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**Neutralizing antibodies to interferon alpha and circulating Interferon in chronic hepatitis C patients non-responding to pegylated interferon plus ribavirin re-treated by Peg-Interferon alpha-2a and ribavirin (ANRS HC16 GAMMATRI substudy\*)**

Philippe Halfon, Sophie Pérusat, Marc Bourlière, Jean-Pierre Bronowicki, Pascale Trimoulet, Yves Behnamou, Vincent Leroy, Patrick Marcellin, Juliette Foucher, Guillaume Pénaranda, et al.

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**Neutralizing antibodies to interferon alpha and circulating Interferon in chronic hepatitis C patients non-responding to pegylated interferon plus ribavirin re-treated by Peg-Interferon alpha-2a and ribavirin (ANRS HC16 GAMMATRI substudy\*)**

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Keywords:	second line treatment, neutralizing antibody, hepatic C, non-responders



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3 **Neutralizing antibodies to interferon alpha and circulating interferon in patients**  
4 **with chronic hepatitis C non-responding to pegylated interferon plus ribavirin re-**  
5 **treated by pegylated interferon alpha-2a and ribavirin (ANRS HC16 GAMMATRI**  
6 **substudy\*)**  
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30 **Running Head:** Gammatri study  
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43 **List of Abbreviations:**

44 PEG: pegylated; IFN: interferon; RIBA, ribavirin; NR: non-responders to a second line  
45 treatment.  
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## Abstract

A lack of antiviral response in patients with chronic hepatitis C treated with pegylated (PEG)-interferon (IFN)  $\alpha$ -2a + ribavirin (RIBA) may be explained by neutralizing antibodies to IFN  $\alpha$ -2a. The aim of the study was to assess neutralizing antibodies to IFN  $\alpha$ -2a and IFN levels in non-responder patients who were re-treated by PEG IFN  $\alpha$ -2a and RIBA for 12 weeks.

Non-responders to a first-line treatment of PEG IFN  $\alpha$ -2a + RIBA were included for treatment with PEG IFN  $\alpha$ -2a (180 $\mu$ g/week) + RIBA (1000 mg/d if <75 kg, 1200 mg otherwise) for 48 weeks. HCV RNA was measured at week 12. IFN levels and neutralizing antibodies to IFN  $\alpha$ -2a were measured retrospectively on stored sera at baseline and weeks 4 and 12, using a quantitative sandwich ELISA for neutralizing antibodies to IFN  $\alpha$ -2a.

Twenty-three patients were non-responders and 19 patients were responders at week 12 of the initial phase of the second-line treatment. Non-responders and responders did not differ statistically: baseline age (median age 47 vs. 50 years), HCV RNA (median 6.8 vs. 6.4 log<sub>10</sub> copies/ml), gender (70% vs. 73% males), genotype (genotype 1: 91% vs. 80%). The median IFN  $\alpha$ -2a levels (pg/ml) at weeks 0, 4, and 12 (interquartile range) did not differ between the 19 responders to initial phase of second line treatment and the 23 non-responders :< 3.3 (<3.3- 371.4), 1457.3 (106.8- 3284.8), and 1652 (90.8- 5000); 84.5 (3.3- 277.4), 1407.4 (120.2- 2443.4), and 1620.1 (120.2- 2287.1) respectively.

Among non-selected consecutive non-responder patients, re-treatment with PEG IFN  $\alpha$ -2a + RIBA is associated with virological response regardless of the presence of antibody-mediated resistance to conventional IFN treatment.

**Key words:** Second line treatment; Neutralizing antibody; Hepatitis C virus; Non-responders

## 1 INTRODUCTION

2 Chronic hepatitis C is a major cause of liver disease, with worldwide prevalence  
3 estimated at 3% [WHO., 2000]. Only a minority of those infected spontaneously clear  
4 HCV. Of patients who become chronic carriers, 70–80% may progress to cirrhosis and  
5 hepatocellular carcinoma [Hoofnagle, 2002; Micallef et al., 2006; Thomas and Seeff,  
6 2005].

7 Response to interferon (IFN)  $\alpha$ -2a and ribavirin (RIBA) combination therapy for the  
8 treatment of chronic hepatitis C viral (HCV) infection varies largely among patients.  
9 Indeed, with a combination of pegylated (PEG) IFN  $\alpha$ -2a or  $\alpha$ -2b plus RIBA for 24 or 48  
10 weeks depending on genotype, currently the standard of care for patients with chronic  
11 hepatitis C, up to 50-60% of patients are non-responders or experience relapse after  
12 treatment ends [Seeff, 2002]. Many hypotheses have been proposed to explain the  
13 absence of response to IFN. Recent data suggest that host genetics may be useful for  
14 predicting drug response, and they also support the notion that IL28B could play a role  
15 in the treatment of HCV and in other diseases treated with IFN [Ge et al., 2009;  
16 McCarthy et al.; Rauch et al., 2010; Suppiah et al., 2009; Thomas et al., 2009].

17 A possible explanation for the lack of response is the development of neutralizing  
18 antibody to interferon, as shown in case series [van der Eijk et al., 2006]. There have not  
19 been reports so far on large unselected series of re-treated patients to study formally the  
20 association between neutralizing antibody and the response to treatment.

21 In the setting of a clinical trial, relationships were assessed between either IFN  
22 (endogenous or not) or neutralizing antibody to IFN  $\alpha$ -2a levels and virological response  
23 to a second-line treatment with PEG IFN  $\alpha$ -2a + RIBA.

## 24 PATIENTS AND METHODS

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26  
27 *Patients:* In a phase II, multicentric, non-comparative trial (ANRS HC16 GAMMATRI),  
28 patients received PEG IFN  $\alpha$ -2a (180 $\mu$ g/week) in combination with RIBA (1000 mg/d if  
29 <75 kg, 1200 mg otherwise) for 48 weeks. IFN gamma-1b was added to this  
30 combination if patients did not experience a virological response at the 12-week visit,  
31 defined as a decrease in HCV RNA greater than 2 log<sub>10</sub> copies/ml relative to the

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3 32 baseline. The current study included serum samples during the first 12 weeks when  
4  
5 33 patients received PEG IFN  $\alpha$ -2a and RIBA exclusively.

6  
7 34 Adult patients (18-70 years) were eligible if they had chronic hepatitis C, were non-  
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9 35 responders to a previous treatment with PEG IFN and RIBA (at least 12 weeks without  
10  
11 36 treatment change and stopped at least 3 months before enrolment), had a Metavir  
12  
13 37 histological score  $\geq$ F2, and signed a written informed consent. The protocol was  
14  
15 38 approved by the ethics committee of Haut Lévêque hospital (Pessac, France), and was  
16  
17 39 conducted in compliance with the Declaration of Helsinki.

18  
19 40 For this study, the definition of treatment response was a decrease in HCV RNA greater  
20  
21 41 than 2 log<sub>10</sub> copies/ml at week 12 relative to baseline values. Another criterion of  
22  
23 42 treatment response was undetectable HCV RNA at week 12 or 28.

24  
25 43 *Measurements:* HCV RNA was measured at week 12 using TaqMan PCR (Roche  
26  
27 44 Diagnostics) (lower limit of detection 15 UI/ml) in a central laboratory to assess early  
28  
29 45 response. IFN levels and neutralizing antibody to IFN  $\alpha$ -2a were retrospectively  
30  
31 46 measured on stored sera at baseline and weeks 4 and 12. Measurements were  
32  
33 47 performed by technicians who were blinded to the individual response to treatment.  
34  
35 48 Neutralizing antibodies to IFN  $\alpha$ -2a were determined using a quantitative sandwich  
36  
37 49 enzyme-linked immunosorbent assay (ELISA) for antibodies directed to IFN  $\alpha$ -2a  
38  
39 50 (Bender Med- Systems Diagnostic) with a limit of detection at 1.38 ng/ml. IFN  $\alpha$ -2a  
40  
41 51 levels were measured using a quantitative sandwich enzyme-linked immunosorbent  
42  
43 52 assay (ELISA) for IFN  $\alpha$ -2a (Bender Med- Systems Diagnostic) with a limit of detection  
44  
45 53 at 3.3 pg/ml. For the computation of statistical parameters, undetectable values were  
46  
47 54 assigned a value of zero.

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49 55 *Statistical analysis:* Fisher's exact or Chi-square tests were used to compare  
50  
51 56 proportions. The non-parametric Wilcoxon test was used to compare medians. All  
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53 57 calculations were assessed using SAS V9.1.3 software (SAS Institute Inc. Cary, NC)

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## 58 59 60 **RESULTS**

61  
62 61 Twenty-three patients were non-responders and 20 patients were responders at week  
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64 62 12 to the initial phase of second-line treatment. Levels of IFN  $\alpha$ -2a and neutralizing

antibody to IFN  $\alpha$ -2a could be measured in all the patients, except for one responder who had no stored serum available and was excluded from the analysis. Non-responders and responders to the initial phase of the second-line treatment did not differ statistically: baseline age (median age 47 vs. 50 years), HCV RNA (median 6.8 vs. 6.4 log<sub>10</sub> copies/ml), gender (70% vs. 73% males), genotype (genotype 1: 91% vs. 80%).

The median IFN  $\alpha$ -2a levels (pg/ml) at weeks 0, 4, and 12 (interquartile range) did not differ between the 19 responders to initial phase of second line treatment and the 23 non-responders :< 3.3 (<3.3-371.4), 1457.3 (106.8-3284.8), and 1652 (90.8-5000); 84.5 (3.3-277.4), 1407.4 (120.2-2443.4), and 1620.1 (120.2-2287.1) respectively. Levels reached at weeks 4 and 12 were in the therapeutic range and indicated exposure to exogenous IFN. Neutralizing antibody levels were detectable among 3/19 responders (16%) vs. 5/23 non-responders (22%) (p=0.70) at week 0, among 6/19 responders (32%) vs. 6/23 non-responders (26%) (p=1.0) at week 4, and among 7/19 responders (37%) vs. 6/23 non-responders (26%) at week 12 (p=0.73) (**Table 1**). Of note in one responder, neutralizing antibody to IFN  $\alpha$ -2a was present at week 0 and week 4 but not at week 12.

Among 5 of the 6 non-responders with neutralizing antibody to IFN  $\alpha$ -2a, neutralizing antibody levels were detectable from week 0 to week 12, and for one of the non-responders they were detectable at week 4 and week 12. All responders and non-responders who had neutralizing antibody to IFN  $\alpha$ -2a had undetectable endogenous IFN at week 0. There was no association between the presence of neutralizing antibody to IFN  $\alpha$ -2a and endogenous IFN; only one patient who had endogenous IFN at baseline had neutralizing antibody to IFN  $\alpha$ -2a. In addition, there was no significant association between the presence of endogenous IFN  $\alpha$ -2a at baseline without any exogenous IFN  $\alpha$ -2a and response to treatment (p=0.057).

At week 28, nine patients had undetectable HCV RNA: three of the patients had neutralizing antibody to IFN- $\alpha$  at week 12 and one had neutralizing antibody to IFN  $\alpha$ -2a at week 0 and week 4. In three responders who had undetectable HCV RNA at week 28, endogenous IFN  $\alpha$ -2a was found at the baseline but no exogenous IFN  $\alpha$ -2a, but these three patients were not the same as the three patients with neutralizing antibody to IFN  $\alpha$ -2a.



## 94 Discussion

95 The present study, which is the largest study examining unselected re-treated patients,  
96 showed no association between the response to a 12-week PEG IFN  $\alpha$ -2a + RIBA  
97 session and neutralizing antibody to IFN  $\alpha$ -2a. It was reported previously that if there is  
98 an association of the presence of neutralizing antibody to IFN  $\alpha$ -2a and the absence of  
99 response to IFN  $\alpha$ -2a, virological response may be restored or induced in hepatitis C-  
100 infected patients (patients who might develop an antibody-mediated resistance to  
101 conventional recombinant IFN treatment) [van der Eijk et al., 2006]. The study by Van  
102 der Eijk et al. (2006) did not describe the criteria for patient selection. In the present  
103 study only few non-responders had neutralizing antibodies to IFN  $\alpha$ -2a. Thus the  
104 absence of neutralizing antibodies to IFN  $\alpha$ -2a might not explain the absence of  
105 response.

106 A higher proportion of patients harboring neutralizing antibodies to IFN (39%) was found  
107 in the present study compared with the study by Leroy et al. [Leroy et al., 1998] (3 to  
108 9%); but Leroy et al. (1998) did not find a relationship with initial response but rather with  
109 breakthrough.

110 It has been suggested recently that host genetics might be more useful for predicting  
111 drug response, as genome-wide association studies have reported associations  
112 between IL28B single polynucleotide polymorphisms and the response to antiviral  
113 treatment in individuals infected with HCV genotype 1. In these studies, eight single  
114 polynucleotide polymorphisms (rs8105790, rs12979860, rs11881222, rs8103142,  
115 rs28416813, rs4803219, rs8099917, and rs7248668) of IL28B were analyzed, with a  
116 view to identifying associations with the response to PEG IFN  $\alpha$ -2a and RIBA [Ge et al.,  
117 2009; McCarthy et al.; Rauch et al., 2010; Suppiah et al., 2009; Thomas et al., 2009].

118  
119 Isolated cases have been observed but might well be explained by a selection bias of  
120 very specific cases in single centers. Among re-treated patients, second-line treatment  
121 with PEG IFN  $\alpha$ -2a + RIBA yields a virological response in the presence of antibody-  
122 mediated resistance to conventional IFN treatment, and neutralizing antibody to IFN  $\alpha$ -  
123 2a cannot be considered as a predictor of a virological response among these patients.  
124 Nevertheless, there might be circumstances or populations behaving differently. Indeed,



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3 125 Jorns et al. [Jorns et al., 2006] showed that anti-IFN  $\alpha$ -2a antibodies had already  
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5 126 developed after the first of four unsuccessful IFN therapies, suggesting that neutralizing  
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7 127 antibodies may have contributed to the failure of previous IFN treatment lines.

8  
9 128 However, would the results be the same if natural IFN were used for the second-line  
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11 129 therapy instead of recombinant IFN? Indeed, there are several indications that  
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13 130 neutralizing antibodies to recombinant IFNa do not redevelop during natural IFN re-  
14  
15 131 treatment and, when still present, they are not able to neutralize all subtypes present in  
16  
17 132 the mixture of natural IFN preparations [Antonelli et al., 1991; Antonelli et al., 1996; Berg  
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19 133 et al., 2001; Milella et al., 1993; Milella et al., 1995; Roffi et al., 1995]. An association of  
20  
21 134 neutralizing antibodies with treatment failure was reported previously [Basso et al.,  
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23 135 2007; Cornberg et al., 2006; Poynard et al., 2009; Sherman et al., 2006; Shiffman, 2004;  
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25 136 Taliani et al., 2006; Veldt et al., 2003]. Several studies showed that re-treatment with  
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27 137 natural IFN was then able to restore or induce a virological response in hepatitis C  
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29 138 patients who developed an antibody-mediated resistance to conventional recombinant  
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31 139 IFN treatment [Antonelli et al., 1991; Antonelli et al., 1996].

32  
33 140 In conclusion, this study shows that neutralizing antibodies to IFN  $\alpha$ -2a can be found at  
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35 141 similar levels in patients who respond and who do not respond to a re-treatment by PEG  
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37 142 IFN  $\alpha$ -2a + RIBA. Other mechanisms, such as host genetic or immunological factors,  
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39 143 need to be identified and investigated in unbiased studies to explain most treatment  
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41 144 failures among patients infected chronically by HCV and who adhered to their therapy.  
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## Appendix

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Table 1: Endogenous IFN- $\alpha$ , presence of neutralizing antibody to IFN- $\alpha$ , and treatment response at week 12; analysis of 42 patients of the ANRS HC16 GAMMATRI trial

		HCV RNA decrease > 2log <sub>10</sub> at week 12				
		yes (responders)		no (non-responders)		
<b>Neutralizing antibody to IFN-<math>\alpha</math></b>						
Week 0						
	> 1.38 ng/ml (%)	3	(16)	5	(22)	p=0.700
	Median (IQR*)	0	(0-0)	0	(0-0)	
Week 4						
	> 1.38 ng/ml (%)	6	(32)	6	(26)	p=1.000
	Median (IQR*)	0	(0-53)	0	(0-81)	
Week 12						
	> 1.38 ng/ml (%)	7	(37)	6	(26)	p=0.057
	Median (IQR*)	0	(0-124)	0	(0-33)	
<b>Interferon <math>\alpha</math>-2a levels</b>						
Week 0						
	> 3.3 pg/ml (%)	4	(21)	12	(52)	p=0.057
	Median (IQR*)	0	(0-0)	85	(0-181)	
Week 4						
	> 3.3 pg/ml (%)	18	(95)	21	(91)	
	Median (IQR*)	1457	(876-1722)	1407	(960-1903)	
Week 12						
	> 3.3 pg/ml (%)	19	(100)	22	(96)	
	Median (IQR*)	1652	(1147-2633)	1620	(1297-1785)	

Abbreviation: IQR = interquartile range