Characterization of B-cell subset changes following treatment of neuromyelitis optica spectrum disorder (NMOSD) with inebilizumab: results from the N-MOmentum study

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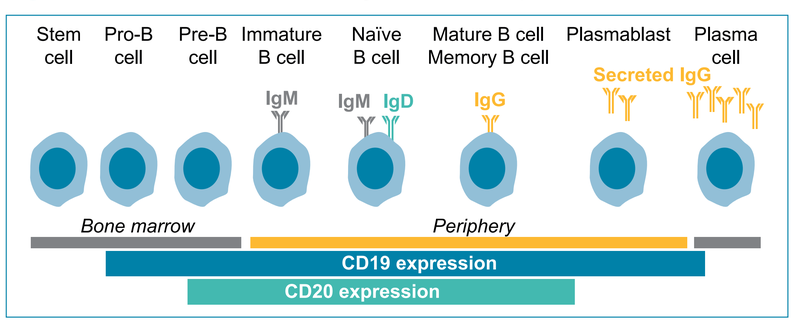
INTRODUCTION

- Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune, central nervous system disorder that is strongly associated with B-cell-mediated immunopathology.[1]
- Serum autoantibodies against the astrocyte water channel protein, aquaporin-4-immunoglobulin G (AQP4-IgG), are pathogenic in NMOSD and are detected in ~75% of patients.[2]
- B cells may contribute to NMOSD through:[2]
  - pathological autoantibody production
  - pro-inflammatory cytokine secretion
  - B-cell-antigen presentation, supporting T-cell-mediated autoimmunity.
- Inebilizumab is a humanized, B-cell-depleting, anti-CD19 antibody shown to reduce adjudicated NMOSD attacks in the phase 2/3 N-MOmentum study.[3]
  - Inebilizumab reduced the risk of adjudicated NMOSD attacks by 72.8% compared with placebo (hazard ratio [HR], 0.272; 95% confidence interval, 0.150–0.496; $p < 0.0001$).
- Anti-CD19 antibodies recognize and deplete a wider range of lymphocytes exclusively from the B-cell lineage than anti-CD20 antibodies (**Figure 1**).[3]

Figure 1. CD19: a differentiated target for B-cell depletion.



CD19 is expressed on a wide range of B cells.

OBJECTIVE

- To characterize the relationship between peripheral blood B-cell lineage subsets and adjudicated NMOSD attacks in patients treated with inebilizumab in the phase 2/3 N-MOmentum study.

METHODS

Study design

- Participants in N-MOmentum were randomly assigned 3:1 to intravenous inebilizumab 300 mg or placebo, with dosing on days 1 and 15.[3]
  - The randomized controlled period (RCP) for each participant was up to week 28 or until occurrence of an adjudication committee-determined attack.[3]
  - Blood samples were collected during study visits at baseline and at weeks 1, 2, 4, 8, 12, 16, 22 and 28 to assess B-cell counts, and at baseline and weeks 2, 4, 8, 12, 16 and 28 for plasma cell gene expression.[3]

Evaluation of B-cell pharmacodynamics

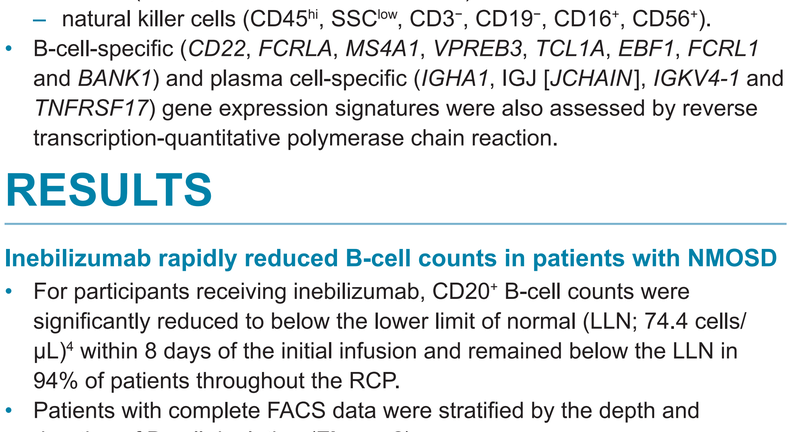
- The impact of inebilizumab treatment on peripheral blood mononuclear cell subsets was assayed by fluorescence-activated flow cytometry (FACS) at regular intervals during the RCP. B-cells were enumerated using CD20 as a lineage FACS marker, as bound inebilizumab interferes with the CD19-based FACS assay. The subsets measured included:
  - B cells
    - $CD20^+$ B cells ($CD45^{hi}$ [$CD3^-$, $CD56^-$, $CD14^-$], $CD33^-$, $CD20^+$)
    - immature transitional B cells ($CD45^{hi}$ [$CD3^-$, $CD14^-$, $CD56^-$], $CD20^+$, IgD^+, $CD27^-$, $CD38^{hi}$)
    - naïve B cells ($CD45^{hi}$ [$CD3^-$, $CD14^-$, $CD56^-$], $CD20^+$, IgD^+, $CD27^-$, $CD38^{dim/-}$)
    - memory B cells ($CD45^{hi}$ [$CD3^-$, $CD14^-$, $CD56^-$], $CD20^+$, $IgD^{+/-}$, $CD27^+$)
  - plasmablasts/plasma cells (in a subset of patients only; $CD45^{hi}$ [$CD3^-$, $CD14^-$, $CD56^-$], $CD27^+$, HLA-$DR^{hi/low}$, $CD38^+$)
  - T cells ($CD45^{hi}$, $CD3^+$ and $CD4^+$ or $CD8^+$)
  - natural killer cells ($CD45^{hi}$, SSC^{low}, $CD3^-$, $CD19^-$, $CD16^+$, $CD56^+$).
- B-cell-specific (*CD22*, *FCRLA*, *MS4A1*, *VPREB3*, *TCL1A*, *EBF1*, *FCRL1* and *BANK1*) and plasma cell-specific (*IGHA1*, IGJ [*JCHAIN*], *IGKV4-1* and *TNFRSF17*) gene expression signatures were also assessed by reverse transcription-quantitative polymerase chain reaction.

RESULTS

Inebilizumab rapidly reduced B-cell counts in patients with NMOSD

- For participants receiving inebilizumab, $CD20^+$ B-cell counts were significantly reduced to below the lower limit of normal (LLN; 74.4 cells/μL)[4] within 8 days of the initial infusion and remained below the LLN in 94% of patients throughout the RCP.
- Patients with complete FACS data were stratified by the depth and duration of B-cell depletion (**Figure 2**).
  - 'Stable depleters' (118/161, 73% of treated patients) had B-cell counts continuously lower than 5 cells/μL during the RCP.
  - 'Partial depleters' (15/161, 9% of treated patients) had B-cell counts below the LLN but greater than 5 cells/μL throughout the RCP.
  - 'Early repleters' (28/161, 17% of treated patients) showed initial B-cell depletion but repleted to greater than 5 cells/μL before the final RCP visit.

Figure 2. $CD20^+$ B-cell-depletion kinetics in patients treated with inebilizumab.

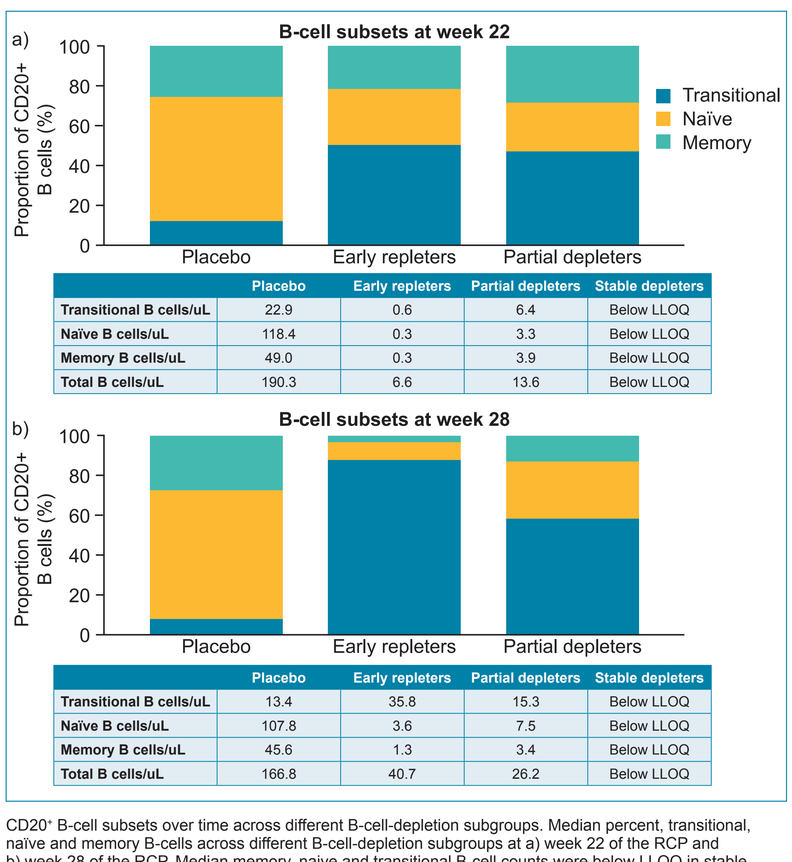


Inebilizumab-treated patients were placed into three subgroups based on the kinetics and depth of B-cell depletion: stable depleters (< 5 cells/μL), partial depleters (less than lower limit of normal but > 5 cells/μL) and early repleters (initial B-cell depletion with partial repletion to > 5 cells/μL before the last visit). The shaded region represents the interquartile range. Line thickness is proportional to the number of samples in each group.
RCP, randomized controlled period.

Transitional, naïve and memory B cells depleted to <1 cell/μL in stable depleters; transitional and naïve B cells made up the majority of reconstituting cells in the early repleter subgroup

- Early repletion of B cells was associated with the emergence of transitional and naïve B cells (**Figure 3**).
  - Although B-cell counts were partially repleted at the end of the RCP in the treated group, they were substantially lower than that of the placebo group.

Figure 3. Enumeration of B-cell subsets during RCP.



	Placebo	Early repleters	Partial depleters	Stable depleters
Transitional B cells/uL	22.9	0.6	6.4	Below LLOQ
Naïve B cells/uL	118.4	0.3	3.3	Below LLOQ
Memory B cells/uL	49.0	0.3	3.9	Below LLOQ
Total B cells/uL	190.3	6.6	13.6	Below LLOQ

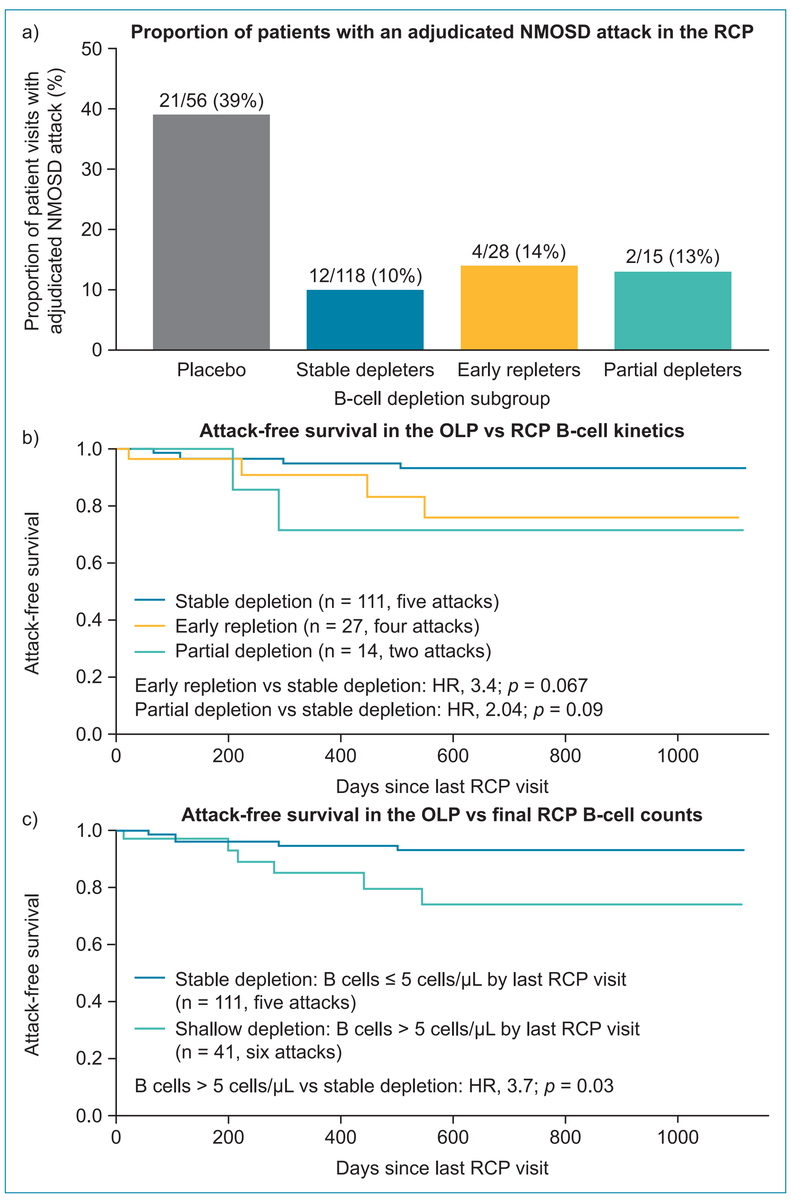
	Placebo	Early repleters	Partial depleters	Stable depleters
Transitional B cells/uL	13.4	35.8	15.3	Below LLOQ
Naïve B cells/uL	107.8	3.6	7.5	Below LLOQ
Memory B cells/uL	45.6	1.3	3.4	Below LLOQ
Total B cells/uL	166.8	40.7	26.2	Below LLOQ

CD20+ B-cell subsets over time across different B-cell-depletion subgroups. Median percent, transitional, naïve and memory B-cells across different B-cell-depletion subgroups at a) week 22 of the RCP and b) week 28 of the RCP. Median memory, naive and transitional B-cell counts were below LLOQ in stable depleters and are therefore not included in the barplot.
LLOQ, lower limit of quantification; RCP, randomized controlled period.

B-cell-depletion kinetics in the RCP were associated with attacks in the open-label phase

- In patients receiving inebilizumab, there was no clear relationship in the RCP between the frequency of adjudicated NMOSD attacks and $CD20^+$ B-cell depletion (stable depleters: 12/118 [10%]; partial depleters: 2/15 [13%]; early repleters: 4/28 [14%]) (**Figure 4a**).
- However, in the open-label phase, stable depleters (< 5 cells/μL by last RCP visit) were more than three times less likely to have an adjudicated attack than early repleters (HR, 3.4; $p = 0.067$) (**Figure 4b**), two times less likely than partial depleters (HR, 2.04; p =0.09) (**Figure 4b**) and three times less likely than shallow depleters (> 5 cells/μL by last RCP visit [early repleters and partial depleters combined]; HR, 3.7; $p = 0.03$) (**Figure 4c**).

Figure 4. Proportion of patients across B-cell-depletion subgroups who experienced an adjudicated NMOSD attack in the RCP. B-cell-depletion kinetics in the RCP are associated with attacks in the OLP.

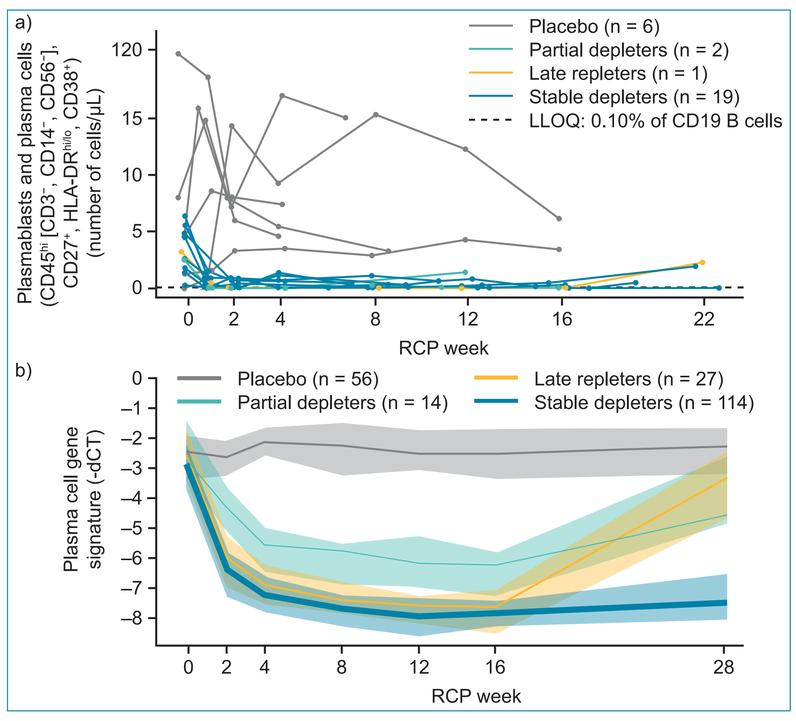


a) Statistical significance of the proportion of patients who experienced adjudicated attacks across different B-cell-depletion subgroups was assessed using the Fisher's exact test and was not significant (ER vs SD, OR = 1.5, p = ns; PD vs SD, OR = 1.4, p = ns). Cox regression analysis of attack-free survival in the RCP by B-cell-depletion subgroup was not significant. $CD20^+$ B-cell counts were also treated as a time-varying covariate in Cox regression analysis of attack-free survival and were not significantly associated with attacks. b) Kaplan–Meier plot of time until first adjudicated OLP attack in inebilizumab-treated patients with stable B-cell depletion, early B-cell repletion and partial B-cell depletion from each patient's last RCP visit. c) Kaplan–Meier plot of time from last RCP visit until first adjudicated OLP attack in inebilizumab-treated patients with > 5 $CD20^+$ B cells/μL at their last RCP visit (early repletion and partial depletion combined) versus those with < 5 $CD20^+$ B cells/μL (stable depletion). Statistical significance of the HR was assessed using Wald's test on coefficients calculated using Cox regression.
HR, hazard ratio; NMOSD, neuromyelitis optica spectrum disorder; ns, not significant; OLP, open-label phase; OR, odds ratio; RCP, randomized controlled period.

Plasmablast and plasma cell counts, and plasma cell gene signatures were reduced in the inebilizumab group

- Plasmablast and plasma cell counts measured by FACS were reduced in patients receiving inebilizumab compared with those receiving placebo (**Figure 5a**).
- The expression signature of plasma cell-specific genes is a robust, sensitive and accurate measure of plasma cells in clinical samples.[5]
  - Plasma cell gene signatures were significantly lower in inebilizumab-treated patients than in patients receiving placebo (**Figure 5b**).

Figure 5. Longitudinal plasmablast and plasma cell counts, and plasma cell gene signatures.

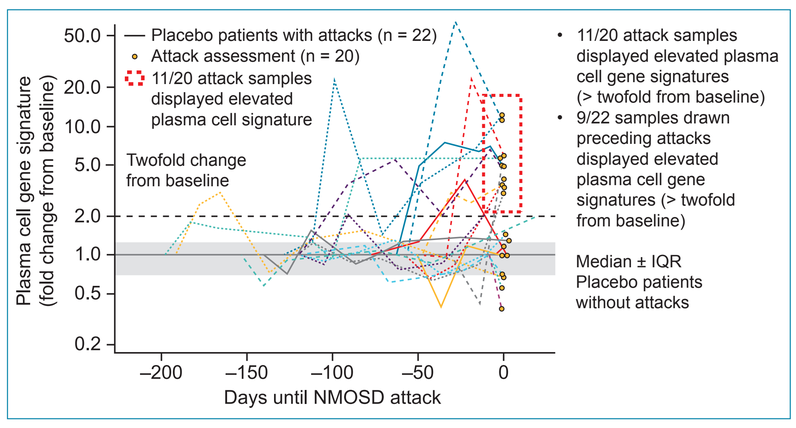


a) Plasmablast and plasma cell counts during the RCP in inebilizumab-treated patients across B cell depletion subgroups. LLOQ for plasma cell and plasmablast counts was established at 0.10 % of CD19 B cells. Samples below LLOQ were imputed at 0.05 cells/μl. b) Median plasma cell gene signature in inebilizumab- and placebo-treated patients over time during the RCP. Shaded region represents IQR. Statistical significance between treatment groups was assessed using the Mann–Whitney U test (*$p < 0.001$). dCT, delta cycle threshold; IQR, interquartile range; LLOQ, lower limit of quantification; RCP, randomized controlled period.

Plasma cell gene signatures increased with adjudicated NMOSD attacks

- In 11/20 attack samples (55%) from the placebo group, plasma cell gene signatures were more than twofold greater than at baseline during adjudicated NMOSD attacks (**Figure 6**).
  - In patients receiving inebilizumab, there were no observable increases in plasma cell gene signature in attack-related samples.

Figure 6. Plasma cell gene signatures in patients in the placebo group who had an adjudicated NMOSD attack.



Plasma cell gene signatures leading to adjudicated NMOSD attacks in the RCP. Profile plot of plasma cell gene signature in placebo group in the 22 patients who experienced NMOSD attacks are plotted in colour. The 20 attack assessments for which the plasma cell gene signature was measured (2 attack samples were unavailable) are highlighted with yellow dots. Significance of increases from baseline to attack in placebo cohort were assessed using Wilcoxon-signed rank test ($p < 0.001$). Plasma cell signature was significantly associated with attack when treated as a time varying covariate in Cox-regression (HR: 1.43, $p = 0.01$).
IQR, interquartile range; NMOSD, neuromyelitis optica spectrum disorder; RCP, randomized controlled period.

CONCLUSIONS

- Compared with placebo, inebilizumab treatment resulted in specific, rapid and durable depletion of B cells and plasmablasts/plasma cells in patients with NMOSD.
- Adjudicated NMOSD attack frequency was not increased in early B-cell-reconstituting or partially depleted participants, compared with stable depleters during the randomized controlled period.
- Patients with B cell counts consistently <5 cells/μL were less likely to have an NMOSD attack during the open label period.
- In 55% of the placebo-treated patients tested, an increase in plasma cell gene signature occurred within 1 week of an NMOSD attack.
  - Further studies are needed to decipher the complex relationship between B-cell depletion and therapeutic efficacy.

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