including older age, high viral load, and a high proportion of IL28B genotype CT in the null responders.

Detectable HCV RNA was cleared rapidly; viral suppression was greater at all timepoints than reported results with alfa/RBV combined with telaprevir or TMC435 in genotype 1 null responders.[4, 20] The slightly greater early viral suppression in ineligible/intolerant patients may reflect the higher frequency of IL28B CC genotype in this group. In the overall population, early virologic response was greater in patients with CC genotype, although this difference disappeared by week 12. Potentially, CC genotype may increase early viral suppression by increasing responsiveness to endogenous interferons that are released as a result of the rapid antiviral activity of the dual DAA therapy, allowing reversal of HCV-induced immunosuppression.[21]

These results in patients with HCV genotype 1b differ from those reported for genotype 1a. In a similar study of US/European null responders, 2/9 patients with genotype 1a achieved SVR with daclatasvir + asunaprevir dual therapy, compared with 10/10 patients with genotype 1a who received quadruple therapy combining daclatasvir and asunaprevir with alfa/RBV.[8] This difference suggests that viral genotype can influence responses to DAA regimens, and outcomes can be optimized by individualized therapy that considers viral genotype.

The two populations included in this study represent substantial numbers of patients worldwide. Approximately 10% of HCV genotype 1-infected patients receiving alfa/RBV have a null response.[22] The cumulative prevalence of alfa/RBV null responders and the frequent failure of retreatment with current regimens together suggest that a large population of null responders is awaiting improved therapies. The population of alfa/RBV ineligible or intolerant patients has not been studied extensively but may be substantial. In the IDEAL study, 23.2% of the 4469 patients

screened were considered ineligible for alfa/RBV therapy; of these, 30.3% had hematologic or psychiatric conditions that may not preclude DAA-only regimens.[23] In registration trials, 9.7% to 14% of patients receiving alfa/RBV discontinued study treatment due to intolerance.[24, 25] Moreover, these clinical trial data are likely to underestimate the true size of the ineligible and intolerant populations in community practice.

Virologic failures occurred relatively late in therapy after extended periods with undetectable HCV RNA. All seven patients with virologic failure had emergent NS5A and NS3 mutations that together confer high-level resistance to both daclatasvir and asunaprevir *in vitro*.[11, 12] Pretreatment, NS5A-Y93H was detected in five of the seven patients with virologic failure and in five additional patients who achieved SVR, suggesting that pre-existing Y93H is loosely associated with virologic failure but is not an absolute predictor. Pharmacokinetics may also have contributed; nearly all patients with virologic failure had trough plasma concentrations of daclatasvir and asunaprevir below their respective median values. However, SVR was achieved by most patients with trough drug levels below the median, and by several patients who discontinued study treatment after 2–16 weeks. Thus, the relationship of drug exposure to virologic outcome remains uncertain; further study is needed to define on-treatment predictors of outcome and the optimal duration of therapy.

Current data do not fully explain observed differences between the two study populations in rates of virologic failure and SVR. IL28B genotype was the primary difference between the two populations pretreatment. All three breakthroughs occurred in ineligible/intolerant patients with the unfavorable IL28B CT genotype; however, null responders had no breakthroughs despite a much higher frequency of this genotype. Pre-existing resistance-associated polymorphisms and

plasma drug concentrations were similar across populations, but only ineligible/intolerant patients experienced failure. Analysis of baseline parameters failed to identify other factors that may have influenced outcomes. However, these analyses were limited by the relatively small study population and may have been confounded by unreported non-adherence or baseline parameters not quantified absolutely, such as the stage of liver fibrosis. This issue requires further study in larger populations to confirm the apparent difference in outcomes and to identify factors predictive of virologic failure.

The adverse event profile of the study regimen was generally more favorable than that typically observed with alfa/RBV-containing regimens.[26] There were no significant hematologic or psychiatric abnormalities; the most common adverse events were non-specific in nature and generally mild to moderate in intensity. Mild diarrhea was experienced by 26% of study patients, consistent with previous studies of asunaprevir and other drugs of this class.[4, 6, 14] The four observed grade 3/4 ALT elevations resolved with continued therapy or after discontinuation and were not associated with significant clinical events. A role for study drugs in the reported serious adverse events cannot be ruled out except for the gastroenteritis; however, four of the six events resolved spontaneously with continued treatment. The case of hyperbilirubinemia with gastroenteritis was complicated by multiple confounding factors, and the contribution of study drugs is uncertain.[7]

In conclusion, dual oral therapy with daclatasvir and asunaprevir elicited rapid clearance of detectable HCV RNA and achieved high rates of SVR in two difficult-to-treat patient populations. These results confirm initial findings that HCV genotype 1b infections can be cured with daclatasvir combined with asunaprevir, without alfa/RBV.[7, 8] Thus, this regimen has potential to offer

effective treatment to null responders who have previously shown little or no response to alfa/RBV, and to alfa/RBV ineligible/intolerant patients who have no current treatment options. Further research will assess the benefits of this and other DAA combinations in larger and more diverse patient populations, but the promise of all oral and well-tolerated HCV therapy is on the horizon.

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FIGURE LEGENDS

Fig. 1. Patient disposition.

Patient flow through treatment and follow-up is shown. d/c, discontinuation of study medication; SVR_4, SVR_{12} and SVR_{24}, sustained virologic response 4, 12 or 24 weeks posttreatment. [a]On-study follow-up continued to posttreatment week 4; HCV RNA remained undetectable at posttreatment week 24 after study discontinuation, reported as failure for SVR_{24} per statistical protocol requirements; [b]HCV RNA was undetectable at posttreatment week 24 after study discontinuation due to addition of alfa/RBV, reported as failure for SVR per statistical protocol requirements; [c]On-study follow-up to assess SVR continued after discontinuation of study drugs.

Fig. 2. Outcomes by IL28B genotype.

Virologic outcomes at milestone time points are shown for the overall population by IL28B (rs12979860) genotype. End of treatment is week 24 or the last on-treatment visit for patients who discontinued early. RVR, rapid virologic response; cEVR, complete early virologic response; SVR_{12} and SVR_{24}, sustained virologic response 12 or 24 weeks posttreatment.

Fig. 3. HCV RNA levels, individual patients.

Serum HCV RNA levels over time are shown for each patient. Panel A, null responders; panel B, ineligible/intolerant patients. EOT, end of treatment; SVR_{24}, sustained virologic response 24 weeks posttreatment; LLOQ, lower limit of quantitation=15 IU/mL.

Fig. 4. Daclatasvir and asunaprevir trough plasma concentrations.

Available trough plasma concentrations of asunaprevir and daclatasvir for individual patients are plotted and color-coded according to each patient's virologic outcome. Multiple determinations are shown for some patients. *Indicates values from a single patient with documented noncompliance.

Figure 1

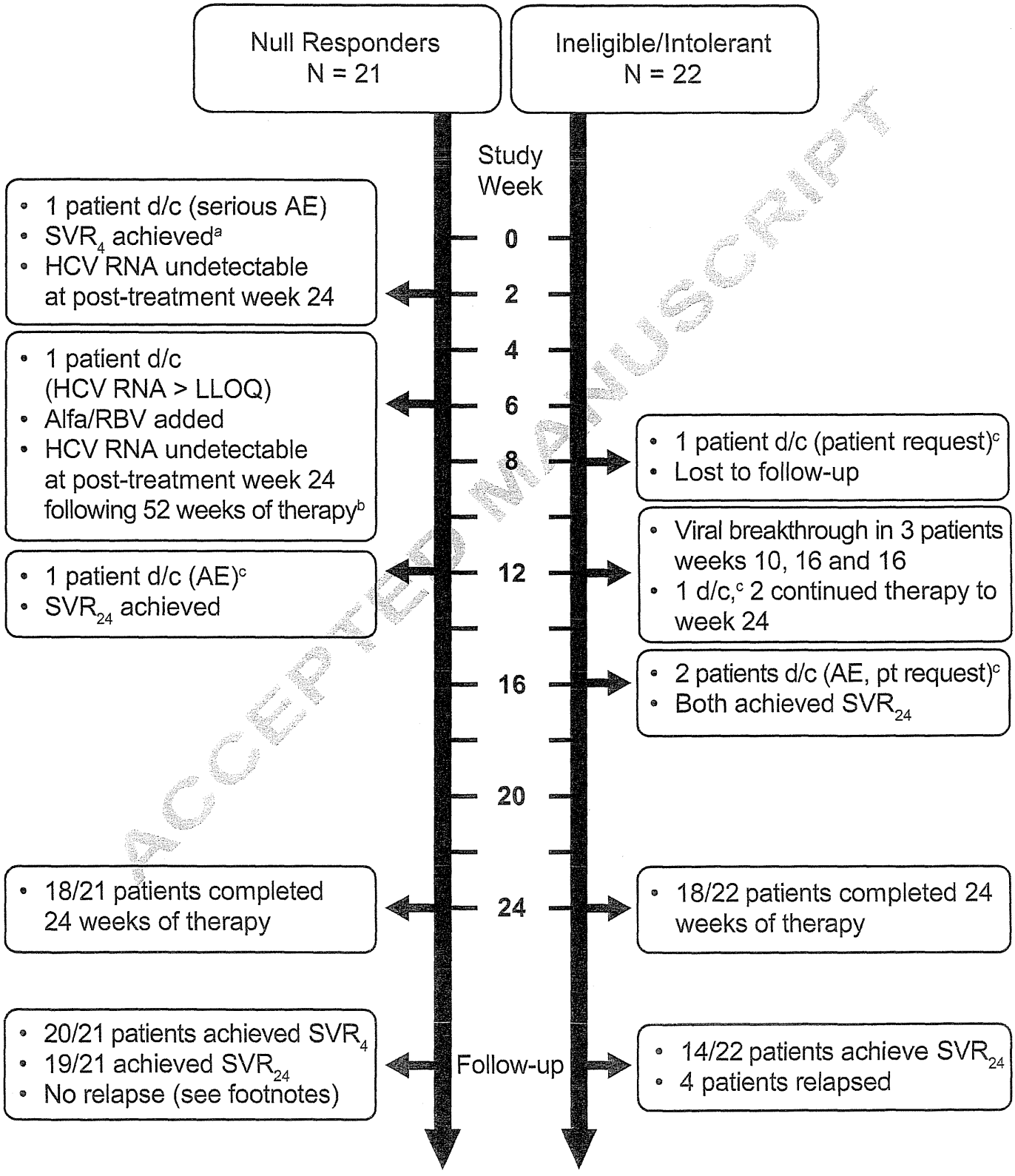


Figure 2

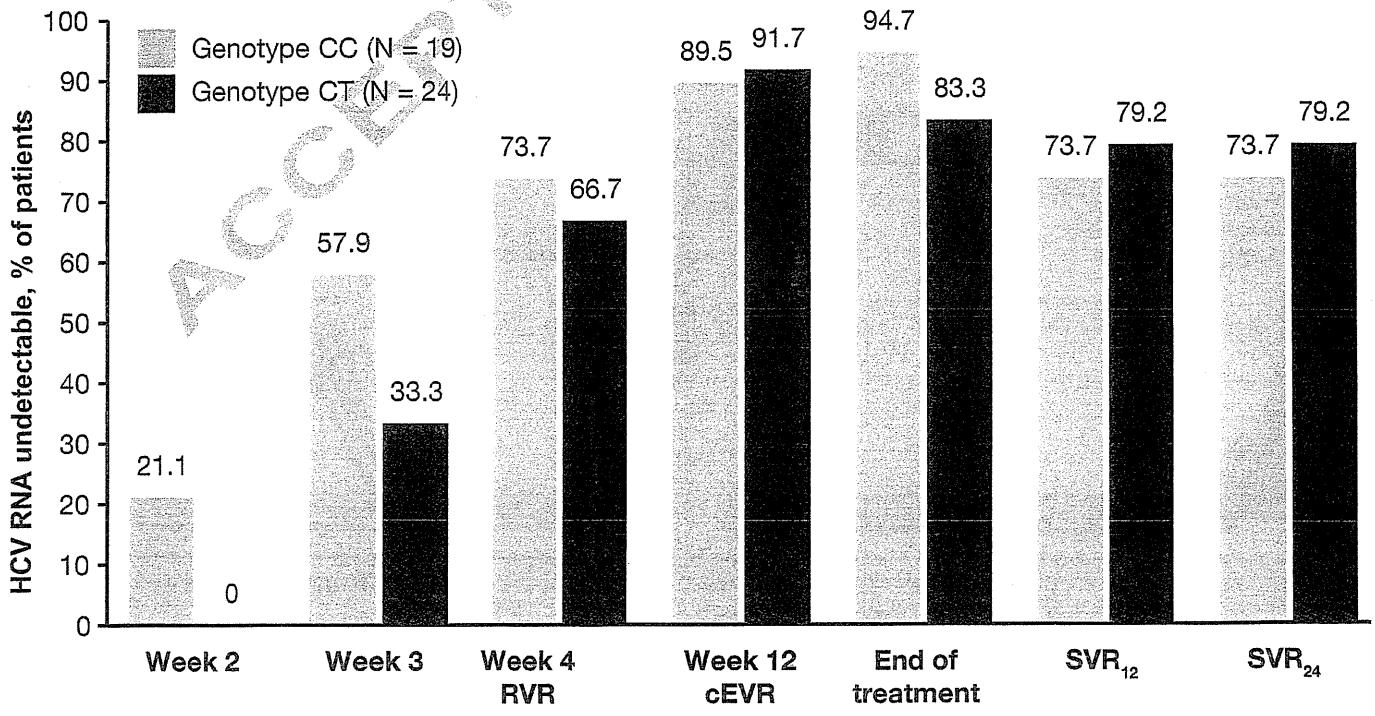
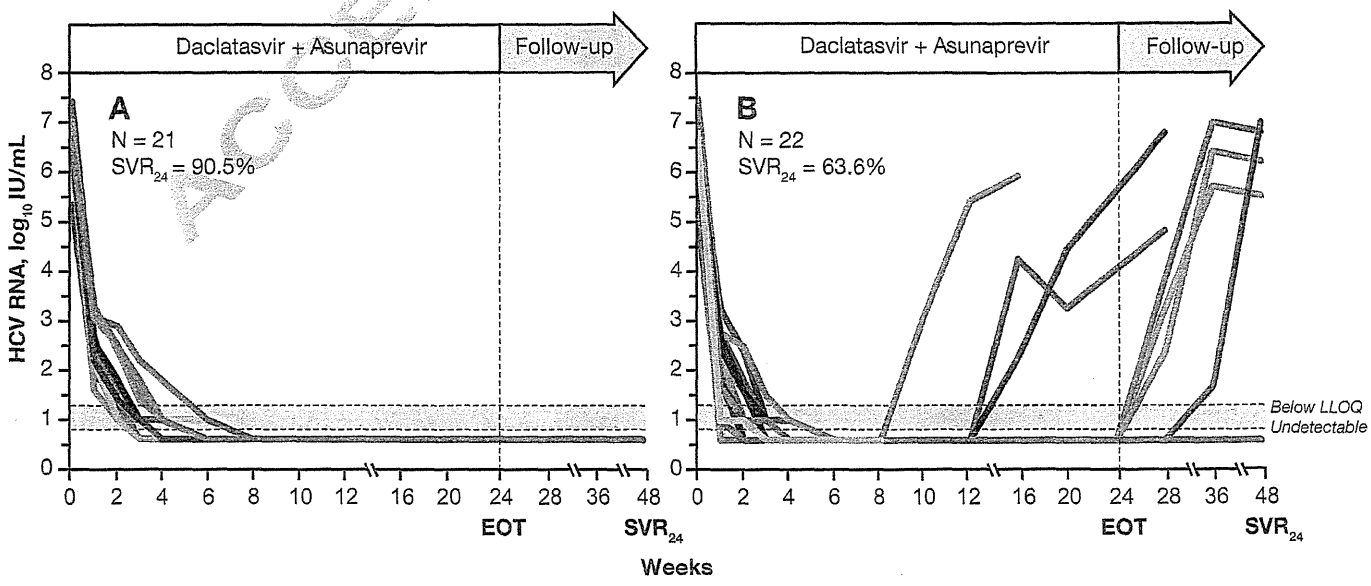
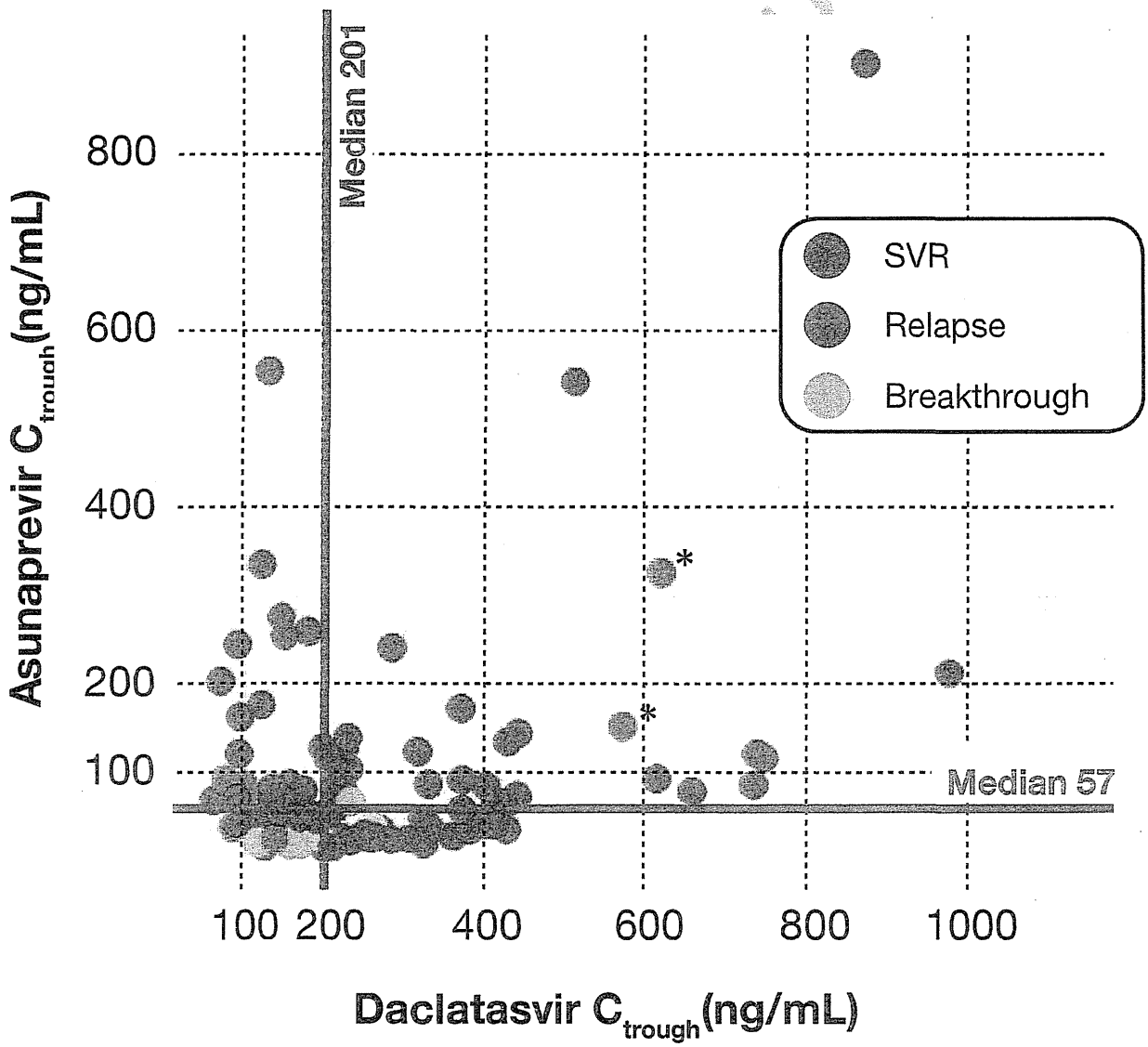


Figure 3



LLOQ, lower limit of quantitation = 15 IU/mL

Figure 4



TABLES

Table 1. Baseline demographic and disease characteristics

Parameter		Null Responders (N=21)	Ineligible/Intolerant (N=22)
Age, median years (range)		61 (31–70)	68 (47–75)
Male, n (%)		8 (38.1)	6 (27.3)
HCV genotype 1b, n (%)		21 (100)	22 (100)
IL28B genotype, n (%)			
(rs12979860)	CT	18 (85.7)	6 (27.3)
	CC	3 (14.3)	16 (72.7)
HCV RNA, mean log_{10} IU/mL (SD)		6.8 (0.47)	6.6 (0.64)
ALT, mean U/L (SD)		57.9 (24.86)	45.7 (25.79)
APRI score			
Score >2, n (%)		3 (14.3)	1 (4.5)
Median (range)		(0.24–3.41)	(0.40–2.79)
Alfa/RBV ineligible, n (%)		na	18 (81.8)
Alfa/RBV intolerant, n (%)		na	4 (18.2)

Table 2. Virologic outcomes

n (%)	Null Responders (N=21)	Ineligible/Intolerant (N=22)
HCV undetectable, week 4 (RVR)	11 (52.3)	19 (86.4)
HCV undetectable, week 12 (cEVR)	19 (90.5)	20 (90.9)
HCV undetectable, end of treatment	19 (90.5)	19 (86.4)
SVR_4	20 (95.2)[1]	15 (68.2)[2]
SVR_{12}	19 (90.5)[1]	14 (63.6)[2]
SVR_{24}	19 (90.5)[1]	14 (63.6)[2]
Viral breakthrough	0	3 (13.6)
Posttreatment relapse	0	4 (18.2)

Intention to treat (missing=failure) analysis. End of treatment is week 24 or last on-treatment visit for patients who discontinued early. RVR, rapid virologic response; cEVR, complete early virologic response; SVR_4, SVR_{12}, and SVR_{24}, sustained virologic response 4, 12 or 24 weeks posttreatment. [1]Two patients discontinued from the study before completion of follow-up. One patient received added alfa/RBV per protocol criteria and is counted as failure for SVR_4, SVR_{12}, and SVR_{24} for DAA-only therapy; one patient had missing HCV RNA data for follow-up weeks 12 and 24 and is counted as failure for SVR_{12} and SVR_{24} per statistical protocol. [2]One patient was lost to follow-up for assessment of SVR_{12} and SVR_{24}.

Table 3. Resistance-associated polymorphisms in patients with virologic failure

Patient			NS5A				NS3	
			L31	Q54	P58	Y93	Q80	D168
Viral Breakthrough	1	Baseline	L/M			Y/H		
		Post-VBT	M		A	H		A
	2	Baseline		Y		Y/H	L	
		Post-VBT	M	Y		H		V
	3	Baseline		Y		H		
		Post-VBT	M	Y		H		V
Posttreatment relapse	4	Baseline			P/S	Y/H		
		Post-relapse	M			H		A
	5	Baseline			L			
		Post-relapse	M		L	H		V/D
	6	Baseline						
		Post-relapse	V			H		V
	7	Baseline				H		
		Post-relapse	V/M			H		V

Table 4. Most frequent adverse events and laboratory abnormalities

Event, n (%)		Null Responders (N=21)	Ineligible/Intolerant (N=22)
Adverse Events Occurring in ≥ 3 Patients in Either Group	Headache	8 (38)	6 (27)
	Nasopharyngitis	6 (29)	8 (36)
	ALT increase	6 (29)	6 (27)
	Diarrhea	9 (43)	2 (9)
	AST increase	6 (29)	4 (18)
	Pyrexia	3 (14)	5 (23)
	Eosinophilia	1 (5)	4 (18)
	Abdominal discomfort	3 (14)	2 (9)
	Malaise	2 (10)	3 (14)
	Constipation	2 (10)	3 (14)
	Back pain	3 (14)	1 (5)
	Decreased appetite	0	3 (14)
Grade 3 or 4 Lab Abnormalities	ALT	2 (10)	2 (9)
	AST	1 (5)	2 (9)
	Lymphocytes	2 (10)	1 (5)
	Phosphorus	1 (5)	1 (5)
	Bilirubin, total	1 (5)	0

	Leukocytes	1 (5)	0