EVALUATION OF IMMUNOGENICITY IN THE NON-INTERVENTIONAL POST-AUTHORIZATION SAFETY STUDY (PASS) FOR THE PROSPECTIVE EVALUATION OF THE SAFETY AND TOLERABILITY PROFILE OF HSA-free SUBCUTANEOUS INTERFERON (scIFN) β-1a IN TREATMENT OF NAÏVE RELAPSING MULTIPLE SCLEROSIS (MS) PATIENTS – STEP STUDY

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OBJECTIVES: Assessment of immunogenicity in a cohort of naïve MS patients starting treatment with HSA-free scIFNβ-1a via the evaluation of developing Binding Antibodies (BAbs) and Neutralizing Antibodies (NAbs). This is one of the aims of the STEP study, a multicenter, non- interventional, study for the safety evaluation of HSA-free scIFNβ-1a.

MATERIALS: 851 samples from 198 patients treated with HSA-free scIFNβ-1a from 27 MS Sites throughout Italy were analyzed by an independent laboratory in Orbassano to detect BAbs and NAbs. The subjects had been evaluated on the first day of drug administration and subsequently every 6 months post-treatment.

BAbs status categorization

- **NEGATIVE**: patients who had never shown a positive result during follow-up
- **POSITIVE**: patients who developed BAbs persisting until the end of follow-up
- **FLUCTUATING**: patients who showed both positive and negative results during follow-up

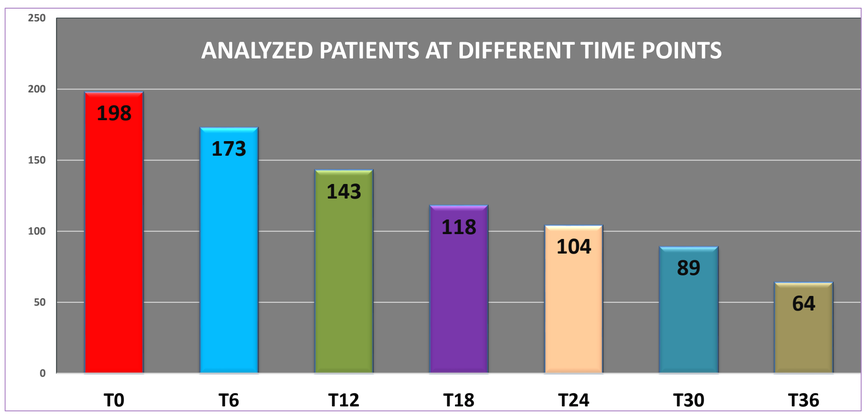
Binding Antibodies (BAbs). An indirect Enzyme-Lynked Immuno-Sorbent Assay (ELISA) has been performed to detect BAbs. The established cut-off was 9 BU (Binding Unit)

Neutralizing Antibodies (NAbs). A Cytopathic Effect Assay (CPE) been performed to detect NAbs. The cut-off was 20 TRU (Ten Fold Reduction Unit). A title >100 TRU indicates no biological activity of the drug.

NAbs status categorization

- **NEGATIVE**: patients who had never shown a positive result during follow-up
- **PERSISTENT POSITIVE**: patients who developed NAbs persisting until the end of follow-up
- **ISOLATED POSITIVE**: patients who showed a single positive result during the follow-up
- **SERO-REVERTED**: patients who, after a period of positivity, showed negative results until the end of follow-up

A total of 198 subjects were analyzed: 25 were evaluated only at start of treatment, 30 completed 6-months follow-up, 25 completed 12 months, 14 completed 18 months, 15 completed 24 months, 25 completed 30 months, 64 completed 36 months.



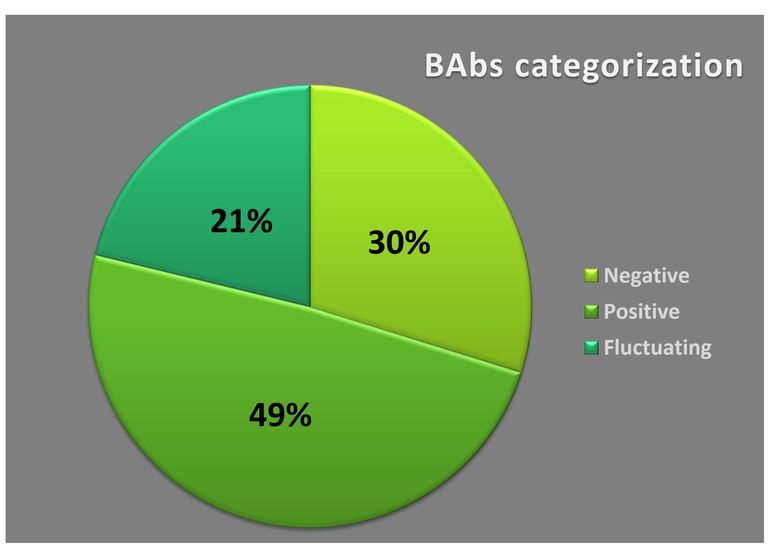
RESULTS: Based on categorization, we identified 59 negative patients, 97 positive and 42 that resulted fluctuating. Only the Positive Babs group was analyzed for NAbs

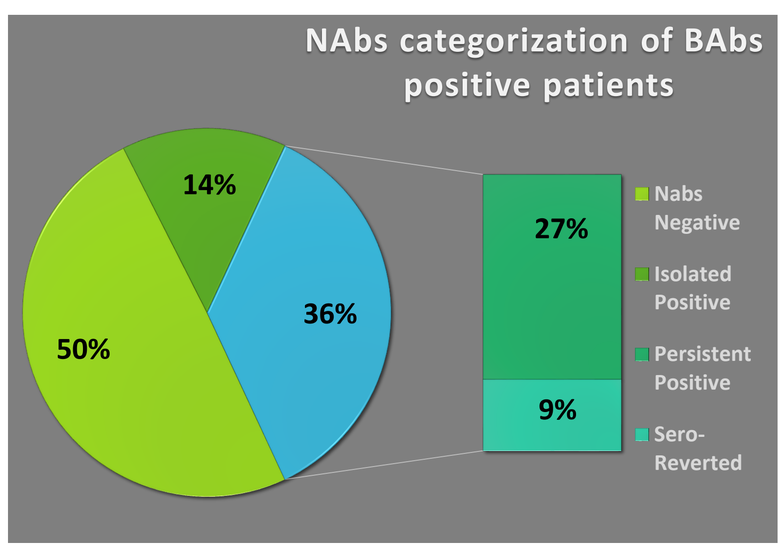
NAbs evaluation in the group of BAbs positive patients showed that half of patients are NAbs Negative and that 25% (9 out of 35) of NAbs Positive became Negative during follow-up. 5 out of 9 Sero-reverted were positive with a title <100 TRU.
In the of BAbs Fluctuant patients, only 2 were Persistent Positive for NAbs (1,01%).

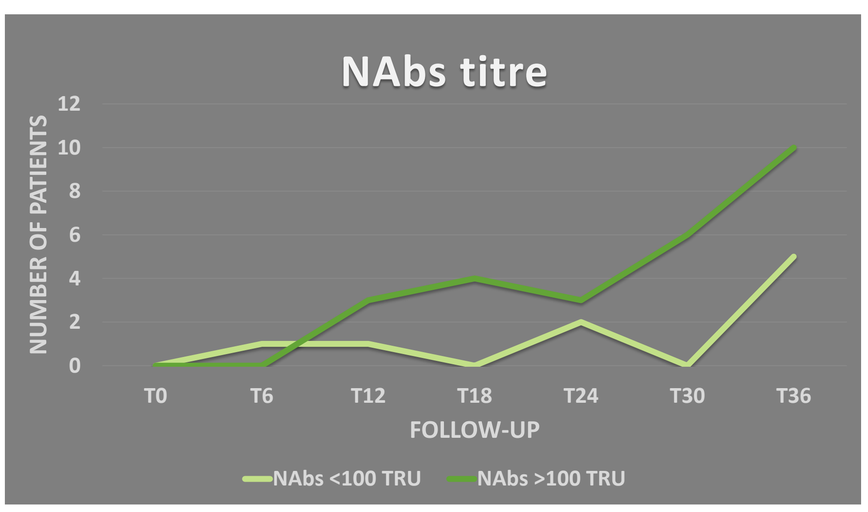
Babs categorization	N° of patients	Percentage
Negative	59	30%
Positive	97	49%
Fluctuating	42	21%

NAbs categorization	N° of patients	Percentage
NAbs Negative	48	50%
Isolated Positive	14	14%
Persistent Positive	26	27%
Sero-Reverted	9	9%

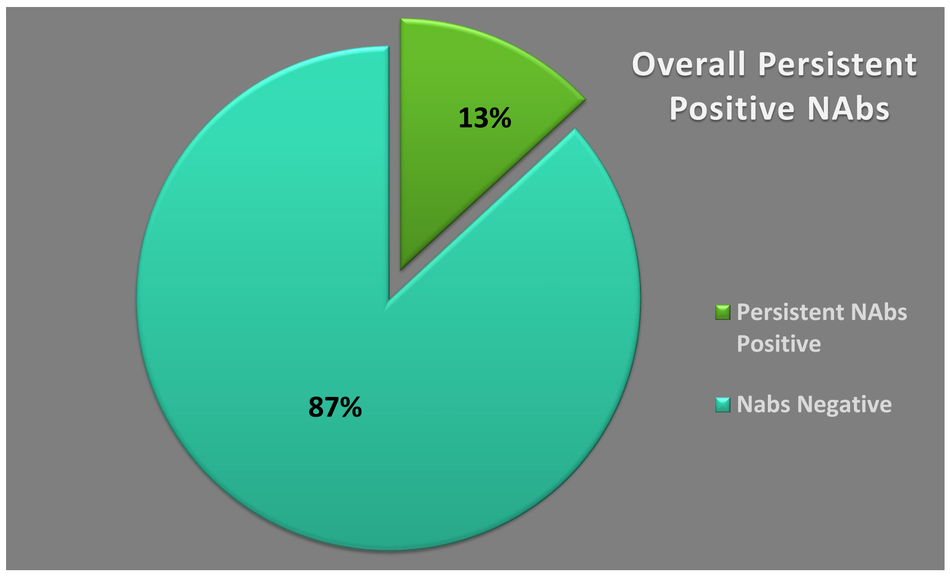
Follow-up	Nabs <100 TRU	Nabs >100 TRU
T0	0	0
T6	1	0
T12	1	3
T18	0	4
T24	2	3
T30	0	6
T36	5	10
TOTAL	9	26







Follow-up	Patients	BAbs positive	NAbs >20 TRU	Sero-reverted	Persistent NAbs Positive	NAbs Negative
T0	25	7	-	-	-	25
T6	30	12	1	-	1	29
T12	25	14	4	-	4	21
T18	14	8	4	-	4	10
T24	15	8	5	1	4	11
T30	25	8	6	3	3	22
T36	64	40	15	5	10	54
TOTAL	**198**	**97**	**35**	**9**	**26**	**172**



This study is a real-world, independent laboratory analysis of HAS-free-Rebif-oriented immunogenicity of MS patients. Results showed that although the HAS-free-Rebif induced the development of antibodies in almost half of all treated patients, the neutralizing activity that could result in reduced treatment efficacy was only detected in a small portion of these patients.

The final analysis of all samples showed that 49% of treated patients developed Binding Antibodies to IFNβ-1a; of those, 35 patients (17,7%) developed Persistent Neutralizing Antibodies, but 9 (4,5%) became Negative after a period of positivity (Sero-reverted group). The real Persistent Positive patients were 26 (13,1%)

CONCLUSION: Results of the STEP study indicate that HSA-free-Rebif is less immunogenic than the previous[1] formulation. In addition, these results confirm the findings of a large, previously conducted phase-3, randomized trial (REFLEX).[2]

References

1. Mikol D, Barkhof, F, Chang, P Coyle PK, Jeffery DR, Schwid SR, Stubinski B, Uitdehaag B; REGARD study group. Lancet Neurol. 2008 Oct;7(10):903-14
2. Comi G, De Stefano N, Freedman MS, Barkhof F, Polman CH, Uitdehaag BM, Casset-Semanaz F, Hennessy B, Moraga MS, Rocak S, Stubinski B, Kappos L. Lancet Neurol. 2012 Jan;11(1):33-41.