# Baseline Serum Neurofilament Light Chain Levels Predict Conversion to McDonald 2005 MS Within 2 years of a First Clinical Demyelinating Event in REFLEX

**J. Kuhle[1], D. Leppert[1], G. Comi[2], N. De Stefano[3], L. Kappos[4], M. S. Freedman[5], D. Issard[6], S. Roy[7]**

[1]Neurologic Clinic and Policlinic, Department of Medicine, Biomedicine & Clinical Research, University Hospital Basel, and University of Basel, Basel, Switzerland; [2]Department of Neurology and Institute of Experimental Neurology, Università Vita-Salute San Raffaele, Ospedale San Raffaele, Milan, Italy; [3]Department of Neurological and Behavioural Sciences, University of Siena, Siena, Italy; [4]Neurologic Clinic and Policlinic, Departments of Clinical Research and Biomedical Engineering, University Hospital Basel, and University of Basel, Switzerland; [5]University of Ottawa and the Ottawa Hospital Research Institute, Ottawa, ON, Canada; [6]Cytel Inc., Geneva, Switzerland; [7]Merck, Aubonne, Switzerland, a division of Merck KGaA, Darmstadt, Germany.

**ACTRIMS-ECTRIMS 2020 Virtual Congress | 11 – 13 September**

# Disclosures

This study was sponsored by Merck KGaA, Darmstadt, Germany.

- **JK**'s institution (University Hospital Basel) has received and exclusively used for research support: consulting fees from Biogen, Novartis, Protagen AG, Roche, and Teva; speaker fees from the Biogen, Genzyme, Merck KGaA (Darmstadt, Germany), Novartis, Roche, and Swiss MS Society; travel expenses from Merck KGaA (Darmstadt, Germany), Novartis, and Roche; and grants from Bayer, Biogen, Celgene, ECTRIMS Research Fellowship Programme, Genzyme, Merck KGaA (Darmstadt, Germany), Novartis, Roche, Swiss MS Society, Swiss National Research Foundation (320030_160221), and the University of Basel.
- **DL** is CMO of GeNeuro.
- **GC** has received consulting and speaking fees from Almirall SpA, Biogen, Celgene Group, Excemed, Forward Pharma, Genzyme corporation, Genzyme Europe, F. Hoffmann-La Roche, MedDay, Merck KGaA (Darmstadt, Germany), Novartis, Roche SpA, Sanofi-Genzyme, Teva Italia Srl, and Teva Pharmaceutical Industries Ltd.
- **NDS** is a consultant for Bayer Schering, Biogen, Merck KGaA (Darmstadt, Germany), Novartis, Roche, Sanofi-Genzyme, and Teva; has grants or grants pending from FISM and Novartis; is on the speakers bureaus of Biogen, Merck KGaA (Darmstadt, Germany), Novartis, Roche, Sanofi-Genzyme, and Teva; and has received travel funds from Merck KGaA (Darmstadt, Germany), Novartis, Roche, Sanofi-Genzyme, and Teva.
- **LK**'s institution (University Hospital Basel) has received in the last 3 years and used exclusively for research support: steering committee, advisory board, and consultancy fees from Actelion, Addex, Bayer HealthCare, Biogen, Biotica, Genzyme, Lilly, Merck KGaA (Darmstadt, Germany), Mitsubishi, Novartis, Ono Pharma, Pfizer, Receptos, Sanofi, Santhera, Siemens, Teva, UCB, and Xenoport; speaker fees from Bayer HealthCare, Biogen, Merck KGaA (Darmstadt, Germany), Novartis, Sanofi, and Teva; support of educational activities from Bayer HealthCare, Biogen, CSL Behring, Genzyme, Merck KGaA (Darmstadt, Germany), Novartis, Sanofi, and Teva; license fees for Neurostatus products; and grants from Bayer HealthCare, Biogen, European Union, Innosuisse, Merck KGaA (Darmstadt, Germany), Novartis, Roche Research Foundation, Swiss MS Society, and Swiss National Research Foundation.
- **MSF** received honoraria or consultation fees from Actelion (Janssen/J&J), Alexion, BiogenIdec, Celgene (BMS), EMD Inc., Canada (an affiliate of Merck KGaA, Darmstadt, Germany), EMD Serono, Inc., USA (an affiliate of Merck KGaA, Darmstadt, Germany), Sanofi-Genzyme, Hoffman La-Roche, Merck KGaA (Darmstadt, Germany), Novartis, Teva Canada Innovation; has received research support unrelated to this study from Sanofi-Genzyme Canada, Hoffman-La Roche, EMD Inc., Canada (an affiliate of Merck KGaA, Darmstadt, Germany); was a member of a company advisory board, board of directors, or other similar group for Actelion (Janssen/J&J), Alexion, Atara Biotherapeutics, Bayer Healthcare, BiogenIdec, Celgene (BMS), Clene Nanomedicine, GRI Bio, Hoffman La-Roche, Magenta Therapeutics, Merck KGaA (Darmstadt, Germany), MedDay, Novartis, Sanofi-Genzyme, Teva Canada Innovation; and has been a participant in a company sponsored speaker's bureau for Sanofi-Genzyme, and EMD Serono, Inc., USA (an affiliate of Merck KGaA, Darmstadt, Germany).
- **DI** is an employee of Cytel Inc., Geneva, Switzerland and has received fees for consultancy services from Merck KGaA, Darmstadt, Germany.
- **SR** is an employee of Merck, Aubonne, Switzerland, a division of Merck KGaA, Darmstadt, Germany.

Medical writing assistance was provided by Sarah Wetherill of inScience Communications, Springer Healthcare Ltd, UK, and was funded by Merck KGaA, Darmstadt, Germany.

The REFLEX study: NCT00404352; the REFLEXION study: NCT00813709

# INTRODUCTION

- Serum Neurofilament light chain (sNfL) is a biomarker of neuronal damage, reflecting disease activity and drug response in patients with MS.[1]
  - Levels of sNfL can be used to predict future disability in established MS.[2]
- sc IFN β-1a has proven efficacy in the treatment of patients with a FCDE.[3]
  - In the REFLEX trial, the onset of CDMS or McDonald MS 2005 was significantly delayed.
- During REFLEX, patients treated with sc IFN β-1a had reduced sNfL levels as early as 6 months post-baseline.[4]

**CDMS**, clinically definite multiple sclerosis; **FCDE**, first clinical demyelinating event; **IFN**, interferon; **MS**, multiple sclerosis; sc, subcutaneous, **sNfL**, serum neurofilament light chain

1. Kuhle J, et al. Neurol. 2019;92:e1007–e1015. 2. Barro C, et al. Brain. 2018;141:2382–2391. 3. Comi G, et al. Lancet Neurol. 2012;11:33–41. 4. Kuhle J, et al. Mult Scler. 2019;25(S2):785.

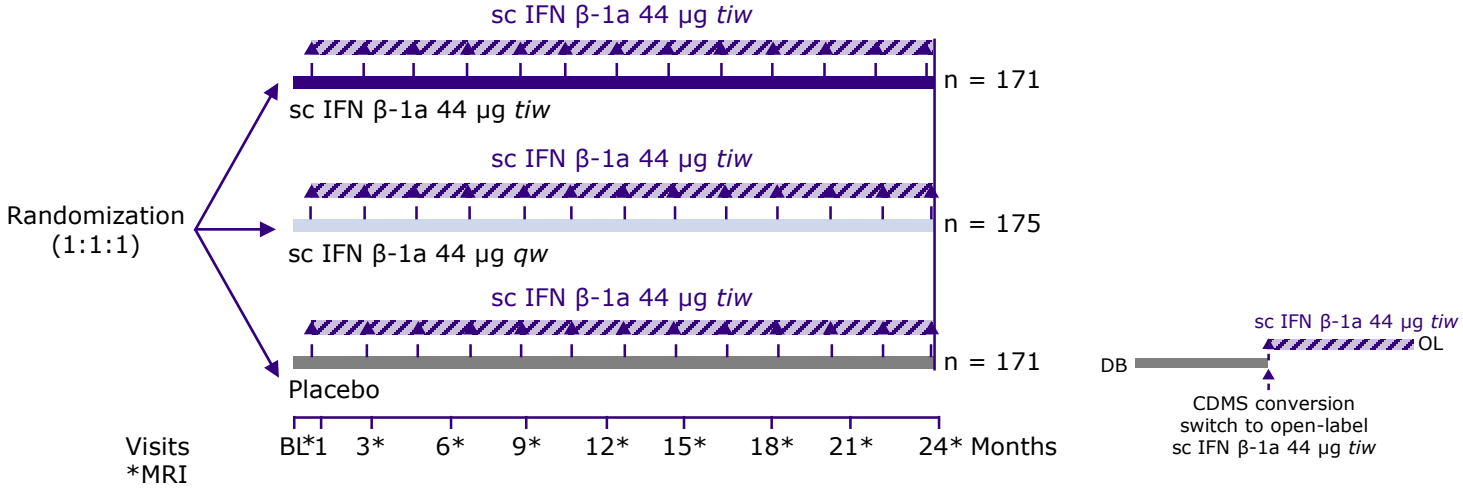
# OBJECTIVES

**Post hoc analysis to assess whether baseline sNfL levels can predict conversion to McDonald 2005 MS in patients with a FCDE receiving sc IFN β-1a in REFLEX.**

**FCDE**, first clinical demyelinating event; **IFN**, interferon; **MS**, multiple sclerosis; **sc**, subcutaneous, **sNfL**, serum neurofilament light chain

# METHODS

## The REFLEX Trial Study Design[1]




**This analysis has been conducted on the double blind period (up to CDMS conversion)**

**Note:** Patients who converted to CDMS during the study were switched to open-label sc IFN β-1a 44 µg tiw *MRI every 3 months. BL, baseline; **CDMS**, clinically definite multiple sclerosis; **CI**, confidence interval; **DB**, double blind; **HR**, hazard ratio; **IFN**, interferon; **M**, month; **MRI**, magnetic resonance imaging; **qw**, once weekly; **OL**, open label; **sc**, subcutaneous; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

1. Comi G, et al. Lancet Neurol. 2012;11:33–41; 2. Disanto G, et al. Ann Neurol. 2017;81:857–870.

# METHODS

## sNfL Analysis

- sNfL levels were analyzed[2] at baseline (M0), M6, M12, and M24.
- M0 sNfL subgroups were defined by median M0 sNfL concentration (26.1 pg/ml)
  - Low M0 sNfL: M0 sNfL ≤ median
  - High M0 sNfL: M0 sNfL > median

## Statistical Analysis

- HRs (95% CI) to determine the factors influencing risk of conversion to McDonald MS were calculated using a univariate Cox's proportional hazard model.
- A stepwise multivariate Cox's proportional hazard model was performed using factors selected from univariate model.
- For both models, variable selection was based on a two-sided Wald test.

**Note:** Patients who converted to CDMS during the study were switched to open-label sc IFN β-1a 44 µg tiw *MRI every 3 months. BL, baseline; **CDMS**, clinically definite multiple sclerosis; **CI**, confidence interval; **DB**, double blind; **HR**, hazard ratio; **IFN**, interferon; **M**, month; **MRI**, magnetic resonance imaging; **qw**, once weekly; **OL**, open label; **sc**, subcutaneous; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

1. Comi G, et al. Lancet Neurol. 2012;11:33–41; 2. Disanto G, et al. Ann Neurol. 2017;81:857–870.

# RESULTS

## Baseline demographics stratified by conversion to McDonald 2005 MS* status

| Characteristic | Placebo (n =140) | sc IFNβ-1a qw (n = 127) | sc IFNβ-1a tiw (n =101) |
|---|---|---|---|
| **Non-converters to McDonald 2005 MS** | | | |
| Age | 33.5 ±7.9 | 32.3 ±8.8 | 31.7 ±8.5 |
| Female, n (%) | 14 (56.0) | 28 (68.3) | 44 (73.3) |
| Baseline sNfL value, median (Q1; Q3) | 15.99 (12.26; 40.50) | 18.82 (10.97; 38.45) | 17.90 (11.48; 29.41) |
| **Converters to McDonald 2005 MS** | | | |
| Age | 30.2 ±7.6 | 30.5 ±7.9 | 30.2 ±8.6 |
| Female, n (%) | 94 (67.1) | 76 (59.8) | 62 (61.4) |
| Baseline sNfL value, median (Q1; Q3) | 27.92 (15.84; 65.59) | 29.20 (18.09; 56.89) | 32.54 (17.18; 62.81) |

Values are mean ±SD unless otherwise specified.*McDonald MS 2005
**IFN**, interferon; **MS**, multiple sclerosis; **Q**, quartile; **qw**, once weekly; **sc**, subcutaneous; **SD**, standard deviation; **tiw**, three times weekly

# RESULTS

## Baseline MRI characteristics stratified by conversion to McDonald 2005 MS* status

| Characteristic | Placebo (n =140) | sc IFNβ-1a qw (n = 127) | sc IFNβ-1a tiw (n =101) |
|---|---|---|---|
| **Non-converters to McDonald 2005 MS** | | | |
| Number of Gd+ lesions at M0 | 0.3 ±0.7 | 0.3 ±0.7 | 0.5 ±1.3 |
| Number of T1 Hypointense Lesions | 1.4 ±1.8 | 2.6 ±4.4 | 4.7 ±6.0 |
| Number of T2 Lesions | 7.8 ±6.3 | 10.6 ±11.1 | 15.3 ±12.2 |
| Normalized Brain Volume ($cm^3$) | 1536.69 ±52.59 | 1542.47 ±75.82 | 1535.37 ±66.13 |
| **Converters to McDonald 2005 MS** | | | |
| Number of Gd+ lesions at M0 | 1.4 ±3.0 | 1.9 ±4.0 | 1.8 ±2.9 |
| Number of T1 Hypointense Lesions | 6.2 ±8.1 | 7.1 ±8.1 | 6.5 ±7.3 |
| Number of T2 Lesions | 23.2 ±20.6 | 28.3 ±22.0 | 26.4 ±21.1 25.8 |
| Normalized Brain Volume ($cm^3$) | 1547.55 ±65.53 | 1534.01 ±64.04 | 1536.72 ±78.96 |

Values are mean ±SD unless otherwise specified.*McDonald MS 2005
**IFN**, interferon; **MS**, multiple sclerosis; **Q**, quartile; **qw**, once weekly; **sc**, subcutaneous; **SD**, standard deviation; **tiw**, three times weekly

# RESULTS

## Univariate analysis of Time to McDonald 2005 MS

| Factor | Factor Level | Parameter Estimate[a] | SE | HR (95% CI)[a] | P value[b] |
|---|---|---|---|---|---|
| Treatment | sc IFN β-1a tiw / placebo<br>sc IFN β-1a qw / placebo | −0.63<br>−0.34 | 0.13<br>0.12 | 0.53 (0.41;0.69)<br>0.71 (0.56;0.91) | <0.001<br>0.006 |
| Age | <30 years / ≥30 years | 0.26 | 0.10 | 1.29 (1.05;1.59) | 0.014 |
| Classification of FCDE | Monofocal / multifocal | −0.39 | 0.10 | 0.68 (0.55;0.83) | <0.001 |
| Steroid use at FCDE | Yes / No | −0.06 | 0.11 | 0.94 (0.75;1.18) | 0.617 |
| Presence of T1 Gd+ lesions at baseline | Yes / No | 0.60 | 0.11 | 1.81 (1.48;2.23) | <0.001 |
| Number of T2 lesions at baseline | | 0.02 | 0.00 | 1.02 (1.02;1.03) | <0.001 |
| Number of T1 Gd+ at baseline | | 0.13 | 0.01 | 1.14 (1.11;1.17) | <0.001 |
| Number of T1 hypointense | | 0.04 | 0.01 | 1.04 (1.02;1.05) | <0.001 |
| Brain volume at baseline | | 0.00 | 0.00 | 1.00 (1.00;1.00) | 0.496 |
| Baseline sNfL subgroup | Low M0 sNfL/<br>High M0 sNfL | −0.54 | 0.11 | 0.58 (0.47;0.72) | <0.001 |

[a]Univariate Cox's proportional hazards model. [b]Two-sided Wald test.

**CI**, confidence interval; **FCDE**, first clinical demyelinating event; **Gd+**, gadolinium-enhancing; **HR**, hazard ratio; **IFN**, interferon; **MS**, multiple sclerosis; **qw**, once weekly; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# RESULTS

## Univariate analysis of Time to McDonald 2005 MS

| Factor | Factor Level | Parameter Estimate[a] | SE | HR (95% CI)[a] | P value[b] |
|---|---|---|---|---|---|
| Treatment | sc IFN β-1a tiw / placebo<br>sc IFN β-1a qw / placebo | −0.63<br>−0.34 | 0.13<br>0.12 | 0.53 (0.41;0.69)<br>0.71 (0.56;0.91) | <0.001<br>0.006 |
| Age | <30 years / ≥30 years | 0.26 | 0.10 | 1.29 (1.05;1.59) | 0.014 |
| Classification of FCDE | Monofocal / multifocal | −0.39 | 0.10 | 0.68 (0.55;0.83) | <0.001 |
| Steroid use at FCDE | Yes / No | −0.06 | 0.11 | 0.94 (0.75;1.18) | 0.617 |
| Presence of T1 Gd+ lesions at baseline | Yes / No | 0.60 | 0.11 | 1.81 (1.48;2.23) | <0.001 |
| Number of T2 lesions at baseline | | 0.02 | 0.00 | 1.02 (1.02;1.03) | <0.001 |

**Baseline sNfL subgroup significantly affected the risk of conversion to McDonald MS in the univariate model.**

| | | | | | |
|---|---|---|---|---|---|
| Baseline sNfL subgroup | Low M0 sNfL/<br>High M0 sNfL | −0.54 | 0.11 | 0.58 (0.47;0.72) | <0.001 |

[a]Univariate Cox's proportional hazards model. [b]Two-sided Wald test.

**CI**, confidence interval; **FCDE**, first clinical demyelinating event; **Gd+**, gadolinium-enhancing; **HR**, hazard ratio; **IFN**, interferon; **MS**, multiple sclerosis; **qw**, once weekly; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# RESULTS

## Multivariate Analysis of Time to McDonald 2005 MS

| Factor | Factor Level | Parameter Estimate[a] | SE | HR (95% CI)[a] | P value[b] |
|---|---|---|---|---|---|
| Treatment | sc IFN β-1a tiw / placebo<br>sc IFN β-1a qw / placebo | −0.80<br>−0.53 | 0.14<br>0.13 | 0.45 (0.34;0.59)<br>0.59 (0.46;0.76) | <0.001<br><0.001 |
| Age | <30 years / ≥30 years | 0.39 | 0.11 | 1.47 (1.19;1.82) | <0.001 |
| Classification of FCDE | Monofocal / multifocal | −0.38 | 0.11 | 0.69 (0.5;0.85) | <0.001 |
| Number of T2 lesions at baseline | | 0.02 | 0.00 | 1.02 (1.01;1.03) | <0.001 |
| Number of T1 Gd+ at baseline | | 0.07 | 0.02 | 1.07 (1.02;1.11) | 0.001 |
| Baseline sNfL subgroup | Low M0 sNfL/<br>High M0 sNfL | −0.26 | 0.12 | 0.77 (0.61;0.97) | 0.024 |

[a]Stepwise multivariate Cox's proportional hazards model. [b]Two-sided Wald test.

**CI**, confidence interval; **FCDE**, first clinical demyelinating event; **Gd+**, gadolinium-enhancing; **HR**, hazard ratio; **IFN**, interferon; **qw**, once weekly; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# RESULTS

## Multivariate Analysis of Time to McDonald 2005 MS

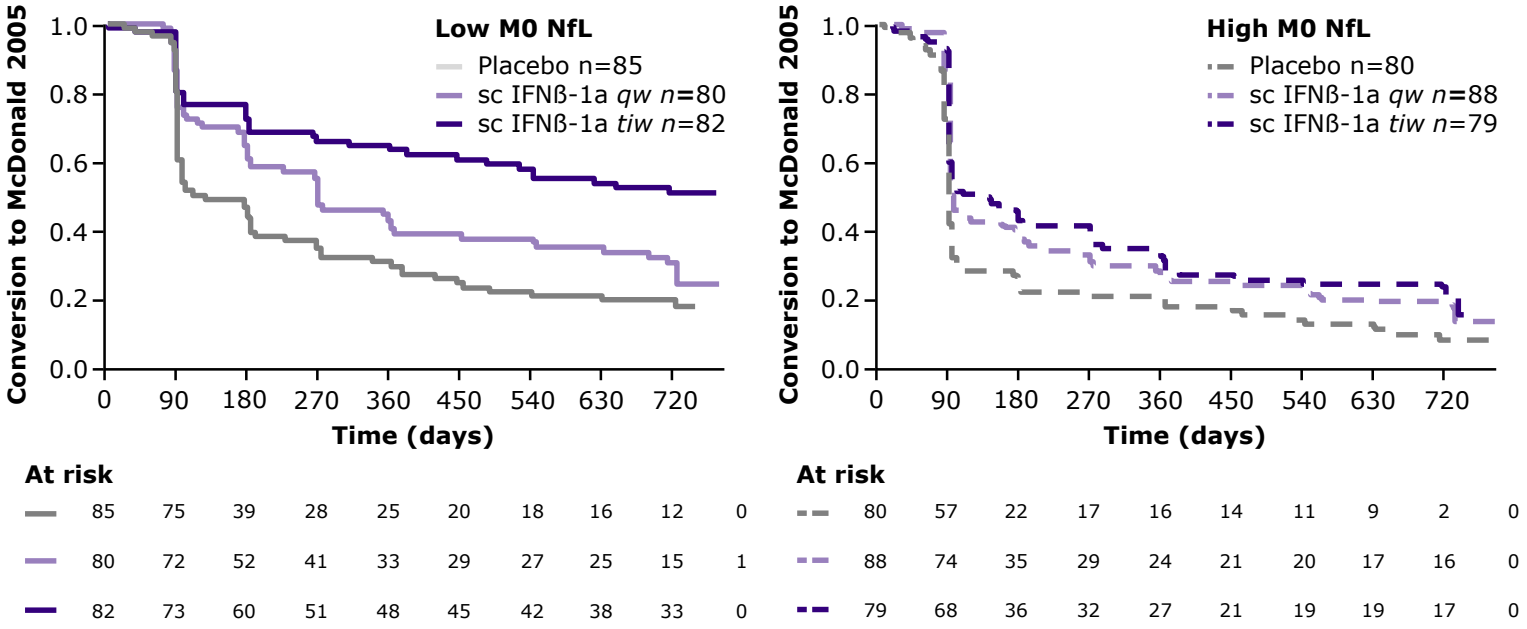
| Factor | Factor Level | Parameter Estimate[a] | SE | HR (95% CI)[a] | P value[b] |
|---|---|---|---|---|---|
| Treatment | sc IFN β-1a tiw / placebo<br>sc IFN β-1a qw / placebo | −0.80<br>−0.53 | 0.14<br>0.13 | 0.45 (0.34;0.59)<br>0.59 (0.46;0.76) | <0.001<br><0.001 |
| Age | <30 years / ≥30 years | 0.39 | 0.11 | 1.47 (1.19;1.82) | <0.001 |
| Classification of FCDE | Monofocal / multifocal | −0.38 | 0.11 | 0.69 (0.55;0.85) | <0.001 |
| Number of T2 lesions at baseline | | 0.02 | 0.00 | 1.02 (1.01;1.03) | <0.001 |
| Number of T1 Gd+ at baseline | | 0.07 | 0.02 | 1.07 (1.02;1.11) | 0.001 |
| Baseline sNfL subgroup | Low M0 sNfL/<br>High M0 sNfL | −0.26 | 0.12 | 0.77 (0.61;0.97) | 0.024 |

**The effect of baseline sNfL subgroup on risk of conversion to McDonald MS was confirmed using a multivariate model.**

**CI**, confidence interval; **FCDE**, first clinical demyelinating event; **Gd+**, gadolinium-enhancing; **HR**, hazard ratio; **IFN**, interferon; **qw**, once weekly; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# RESULTS

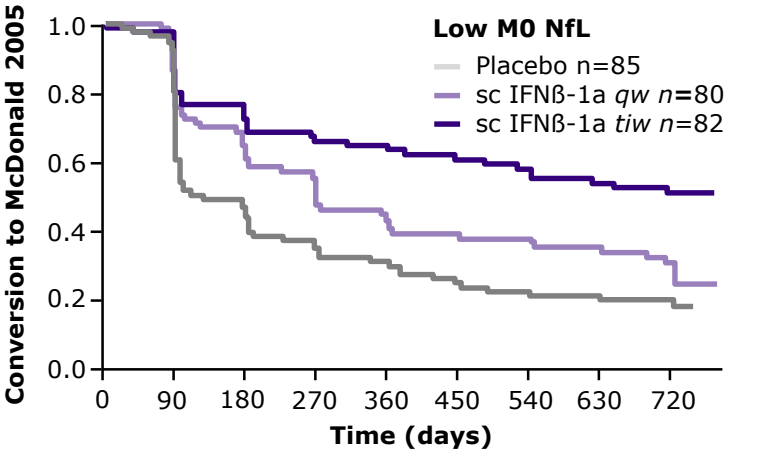
## Time to McDonald 2005 MS by Baseline NfL Subgroup and Treatment Group



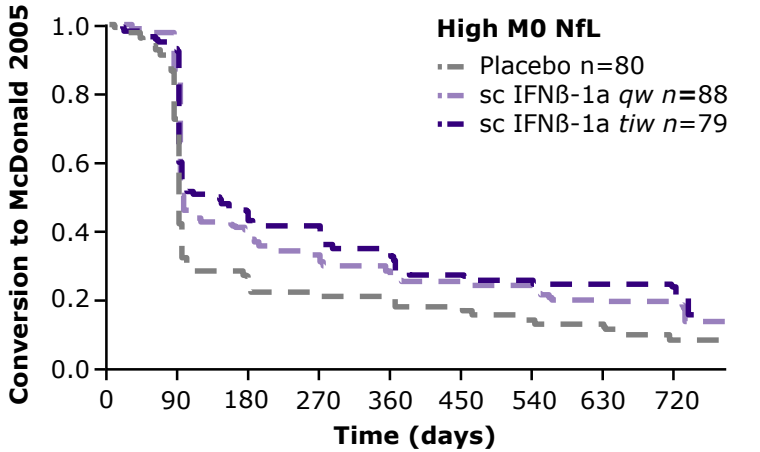

Baseline NfL subgroups were defined using the median baseline NfL value (26.1 pg/mL) as a cut off; low NfL subgroup: NfL value ≤ median M0 NfL value; high NfL subgroup: NfL value > median M0 NfL value.
**BL**, baseline; **CI**, confidence interval; **HR**, hazard ratio; **IFN**, interferon; **qw**, once weekly; **PBO**, placebo; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# RESULTS

## Time to McDonald 2005 MS by Baseline NfL Subgroup and Treatment Group




| | sc IFN-β 1-a qw vs placebo | sc IFN-β 1-a tiw vs placebo |
|---|---|---|
| **HR (95% CI)** | 0.68 (0.48–0.97) | 0.41 (0.27–0.61) |
| ***P* value** | 0.031 | <0.001 |




| | sc IFN-β 1-a qw vs placebo | sc IFN-β 1-a tiw vs placebo |
|---|---|---|
| **HR (95% CI)** | 0.71 (0.51–0.99) | 0.64 (0.45–0.90) |
| ***P* value** | 0.040 | 0.008 |

Baseline NfL subgroups were defined using the median baseline NfL value (26.1 pg/mL) as a cut off; low NfL subgroup: NfL value ≤ median M0 NfL value; high NfL subgroup: NfL value > median M0 NfL value.
**BL**, baseline; **CI**, confidence interval; **HR**, hazard ratio; **IFN**, interferon; **qw**, once weekly; **PBO**, placebo; **sc**, subcutaneous; **SE**, standard error; **sNfL**, serum neurofilament light chain; **tiw**, three times weekly

# CONCLUSIONS

**Higher baseline sNfL levels were associated with an increased risk of conversion to McDonald 2005 MS in patients with a FCDE**

**Age, multifocal disease and number of T1 or T2 lesions at baseline were also confirmed as significant determinants for risk of conversion to McDonald 2005 MS.**

**Conversion to CDMS or McDonald MS was delayed in patients treated with sc IFNβ-1a tiw or qw with both high and low baseline NfL values.**

**FCDE,** first clinical demyelinating event**; IFN**, interferon; **qw**, once weekly; **sc**, subcutaneous **sNfL**, serum neurofilament light chain; **tiw**, three times weekly