBV introduction, and a greater proportion of patients achieving both ETR and SVR24 with, however, no statistical difference compared with arm A.

Although other immunotherapy-based products have been evaluated for HCV treatment,[6,14–17] none have documented the striking effects observed in arm C of this study, regardless of the end point (ie, rapid virologic response, cEVR, ETR, or SVR). Arm C included both TG4040 pretreatment and a higher frequency and longer duration of TG4040 treatment, thus it is difficult to conclude which of

these parameters was most important, possibly both. Of note, in an oncology study, frequent and prolonged administration of a similar MVA-based immunotherapeutic resulted in higher efficacy than a schedule with a lower frequency of injections.[18] On the other hand, we observed only minimal MVA- and HCV-specific cellular immune responses in this study after the introduction of PEG-IFNα/RBV, suggesting that this combination may have had a negative effect on induction and/or detection of T-cell responses, thus potentially decreasing TG4040 efficacy. Although up to 44% of PBMC samples were not available to allow for a full dose-range evaluation, the negative effect of PEG-IFNα could be owing to its known pro-apoptotic and antiproliferative properties.[19–22] This effect possibly combined with the appearance of T-cell escape mutants (which were not analyzed here) could explain why the significantly higher cEVR observed in arm C did not translate into SVR24. TG4040-induced HCV-specific cellular immune responses were observed predominantly in arm C before PEG-IFNα/RBV introduction, mainly against NS3 and NS5B antigens, and this cellular immune response is believed to have potentiated the activity of PEG-IFNα/RBV. Although we were unable to detect a correlation between induced T-cell responses and virologic responses, these data nonetheless are encouraging and support an immunotherapeutic role for TG4040. Given the higher response rates in the TG4040-pretreated arm, TG4040 may be influencing the T-cell response in the liver to a much greater extent than what was detected in circulating PBMCs and/or might activate other nonspecific innate immune effects.

TG4040 in combination with PEG-IFNα/RBV was relatively well tolerated, with the majority of patients experiencing AEs consistent with the known safety profile of both PEG-IFNα/RBV and MVA-based vaccines.[23–25] In 3 patients, an apparently autoimmune-like thrombocytopenia associated with the use of TG4040 and PEG-IFNα/RBV was observed; these events resolved with corticosteroid treatment with or without the use of intravenous immunoglobulins, and represented a consistent pattern of HCV patients with immune thrombocytopenia.[26] Both severe thrombocytopenia and thrombocytopenic purpura also have been reported with PEG-IFNα/RBV alone.[27] Interestingly, the HLA-DRB1*04 allele was identified in the 3 patients with the severe thrombocytopenia. This allele has been associated not only with thrombocytopenic purpura[26] but also other autoimmune disorders such as multiple sclerosis,[28] arthritis,[29] alopecia areata,[30] and autoimmune gastritis.[31] In general, some HLA alleles have been found to be associated with autoimmune and infectious diseases with different mechanisms to explain the associations (eg, linkage disequilibrium with the relevant disease susceptibility gene, preferential presentations of the pathogenic peptide by specific HLA molecules, and molecular mimicry between pathogenic peptides and host-derived peptides). Notably, grade 3 hematologic events were not observed during the first 12 weeks of TG4040 monotherapy before the addition of PEG-IFNα/RBV or in previous phase I studies. These data support the hypothesis that TG4040 could have potentiated a PEG-IFNα–associated thrombocytopenic autoimmune response possibly favored by a shared HLA group predisposing to autoimmune disorders.

Although TG4040 probably will not be combined with PEG-IFNα/RBV in the future, a role for immunotherapeutics in the treatment of HCV infection seems very attractive[7] in a scientific landscape moving toward IFN-free regimens.[32] Regimens combining direct-acting antivirals and RBV are indeed promising, with SVR rates reaching more than 90% in genotype 1 patients, but there is a remaining place for immunomodulators and more particularly in hard-to-treat patients. Immune activation with an immunotherapeutic such as TG4040 may portend a potential long-term advantage in achieving SVR and preventing relapse, as suggested by preliminary evidence of lower T-cell responses in relapser patients,[16,33] particularly when combined with direct-acting antivirals. In summary, this study was a large-scale trial showing an improved virologic response rate with an immunotherapy combined with PEG-IFNα/RBV in chronic hepatitis C. This proof-of-concept study warrants further evaluation in the context of IFN-free regimens. More broadly, this study also illustrates the potential value of viral vector–based immunotherapy for the treatment of chronic infections including viral hepatitis B.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.03.007.

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Conflicts of interest
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Supplementary Materials and Methods

Determination of Neutralizing Anti-MVA Antibody Titers

Neutralizing anti-MVA antibody titers were determined by a biological assay that measured the capacity of patient's serum to neutralize infection of BHK-21 cells (ATCC, Rockville, MD) by a recombinant MVA carrying the green fluorescent protein (MVA-GFP). Briefly, MVA-GFP was incubated with decomplemented patient serum for 1 hour, and then used to infect BHK-21 cells for an additional 16–18 hours at 37°C in a 96-well plate. Each sample was tested in 7 serial 2-fold dilutions including the initial 50-fold diluted serum sample. For each plate, the fluorescence intensity of BHK-21 cells infected with MVA-GFP without serum corresponded to the 100% fluorescence intensity level. The cut-off value corresponding to a neutralizing antibody titer of 50% inhibition of MVA-GFP infection of 66 was established. Patients were considered seropositive for neutralizing anti-MVA antibodies when titers were greater than 66. This cut-off value was based on the mean of the dilution factor plus the Student t test standard deviation from 45 seronegative patients born before 1981 for whom 50% of neutralizing activity was obtained. Titers of less than 50 were replaced by $50/\sqrt{2}$. A mixed model was applied for each arm to analyze the impact of the time on the titers. The Tukey post hoc test then was performed to compare time points.

Analysis of HCV and MVA-Specific Cellular Immune Responses

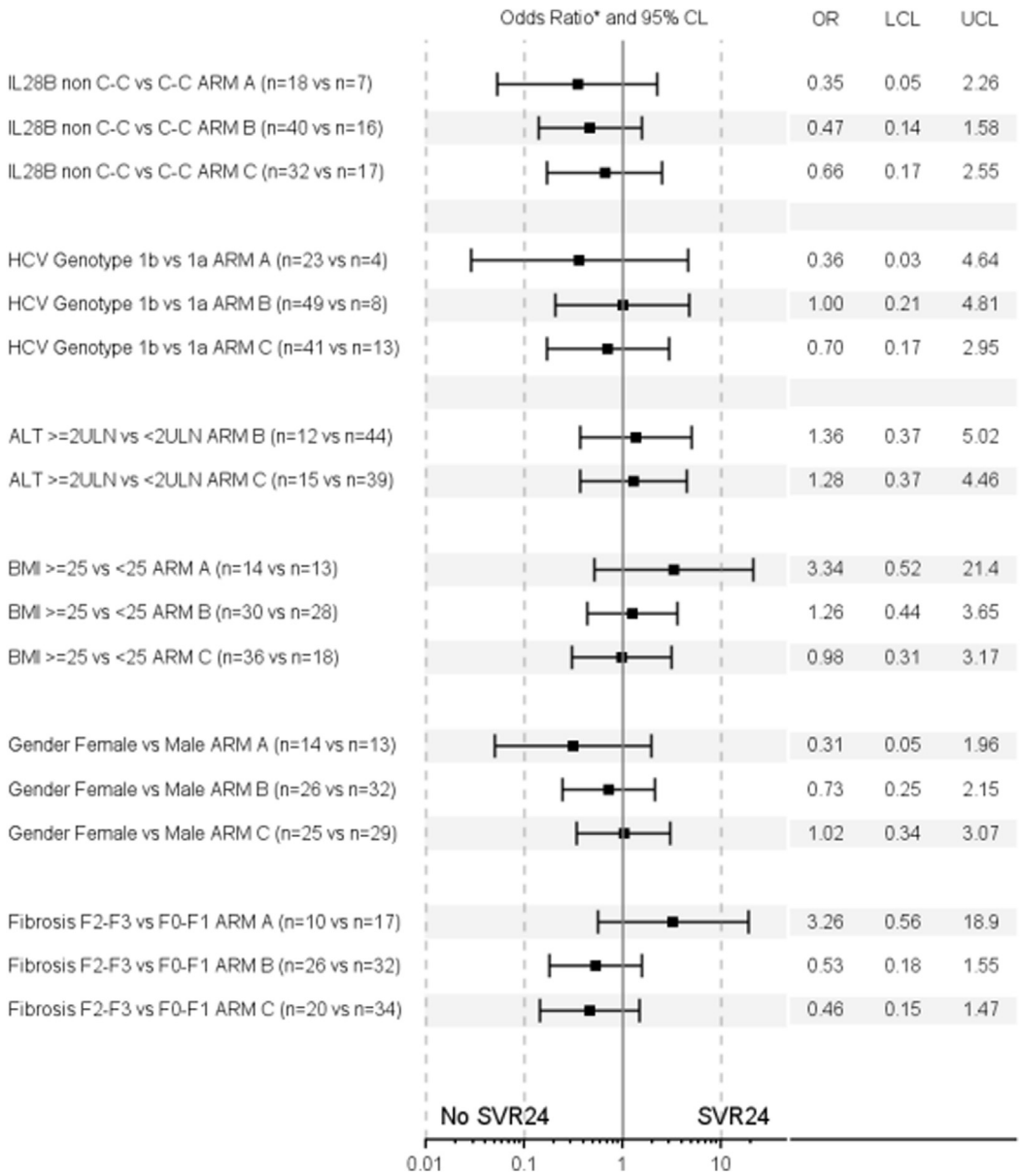
Immune responses were evaluated by IFN-γ ELISpot assays after stimulation with peptide pools using frozen PBMCs. For HCV-specific T-cell responses, 15-mers with 11 amino acids overlap (derived from the vaccine-expressed immunogenic sequences) were used in 5 peptide pools comprising 135 NS3 peptides (split in 2 pools of 70 [NS3/1 pool, N-terminal half], and 65 [NS3/2 pool, C-terminal half] peptides), 56 NS4B peptides were used as a single pool, and 59 NS5A peptides and 119 NS5B peptides were used for immune analysis. For MVA-specific cellular responses, the empty MVA vector (MVATGN33) was used. After overnight resting, PBMC counting and viability was assessed by a cell counter after staining with anti-CD45 and a dead cell marker (7-AAD). Cells then were seeded in human IFN-γ ELISpot plates (Mabtech AB, Hamburg, Germany) and stimulated with HCV peptide pools (0.3 μg/mL), MVATGN33, anti-CD3 antibody, or negative control (culture medium with 0.3% dimethyl sulfoxide) for 40 hours at 37°C, 5% CO_2 in quadruplicate. Spot forming cells were detected following the manufacturer's instructions and counted using an automated ELISpot reader (Immunospot Series 5 Micro ELISpot Analyzer; CTL, Bonn, Germany) and the ImmunoCapture 6.3.3 and ImmunoSpot 5.0.3 Professional Software (Cleveland, OH). For each patient, each antigen, and each time point, positive responses were determined by the Distribution Free Resampling(eq) method.[1] To determine a positive response against a given antigen, a criterion of eligibility was defined. A patient was evaluable for a given antigen if he had at least one time point evaluable before and after treatment. Patients were considered T-cell responders if they experienced a pre-existing T-cell response increase or became positive after treatment.

To evaluate the effect of the treatment on ELISpot T-cell responses, a mixed model was applied on ELISpot data normalized by the negative control, for arms B and C. The following periods were compared: before treatment, during TG4040 pretreatment (for TG4040 pretreatment arm only), during PEG-IFNα/RBV alone (for PR lead-in arm only), during TG4040 in combination with PEG-IFNα/RBV, and after treatment. If several time points were available for a given period, the mean of these time points was performed to consider only one value for each period.

Reference

1. **Moodie Z, Price L**, Gouttefangeas C, et al. Response definition criteria for ELISPOT assays revisited. Cancer Immunol Immunother 2010;59:1489–1501.

Author names in bold designate shared co-first authorship.



Supplementary Figure 1. Forest plot for SVR24 responses according to baseline parameters. Odds ratio (OR), comparing two subgroups, are represented with 95% confidence interval (LCL, Lower Confidence Interval and UCL, Upper Confidence Interval) for each parameter and each treatment arm.

Supplementary Table 1. Summary of Treatment-Emergent Grade 3 or 4 Laboratory Abnormalities

Parameter grade	Arm A, control arm (N = 31)	Arm B, PEG-IFNα/RBV lead-in arm (N = 63)	Arm C, TG4040 pretreatment arm (N = 59)
Hemoglobin			
Grade 3 (<8 g/dL)	0	1 (1.6)	1 (1.7)
Platelet			
Grade 3 (<50.0 to 25.0 $\times$ 10^9/L)	0	0	1 (1.7)
Grade 4 (<25.0 $\times$ 10^9/L)	0	1 (1.6)	2 (3.4)
Leukocytes			
Grade 3 (<2.0 to 1.0 $\times$ 10^9/L)	12 (38.7)	21 (33.3)	21 (35.6)
Grade 4 (<1.0 $\times$ 10^9/L)	0	0	1 (1.7)
Neutrophils			
Grade 3 (<1.0 to 0.5 $\times$ 10^9/L)	13 (41.9)	36 (57.1)	30 (50.8)
Grade 4 (<0.5 $\times$ 10^9/L)	2 (6.5)	3 (4.8)	2 (3.4)
Lymphocytes			
Grade 3 (<0.5–0.2 $\times$ 10^9/L)	6 (19.4)	13 (20.6)	9 (15.3)
Grade 4 (<0.2 $\times$ 10^9/L)	0	0	1 (1.7)
Potassium			
Grade 3 (>6.0 to 7 mmol/L)	0	2 (3.2)	1 (1.7)
International normalized ratio			
Grade 3 (>2.5 $\times$ baseline)	0	0	1 (1.7)
Alanine aminotransferase level			
Grade 3 (>5.0 to 20.0 $\times$ ULN)	1 (3.2)	3 (4.8)	3 (5.1)
Aspartate aminotransferase level			
Grade 3 (5.0–20.0 $\times$ ULN)	0	1 (1.6)	1 (1.7)
Total bilirubin level			
Grade 3 (>3.0 to 10.0 $\times$ ULN)	1 (3.2)[a]	0	0
γ-Glutamyltransferase			
Grade 3 (>5.0–20.0 $\times$ ULN)	1 (3.2)	2 (3.2)	2 (3.4)
Grade 4 (>20.0 $\times$ ULN)	0	0	1 (1.7)
Hyperglycemia			
Grade 3 (>250 to 500 mg/dL)	1 (3.2)	1 (1.6)	0

NOTE. Only patients with laboratory values lower than grade 3 or 4 at baseline that increased to either grade 3 or 4 post-baseline through post-treatment week 24 were considered treatment emergent.

[a]No baseline or screening value was available so we were unable to ascertain whether the abnormality was treatment emergent.