

# Anti-alpha-glucose-based Glycan IgM Antibodies in Patients with a Clinically Isolated Syndrome: Analyses from the **BETAferon®** in Newly Emerging Multiple Sclerosis For Initial Treatment (BENEFIT) Study



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## ABSTRACT

**Background:** To determine whether the baseline measurement of immunoglobulin (IgM) antibodies against alpha-glucose glycans (panel of anti-GAGA and alpha-glucose glycan antibodies) can predict disease activity in patients with a first event suggestive of multiple sclerosis (MS) in the BETAferon® in Newly Emerging multiple sclerosis For Initial Treatment (BENEFIT) study.

**Methods:** The BENEFIT study was designed to evaluate the impact of early- versus delayed-interferon beta-1b (IFNB-1b; Betaferon®) treatment in patients with a first event suggestive of MS. Four hundred and sixty eight patients were randomised to IFNB-1b 250 µg or placebo (5:3), subcutaneously every other day for 2 years or until developing clinically definite MS (CDMS), and were then enrolled in a follow-up phase with IFNB-1b treatment for up to 5 years. Serum samples were taken at baseline and were evaluated for alpha-glucose glycan antibodies according to two different storing/aliquoting protocols (early and delayed frozen samples) by means of an enzyme immunoassay (gMS®; Glycominds). Determination of positive alpha-glucose glycan antibody titres was based on a set of antibodies established by previous studies<sup>1</sup> for prediction of relapse activity. We also explored another extended panel of alpha-glucose glycan antibodies including anti-P63 glycan antibody.

**Results:** Differences in the alpha-glucose glycan IgM antibody measurements of the two sera sets have been detected. Thus, both sample sets were considered, and 180 early frozen and 286 delayed frozen baseline samples were analysed.

**Conclusions:** Serum detection of IgM antibodies to alpha-glucose glycans at baseline may be of value for the prediction of clinical or magnetic resonance imaging (MRI) activity in early MS. Data concerning the predictability of alpha-glucose glycan antibodies for conversion to CDMS, confirmed Expanded Disability Status Scale progression, as well as the association with MRI outcome measures over 5 years will be presented.

## Introduction

- Glycans are predominant surface components of immune cells and give rise to high levels of anti-glycan antibodies such as immunoglobulin (Ig)G, IgM, IgA and IgE. Serum anti-glycan antibodies can potentially interfere with the immune system and can be effective biomarkers in autoimmune diseases such as multiple sclerosis (MS).
- Serum IgM antibodies to a beta N-glycosylated peptide have previously been shown to be increased in patients with relapsing-remitting MS (RRMS).<sup>1</sup>
- The characterisation of the anti-glycan antibody profile in patients with MS demonstrated significantly higher levels of IgM anti-Glc(α1,4)Glc(α) (anti-GAGA4) antibodies in patients with MS compared with those with other neurological diseases.<sup>2-4</sup>
- Furthermore, higher levels of serum anti-alpha-glucose IgM in patients with a first presentation of RRMS predicted time to the next relapse.<sup>2</sup>
- Studies have shown that treatment initiated early after a first event suggestive of MS can delay conversion to clinically definite MS (CDMS).<sup>5-8</sup> –The BETAferon® in Newly Emerging multiple sclerosis For Initial Treatment (BENEFIT) study (NCT00185211) was the first to show that early treatment with interferon beta-1b (IFNB-1b; Betaferon®) delayed the onset of CDMS in patients with clinically isolated syndrome (CIS).<sup>5,6</sup>
- This demonstrates the need for early diagnosis, which may be aided through the assessment of specific biomarkers, to improve management of patients and the monitoring of the effectiveness of treatment in patients with CIS.
- This study explores whether baseline measurement of IgM antibodies against alpha-glucose glycans can predict disease activity in patients with CIS in the BENEFIT study.

## Methods

- BENEFIT comprised a 2-year, multicentre, randomised, double-blind, placebo-controlled, parallel-group, Phase III study with pre-planned analysis after 3 and 5 years of follow-up.<sup>5</sup>
- Eligible patients presented with a first neurological event suggestive of MS that lasted for at least 24 hours, and had an Expanded Disability Status Scale (EDSS) score between 0.0 and 5.0.
- A total of 468 patients were randomised to IFNB-1b 250 µg subcutaneously (sc) every other day (eod) or placebo, for up to 2 years or until CDMS was diagnosed using the modified Poser criteria.<sup>9</sup> Treatment was initiated within 60 days after confirmation of the first clinical event.<sup>5</sup>
- Patients completing the placebo-controlled phase were eligible to enter the pre-planned follow-up phase in which all patients were offered IFNB-1b sc eod for up to 5 years after the first event.
- Serum samples were taken at baseline and were evaluated for alpha-glucose glycan antibodies (anti-GAGA2, anti-GAGA3, anti-GAGA4, anti-GAGA6, anti-alpha-L-Rha, anti-alpha-GlcNAc, anti-P63, anti-P64 and total IgM) according to two different storing/aliquoting protocols (early versus delayed frozen samples, ie, fresh IgM aliquoting versus thawing/freezing aliquoting cycles) by means of an enzyme immunoassay (gMS®; Glycominds):
  - Set A sera samples (delayed frozen; 286 randomly selected samples) were shipped within 3 days after donation under ambient condition and frozen at the central laboratory. For this analysis, the samples were thawed for the first time and prepared according to the Glycominds IgM sera protocol.
  - Set B sera samples (early frozen, 180 samples) were frozen on site prior to shipment to the central laboratory, underwent an additional thaw-freezing cycle and were not prepared according to Glycominds IgM sera protocol.
- A high, positive correlation of repeated anti-GAGA4 measurements was found for Set A samples, but not for Set B samples. Differences were attributed to sample storing/preparation and the deterioration of Set B samples through the additional cycle of thawing/freezing.
- For this reason, only results for Set A samples will be reported regarding the overall analysis of alpha-glucose glycans and their association with clinical/MRI parameters in the BENEFIT study.
- For the purpose of predicting the 'at risk' population among the sample sets, patients were classified as 'positive' or 'negative' according to two classification rules – gMS Classifier 1 and gMS Classifier 2, based on antibody reactivity to two separate types of glycans with no overlap.
- gMS Classifier 1 is based on disaccharides covalently bound via long linker (anti-GAGA2, anti-GAGA3, anti-GAGA4, anti-GAGA6) and was established by Freedman and colleagues<sup>2</sup> for the prediction of 'early relapse (within 24 months)'. The classification rule is as follows:  
 gMS Classifier 1: *Classify patient as 'early relapse', if blood sample is positive regarding anti-GAGA2 OR if blood sample is positive regarding anti-GAGA3 OR if blood sample is positive regarding anti-GAGA4 OR if blood sample is positive regarding anti-GAGA6, where positivity means that the respective anti-GAGA value is greater than a certain threshold (ie, mean plus a certain coefficient, times the standard deviation of the respective anti-GAGA observations in the sample set).*
- gMS Classifier 2 is based on a polymer with repeating units of alpha 3 glucose and alpha 6 glucose (anti-P63) and age at CIS, and was constructed based on the BENEFIT 2-year data of placebo group patients using only statistical methods such as penalised logistic regression and cross-validation for the prediction of 'early CDMS (within 24 months)'. Construction and exploration of this classifier in patients up to year 5 were based partly on the same data. The classification rule is as follows:  
 gMS Classifier 2: *Classify patient as 'early CDMS', if the value of a function involving the patient's age at screening and anti-P63 (based on logistic regression) is greater than a certain threshold.*
- Statistical analyses were performed to explore the effect of each gMS classifier on (eg, how well they 'predict') clinical and MRI activity in patients from the BENEFIT study based on the subjects' 'initial immunoreactivity'.

- Time to CDMS, time to McDonald MS, and time to confirmed EDSS progression were analysed by the log-rank test and Cox proportional hazard regression including age, gender, onset of disease, steroid use at first event, effect of treatment in IFNB-1b, gadolinium-enhancing lesions, and number of T2 lesions as covariates.
- MRI variables and annualised relapse rate (ARR) were analysed by non-parametric analysis of covariance (ANCOVA) and generalised linear Poisson regression, respectively.

## Results

### gMS Classifier 1

- gMS Classifier 1 used to predict the next relapse within 24 months in patients with CIS demonstrated 21.35% sensitivity, 82.78% specificity and 62.45% accuracy.
- gMS Classifier 1 had no significant predictive value regarding time to CDMS (Figure 1a) and time to McDonald MS (Figure 1b).

Figures 1a, b and c. Impact of gMS Classifier 1 on time to CDMS (1a), time to McDonald MS (1b) and time to confirmed EDSS progression (1c) up to year 5

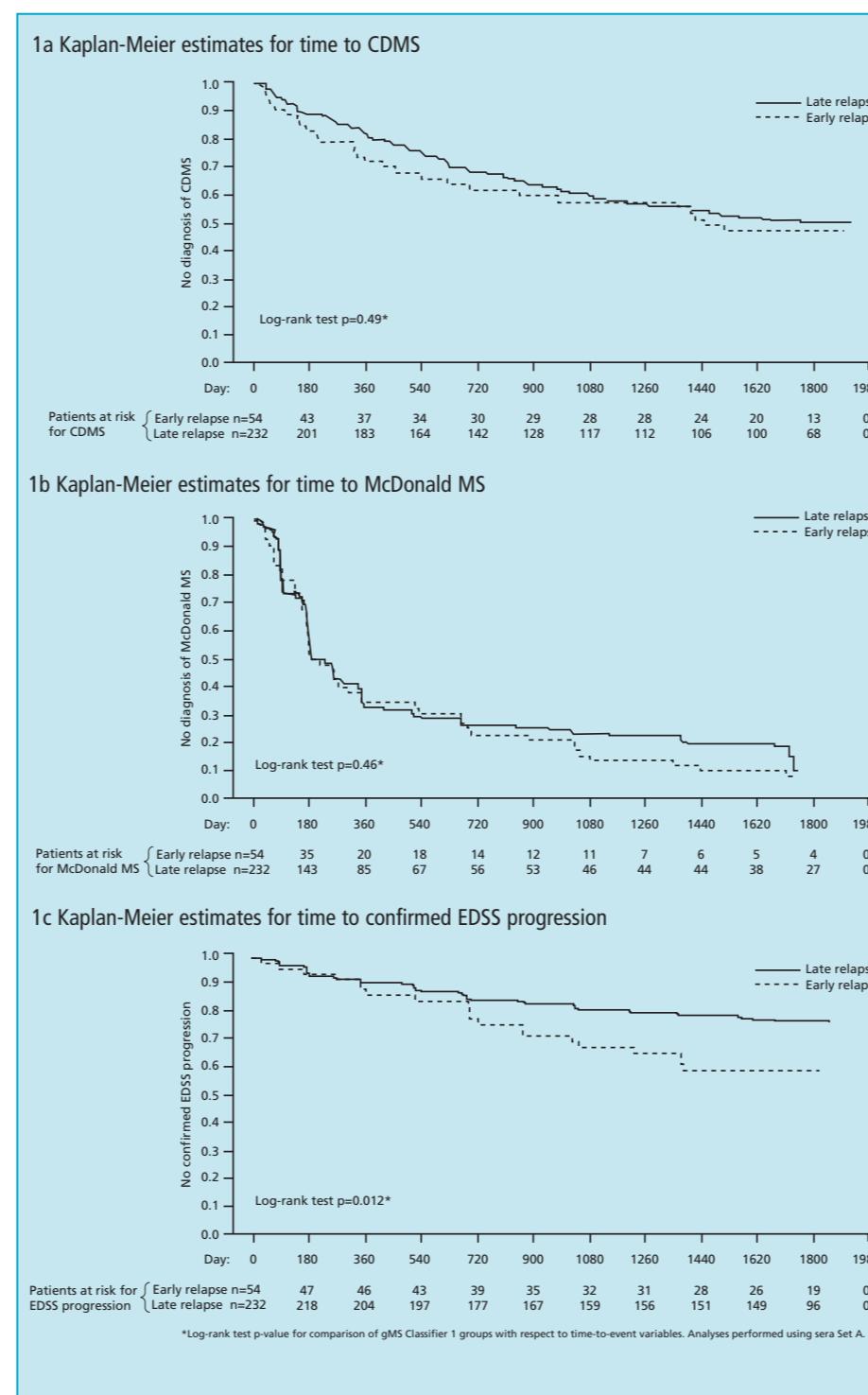


Table 1. Estimates of the impact of gMS Classifier 1 on various clinical time-to-event variables\*

Time-to-event outcome	Parameter		Hazard ratio		
	Estimate	SE	Estimate	95% CI	p-value
Time to CDMS	0.23	0.22	1.26	0.82–1.94	0.295
Time to McDonald MS	0.09	0.16	1.10	0.80–1.51	0.577
Time to confirmed EDSS progression	0.72	0.27	2.05	1.20–3.51	0.009

\*Using the Cox proportional hazard regression model with additional covariates (analyses performed using sera Set A). SE, standard error; CI, confidence interval; CDMS, clinically definite multiple sclerosis; EDSS, Expanded Disability Status Scale.

- gMS Classifier 1 had some impact on time to confirmed EDSS progression in the subpopulation of CIS patients from the BENEFIT study as assessed by log-rank test (Figure 1c; p=0.012) and Cox proportional hazard regression (Table 1; p=0.009). At year 5, according to Kaplan-Meier estimates, 23.3% of the CIS patients classified as 'late relapse' in comparison to 41.8% of the CIS patients classified as 'early relapse' had developed confirmed EDSS progression (Figure 1c).
- In the subpopulation of BENEFIT patients in sample Set A, IFNB-1b treatment also had a significant effect on time to EDSS progression based on the Cox proportional hazard regression model (p=0.0180) in contrast to results based on the full analysis set.
- The effect of gMS Classifier 1 on time to confirmed EDSS progression was diminished if the lowest EDSS score obtained at screening and baseline was used as an additional covariate.
- Further exploratory analyses showed that gMS Classifier 1 had no effect on ARR and MRI parameters including absolute change in T2 or T1 hypointense lesion volume and percentage change in brain volume at 5 years.

Figures 2a, b and c. Impact of gMS Classifier 2 on time to CDMS (2a), time to McDonald MS (2b) and time to confirmed EDSS progression (2c) up to year 5

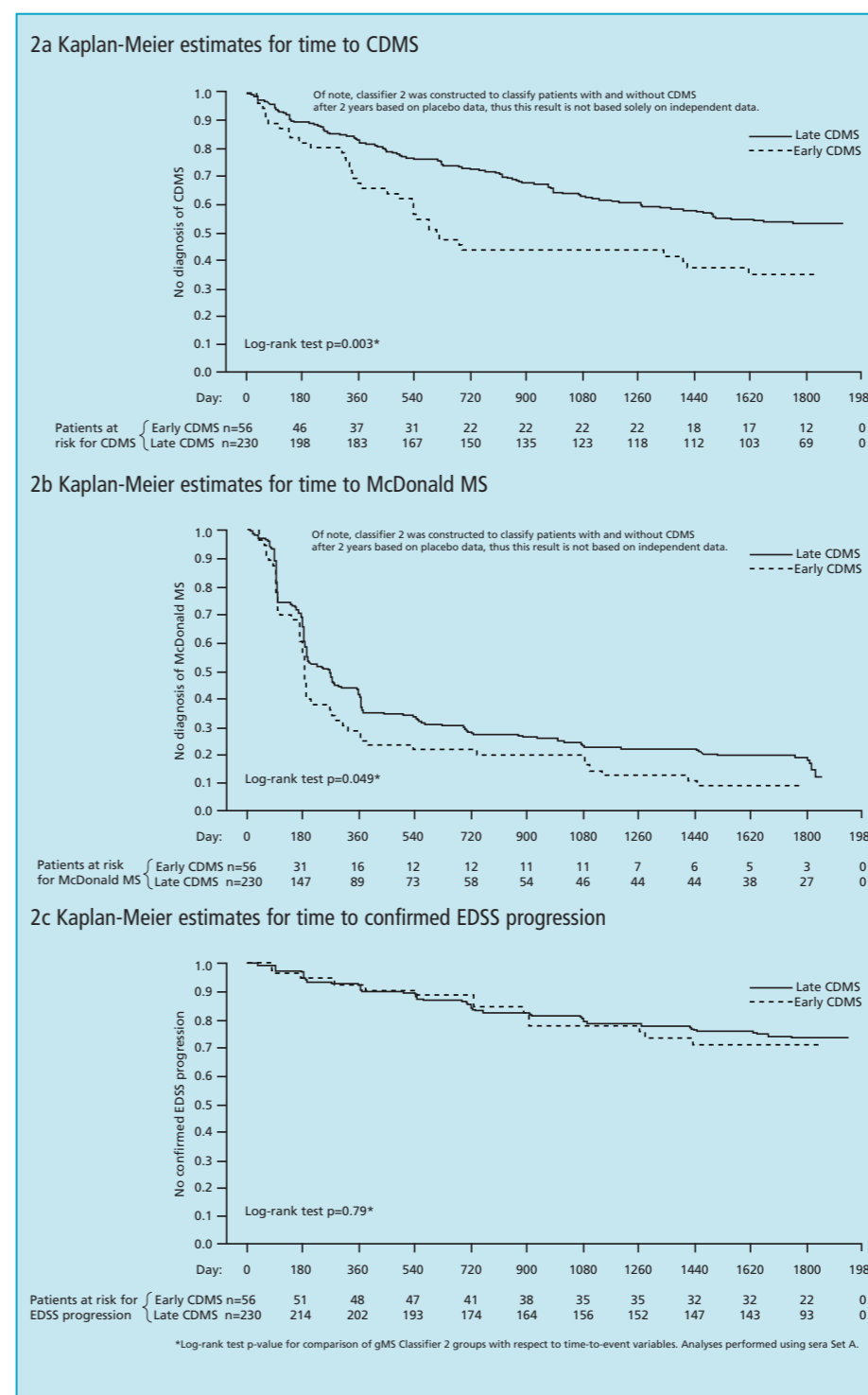


Table 2. Estimates of the impact of gMS Classifier 2 on various clinical time-to-event variables\*

Time-to-event outcome	Parameter		Hazard ratio		
	Estimate	SE	Estimate	95% CI	p-value
Time to CDMS	0.37	0.22	1.44	0.94–2.21	0.094
Time to McDonald MS	0.12	0.17	1.13	0.80–1.58	0.493
Time to confirmed EDSS progression	0.14	0.33	1.15	0.60–2.21	0.669

\*Using the Cox Proportional hazard regression model with additional covariates (analyses performed using sera Set A). SE, standard error; CI, confidence interval; CDMS, clinically definite multiple sclerosis; EDSS, Expanded Disability Status Scale.

### gMS Classifier 2

- Using the behaviour of the placebo group to construct gMS Classifier 2 to predict early CDMS within 24 months in placebo patients, it demonstrated 32.61% sensitivity at a required specificity of at least 90% (achieved value: 90.71%) and 64.00% accuracy.
- As expected (due to the usage of partly the same data for construction and exploration) gMS Classifier 2 had some predictive value for the time to CDMS (Figure 2a, Table 2) up to 5 years.
- gMS Classifier 2 as assessed by the log-rank test had some impact on time to McDonald MS (Figure 2b) but no effect when analyzed by Cox proportional hazard regression model (Table 2). No effect was found on time to confirmed EDSS progression (Figure 2c, Table 2) up to 5 years.
- In the evaluated populations, gMS Classifier 2 had no effect on ARR and MRI parameters including absolute change in T2 or T1 hypointense lesion volume and percentage change in brain volume at 5 years.

## Summary/Conclusions

### gMS Classifier 1

- gMS Classifier 1 derived in order to predict the next relapse within 24 months in patients with first presentation of MS had no predictive value regarding time to CDMS, and no impact on time to McDonald MS, ARR and MRI parameters up to 5 years in a distinct subpopulation of the BENEFIT study.
- gMS Classifier 1 had a statistically significant impact on time to confirmed EDSS progression up to 5 years after CIS in the investigated subpopulation of the BENEFIT study.
- These results were based on exploratory analyses in a distinct subpopulation of the BENEFIT study and require an independent cohort for validation.

### gMS Classifier 2

- gMS Classifier 2 (based on anti-P63 antibody plus age at CIS), was constructed based on the placebo data of a distinct subpopulation of the BENEFIT study in order to predict CDMS within 24 months in patients with CIS, had a some predictive value regarding time to CDMS. The result of the exploratory analysis could be expected as the placebo data were used for both, the construction of the classifier and its exploration.
- gMS Classifier 2 provided limited sensitivity (33%) to predict early CDMS within 24 months at a predefined minimum specificity of 90%.
- gMS Classifier 2 had no impact on time to McDonald MS (based on the Cox proportional hazard regression model), time to confirmed EDSS progression, ARR and MRI parameters up to 5 years after CIS.
- An independent cohort is required for validation of this classifier.

## REFERENCES

- Lolli F, Mazzanti B, Pazzagli M, et al. The glycopeptide CSF114(Glc) detects serum antibodies in multiple sclerosis. *J Neuroimmunol* 2005;167:131–137.
- Freedman MS, Laks J, Dotan N, et al. Anti-alpha-glucose based glycan IgM antibodies predict relapse activity in multiple sclerosis after the first neurological event. *Mult Scler* 2009;15:422–430.
- Schwarz M, Spector L, Gortler M, et al. Serum anti-Glc (alpha1,4) Glc(alpha) antibodies as a biomarker for relapsing-remitting multiple sclerosis. *J Neurol Sci* 2006;244:59–68.
- Brettschneider J, Jaskowski TD, Tumani H, et al. Serum Anti-GAGA4 IgM antibodies differentiate relapsing remitting and secondary progressive multiple sclerosis from primary progressive multiple sclerosis and other neurological diseases. *J Neuroimmunol* 2009; In press.
- Kappos L, Polman CH, Freedman MS, et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology* 2006;67:1242–1249.
- Kappos L, Freedman MS, Polman CH, et al. Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. *Lancet* 2007;370:389–397.
- Jacobs LD, Beck RW, Simon JH, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. *N Engl J Med* 2000;343:898–904.
- Comi G, Filippi M, Barkhof F, et al. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. *Lancet* 2001;357:1576–1582.
- Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983;13:227–231.