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Effect of Neutralizing Antibodies on Pharmacodynamic Biomarkers of Subcutaneous Interferon β -1a in REFLEX and REFLEXION

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INTRODUCTION

- Several pharmacodynamic biomarkers have been described in patients with MS treated with IFN β , each with variable degrees of evidence.¹⁻³
- Non-responders to IFN β often produce neutralising antibodies, which are expected to diminish pharmacodynamic biomarker response to treatment.⁴
- In the REFLEX trial and its extension REFLEXION, approximately 15% of patients treated with sc IFN β -1a developed NAbs over 5 years.^{5,6}



OBJECTIVES

To evaluate the effect of NAbS on candidate pharmacodynamic biomarkers of long-term sc IFN β -1a treatment in a large patient cohort.



METHODS

Study Population

- Serum samples from 507 REFLEX patients and 287 patients who continued into REFLEXION were available for analysis.
- sc IFN β -1a qw arm and placebo arm patients who converted to CDMS during the trials switched to open-label sc IFN β -1a tiw and were excluded from this analysis.

REFLEX trial



REFLEXION trial



*Independent variable biomarker expression; fixed effects: biomarker expression at baseline, treatment arm, gender and time; random effect: subject.

CDMS, relapsing and remitting multiple sclerosis; **IFN**, interferon; **IL-1RA**, interleukin-1 receptor antagonist; **IP-10**, interferon- γ inducible protein; **M**, month; **sc**, subcutaneous **SEM**, standard error of the mean; **TRAIL**, soluble TNF-related apoptosis-inducing ligand



METHODS

Sample Analysis

- **REFLEX:** neopterin, TRAIL, IP-10, 2'5'OAS, and IL-1RA levels were measured from samples collected at baseline (M0), M6, M12, M18, and M24.
- **REFLEXION:** samples were collected every 6 months for up to 5 years – neopterin, TRAIL, and IP-10 data are available only.
- **Serum NAb levels** were analyzed in patients from REFLEX and REFLEXION and pharmacodynamic data were stratified by patient NAb status:
 - NAb-positive [≥ 20 neutralizing units/mL] or NAb-negative.

Statistical Analysis

- The pharmacodynamic effect of biomarkers in relation to sc IFN β -1a therapy was evaluated using linear mixed-effect models.*
- Mean (\pm SEM) biomarker levels are reported.

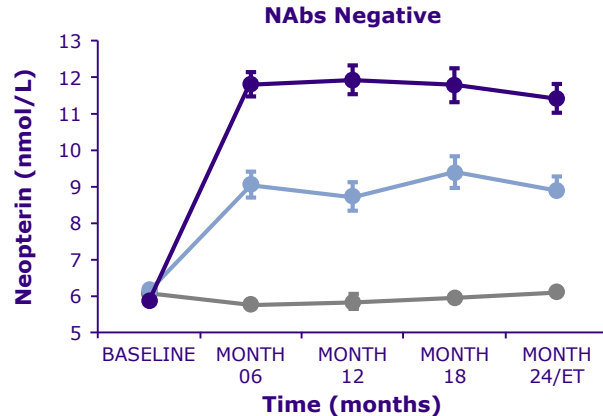
*Independent variable biomarker expression; fixed effects: biomarker expression at baseline, treatment arm, gender and time; random effect: subject.

ODSAS, oral disease activity score; **IP-10**, interferon- γ inducible protein; **M**, month; **sc**, subcutaneous **SEM**, standard error of the mean; **TRAIL**, soluble TNF-related apoptosis-inducing ligand



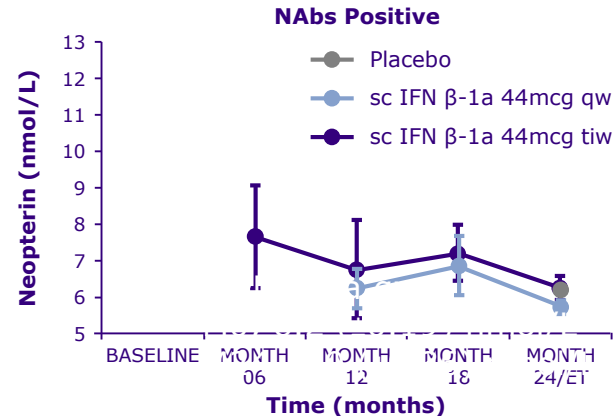
RESULTS - REFLEX

Neopterin in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX



Number of patients, n

●	91	90	92	90	83
●	122	123	109	99	93
●	114	112	104	100	93



Number of patients, n

●	0	0	0	0	1
●	0	0	11	18	18
●	0	3	11	15	20



RESULTS - REFLEX

Neopterin in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX

NAb-negative patients:

- No change was seen with placebo:
 - M0, 6.1 (\pm 0.27) nmol/L
 - M24, 6.1 (\pm 0.17) nmol/L
- Neopterin levels increased with:
 - sc IFN β -1a tiw:
 - M0, 5.9 (\pm 0.20) nmol/L
 - M24, 11.5 (\pm 0.41) nmol/L
 - sc IFN β -1a qw:
 - M0, 6.2 (\pm 0.19) nmol/L
 - M24, 8.9 (\pm 0.36) nmol/L

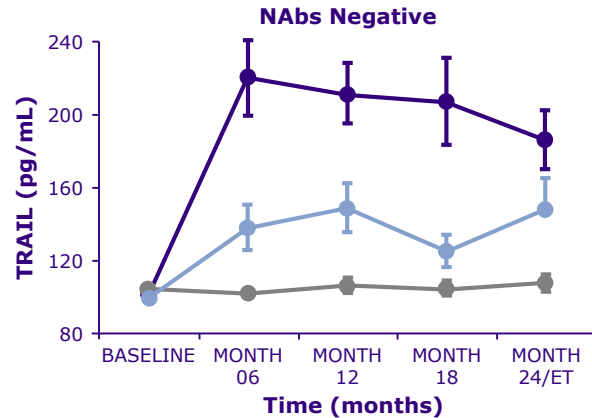
NAb-positive patients:

Levels of neopterin in patients treated with IFN and NAb-positive were similar to those measured for NAb-negative patients who received placebo.



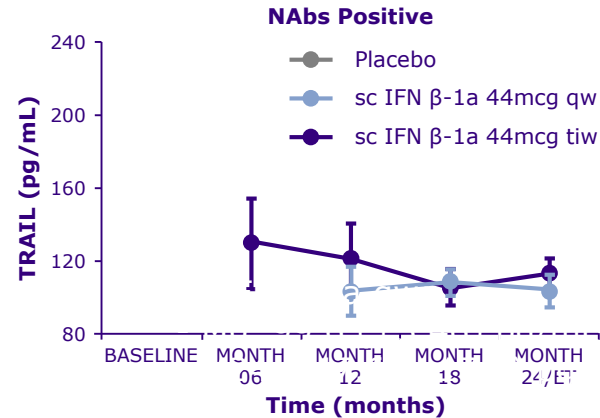
RESULTS - REFLEX

TRAIL in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX



Number of patients, n

Placebo	29	31	31	28	24
sc IFN β -1a 44mcg qw	44	45	39	35	31
sc IFN β -1a 44mcg tiw	34	30	28	26	25



Number of patients, n

Placebo	0	0	0	0	0
sc IFN β -1a 44mcg qw	0	0	4	6	6
sc IFN β -1a 44mcg tiw	0	2	6	7	8

At baseline, no patients fulfilled the criteria for NAb-positive [≥ 20 neutralizing units/mL]

ET, end of trial; IFN, interferon; M, month; NAb, neutralizing antibody; qw, once weekly; sc, subcutaneous; tiw, three times weekly; TRAIL, soluble TNF-related apoptosis-inducing ligand



RESULTS - REFLEX

TRAIL in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX

NAb-negative patients:

- No change was seen with placebo:
 - M0, 103.4 (\pm 3.37) pg/mL
 - M24, 107.0 (\pm 5.25) pg/mL
- Protein expression increased with:
 - sc IFN β -1a tiw:
 - M0, 100.0 (\pm 3.74) pg/mL
 - M24, 185.8 (\pm 16.2) pg/mL
 - sc IFN β -1a qw:
 - M0, 99.6 (\pm 3.46) pg/mL
 - M24, 147.6 (\pm 17.6) pg/mL

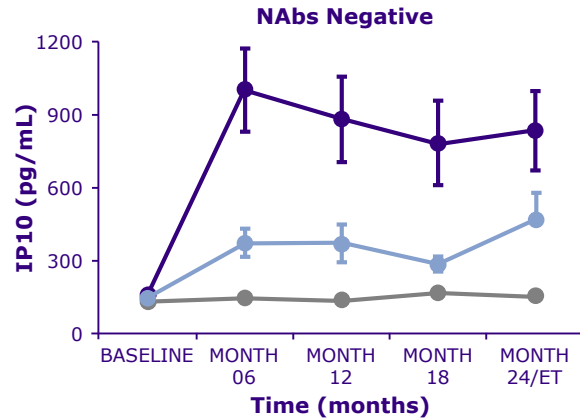
NAb-positive:

Levels of TRAIL in patients treated with IFN and NAb-positive were similar to those measured for NAb-negative patients who received placebo.



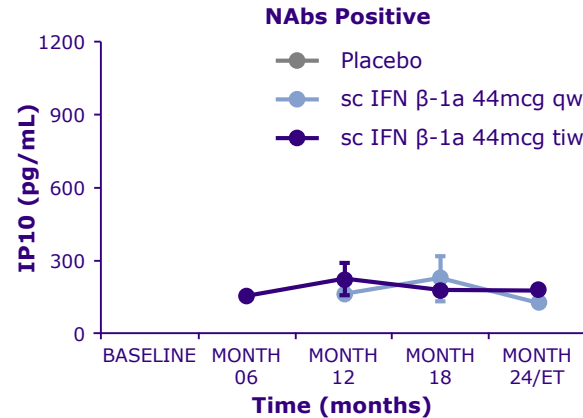
RESULTS - REFLEX

IP-10 in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX



Number of patients, n

●	30	31	31	30	25
●	46	46	38	36	33
●	34	30	28	25	25



Number of patients, n

●	0	0	0	0	0
●	0	0	4	6	6
●	0	2	6	7	8



RESULTS - REFLEX

IP-10 in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX

NAb-negative patients:

- No change was seen with placebo:
 - M0, 133.8 (\pm 9.97) pg/mL
 - M24, 152.0 (\pm 13.5) pg/mL
- Protein expression increased with:
 - sc IFN β -1a tiw:
 - M0, 165.9 (\pm 12.9) pg/mL
 - M24, 834.5 (\pm 166) pg/mL
 - sc IFN β -1a qw:
 - M0, 140.9 (\pm 7.32) pg/mL
 - M24, 469.3 (\pm 106) pg/mL

NAb-positive:

IP-10 protein expression levels in patients treated with IFN and NAb-positive were similar to those measured in NAb-negative patients who received placebo.



RESULTS - REFLEX

2,5-OAS and IL-1RA in Negative or Positive NAb Patients Treated with sc IFN β -1a or Placebo in REFLEX

2'5'OAS REFLEX data



IL-1RA REFLEX data



NAb-Negative:

- Levels of 2'5'OAS and IL-1RA increased in patients treated with sc IFN β -1a tiw and qw versus placebo.

NAb-Positive:

- Biomarker levels in patients treated with IFN and NAb-positive were similar to those measured for NAb-negative patients who received placebo.



CONCLUSIONS

It is known that classical IFN-responsive pharmacodynamic genes do not predict clinical response to IFN β .



In REFLEX and REFLEXION, ~85% of patients were NAb-negative. In these patients, pharmacodynamic biomarkers were upregulated in response to sc IFN β -1a over 5 years.



Reduced pharmacodynamic biomarker expression in NAb-positive patients was consistent with the concept that NAb reduce the pharmacodynamic activity of sc IFN β -1a.