Title:

Natalizumab Treatment Continuum in RRMS: A Systematic Review and Meta-Analysis of Cessation Risks and Dosing Strategies

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Abstract

Background: Natalizumab is a high-efficacy therapy for RRMS. While EID may reduce PML risk, critical gaps persist in understanding post-cessation outcomes, posing urgent clinical challenges for neurologists discontinuing treatment.

Objectives: To compare efficacy/safety of natalizumab SID vs. EID and quantify relapse/disability risk after cessation in adults with RRMS.

Methods: We conducted a systematic review/meta-analysis (PROSPERO: CRD420251103014) following PRISMA guidelines. We searched MEDLINE, Cochrane Library, ClinicalTrials.gov, and other sources (2015–2025) for RCTs and observational studies. Primary outcomes: ARR and disability progression; secondary: PML incidence and post-cessation relapse. Risk of bias was assessed using Cochrane RoB 2.0 and ROBINS-I. Data were synthesized narratively or via meta-analysis, with evidence certainty graded (GRADE).

Results: Twenty-eight studies (n=45,803) were included. The key finding was a substantial 41.7% (95% CI 30.8–53.6%) pooled relapse risk within 12 months of cessation (4 studies, n=857) using a random-effects model. Sensitivity analysis excluding the outlier study (Weinstock-Guttman 2016) showed a relapse risk of 36.9% (95% CI 33.7–40.3%). Narrative synthesis of ARR (9 studies) showed no consistent SID vs. EID difference (median difference 0.00). Meta-analysis of PML risk (3 studies) found no significant difference (RR 0.70, 95% CI 0.20-2.54). Disability outcomes were too heterogeneous for meta-analysis. Evidence certainty was low for most outcomes.

Conclusion: This review demonstrates a substantial 41.7% relapse risk within 12 months of natalizumab cessation, representing the most critical clinical implication. While EID may maintain efficacy comparable to SID, the high withdrawal relapse rate necessitates prompt therapy transition and patient counseling. Treatment decisions must encompass the entire continuum from initiation to discontinuation.

Keywords: Natalizumab, multiple sclerosis, relapsing-remitting, extended interval dosage, treatment discontinuation, meta-analysis, progressive multifocal leukoencephalopathy.

1. Introduction

1.1 Background and Significance

MS is a chronic immune-mediated disease causing neurological disability. Globally affecting 2.8 million people, RRMS accounts for 85-90% of cases and typically presents in early adulthood (1-3). Effective disease-modifying therapy is crucial for managing relapses and disability progression. Hospitalization and large medical expenses are common among patients with severe RRMS, emphasizing the crucial need for effective disease-modifying therapy (4, 5).

Natalizumab, a humanized monoclonal antibody targeting α4-integrin, inhibits lymphocytes from crossing the blood-brain barrier, reducing CNS inflammation and relapse activity (6, 7). Since its FDA approval in 2004, natalizumab has demonstrated significant decreases in relapse frequency and MRI lesion burden in important trials (8, 3, 2). Following reports of progressive multifocal leukoencephalopathy (PML) in 2005, risk management strategies such as patient screening and JC virus surveillance were implemented (9, 10). Despite these developments, questions about optimal dosing regimens and safe withdrawal continue to arise. While standard four-week dose maintains α4-integrin receptor saturation above 80%, extended interval dosing (EID, 5-8 weeks) may reduce PML risk while maintaining efficacy (5). Discontinuation carries a 3-6-month risk of illness reactivation, emphasizing the importance of evidence-based bridging treatments.
However, perhaps the most challenging clinical scenario arises when natalizumab must be discontinued due to PML risk, pregnancy, or patient preference. Limited data exist on the magnitude and timing of relapse risk following cessation, leaving neurologists without clear guidance on managing this high-risk period.

1.2 Current Evidence and Gaps

The phase 2 and pivotal studies consistently shown lower relapse rates and MRI lesion activity (8, 3, 2, 11). Early meta-analyses confirmed efficacy, but were restricted by short follow-up periods, dose variability, and a lack of long-term safety and post-cessation evidence. Nikfar et al. (12) (2010) analyzed four studies (n = 1,407) and discovered a 50% reduction in relapse risk, but the implications for real-world practice remained questionable. Observational studies, such as Salhofer-Polanyi et al. (13) (2014), indicate the danger of rebound following cessation, but variability in design and outcomes prevents definitive guidance. While Rabea et al. (14) (2024) provided valuable insights into natalizumab dosing strategies, their review did not address the critical question of post-cessation outcomes. This gap is particularly concerning because clinical decisions about dosing intervals cannot be separated from withdrawal planning. Recent EMA guidelines specifically recommend that natalizumab treatment plans include protocols for both ongoing dosing AND eventual discontinuation [54]. Neurologists require evidence-based guidance on both optimizing treatment duration AND managing the dangerous period following discontinuation. Our review addresses this urgent need by providing an analysis of post-cessation outcomes while updating the evidence on dosing strategies through 2025. A direct comparison table is provided in the below Table 1.

Table 1. Comparison with Rabea et al. (2024)

ASPECT	RABEA ET AL. (2024)	CURRENT REVIEW
Primary Focus	Dosing strategies (SID vs EID)	Dosing strategies AND cessation outcomes
Post-cessation Data	Not included	Comprehensive synthesis (n = 857; 41.7% pooled relapse risk at 12 months; time-to-relapse analysis)
Study Coverage	Up to June 2023	Extended to 2025 (8 additional studies including 4 new RCTs/observational studies)
PML Risk Analysis	Not stratified by duration	Duration-stratified analysis (>24 vs. <24 months); incorporated person-year data
Real-World Evidence	Registry-based	45,803 patients (TOUCH, MSBase, TYSEDMUS registries + RCTs)
Clinical Scope	Dosing optimization	Treatment continuum framework (initiation → dosing → cessation → transition)
Key Novel Contribution	EID efficacy/safety equivalence	Quantified post-cessation relapse risk + integrated treatment continuum analysis
Practice Implications	Dosing interval selection	Clinical pathway guidance for EID use, cessation timing, and transition strategies

1.3 Rationale for this Review

While natalizumab's efficacy is well-established, critical gaps persist in understanding post-discontinuation outcomes. Real-world evidence highlights substantial rebound risk, but comprehensive data on the magnitude and timing of relapse after cessation have been lacking. This review addresses the most urgent clinical question in natalizumab management: what happens when we stop treatment? We provide a systematic analysis of post-cessation outcomes including 857 patients across 4 studies. By examining the complete treatment continuum from initiation through discontinuation, we provide neurologists with integrated evidence needed for clinical decision-making.

1.4 Objectives

This systematic review and meta-analysis aims to provide comprehensive evidence on natalizumab treatment in adults with RRMS, with a primary focus on post-cessation outcomes and updated analysis of dosing strategies. Using a PICO framework, the objectives are:

- To quantify the absolute risk, timing, and severity of relapse and disability progression following treatment cessation (primary focus).
- To compare the efficacy of standard-interval dosing (SID) versus extended-interval dosing (EID) on annualized relapse rate (ARR), disability progression, and MRI outcomes.
- To evaluate safety profiles, particularly the incidence of PML.
- To synthesize real-world and trial evidence from 2015 onward.
- To support clinical decision-making by offering evidence on dose optimization and cessation procedures.

2. Methods

2.1 Protocol and Registration

This systematic review was conducted according to a predefined protocol registered on PROSPERO (CRD420251103014). The protocol specified the search strategy, eligibility criteria, outcomes, risk-of-bias assessment methods, and synthesis approach. The review is reported in accordance with the PRISMA 2020 statement (15).

2.2 Eligibility Criteria

Studies were selected based on the PICO framework:

- Population: Adults (≥18 years) with relapsing-remitting multiple sclerosis (RRMS) treated with natalizumab monotherapy.
- Intervention: 1) Standard-interval dosing (SID) of natalizumab (every 4 weeks), or 2) Cessation of natalizumab therapy.
- Comparator: 1) Extended-interval dosing (EID; infusion intervals typically >4 weeks, up to 8 weeks), or 2) For cessation studies, either continuation of natalizumab or no active comparator (natural history).
- Outcomes:The primary outcomes were the annualized relapse rate (ARR) and disability progression (confirmed EDSS deterioration). Secondary outcomes included No Evidence of Disease Activity (NEDA-3), relapse risk after cessation, MRI activity, and safety outcomes, specifically the incidence of progressive multifocal leukoencephalopathy (PML).
- Study Designs: Randomized controlled trials (RCTs), prospective and retrospective cohort studies, and case series (for cessation results) were all included. Studies were omitted if they were reviews, conference abstracts without full data, addressed pediatric populations, coupled natalizumab with other disease-modifying treatments, or did not provide relevant outcomes. To reflect current practice, the publishing date range began on January 1, 2015. While the protocol specified studies from 2015 onward, one study (16)) was first published online in November 2014 but assigned to a 2015 journal issue; given its direct relevance, it was retained. This represents a minor deviation from the protocol. Only English-language publications were included.

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2.3 Information Sources

We searched MEDLINE, Cochrane Library, and ClinicalTrials.gov from 2015-2025, with additional studies identified through reference screening and author contact.
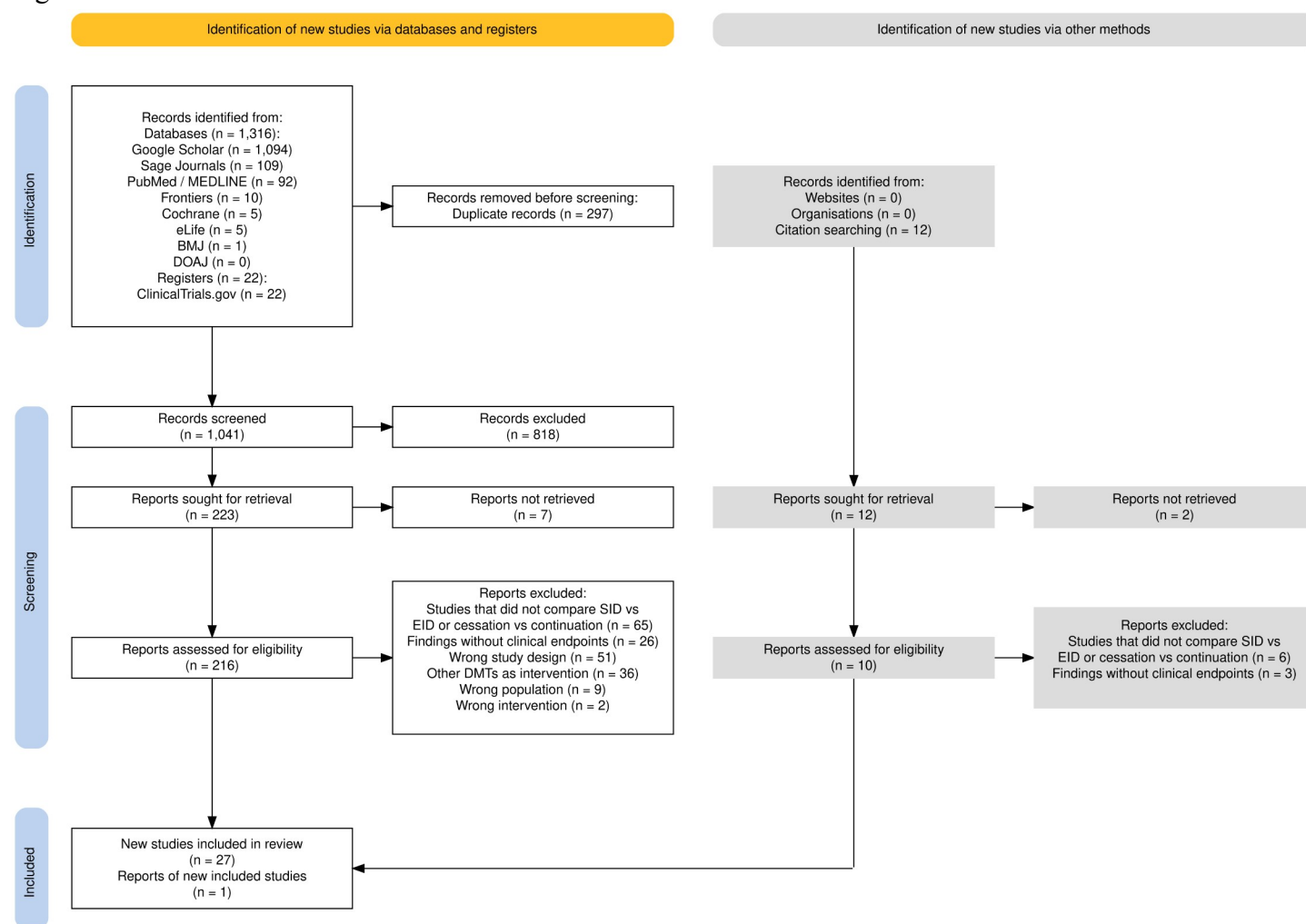
2.4 Search Strategy

Search strategies utilized a combination of controlled vocabulary (e.g., MeSH terms) and keywords related to "natalizumab," "multiple sclerosis," "extended interval dosing," "standard interval dosing," "cessation," "relapse," "disability progression," and "PML." Search filters limited results to human studies and English language, with a publication date from January 1, 2015. The search strategy was tailored for each database and is available on PROSPERO protocol registration.

2.5 Study Selection

Search results were imported into Zotero 6.0.30 for duplicate removal. Title/abstract screening and subsequent full-text assessment were performed independently by two reviewers (V.S., M.B.) using Rayyan QCRI (17) . Conflicts were resolved through discussion or by a third reviewer (D.J.). The study selection process is detailed in the PRISMA flow diagram (18) (Figure 1).

Figure 1: PRISMA flow chart

2.6 Data Collection Process

Data extraction was conducted independently by two reviewers (V.S., D.J.) using a standardized, piloted data extraction form in LibreOffice and Word. Disagreements were resolved by consensus or third-party adjudication (M.B.). Study authors were contacted for clarification or additional data when reported information was incomplete.

2.7 Data Items

Extracted data included:

- Study characteristics (design, country, funding, conflicts of interest).
- Participant characteristics (sample size, demographics, baseline disease characteristics).
- Intervention and comparator definitions (specific EID interval, cessation reason, post-cessation management).
- Outcome data for all pre-specified efficacy and safety endpoints, including effect estimates, measures of dispersion, and raw counts/denominators where available.

2.8 Risk of Bias in Individual Studies

The risk of bias was assessed independently by two reviewers (V.S., M.B.).

- RCTs (n = 3) were evaluated using the Cochrane RoB 2.0 tool (19).
- Non-randomized studies (n = 24) were assessed using the ROBINS-I tool (20).
- Case series (n = 1) were appraised using the Joanna Briggs Institute (JBI) critical appraisal checklist (21).

Results are summarized graphically in Supplementary Figure 1A (RCTs) and Supplementary Figure 1B (non-randomized studies), which were generated using the robvis visualization tool (22). Detailed domain-level judgments for all studies are provided in Supplementary Table 1.

2.9 Synthesis Methods

Given that clinical decisions about dosing intervals cannot be separated from withdrawal planning in practice, we adopted a comprehensive approach examining both aspects of natalizumab management. This integrated perspective reflects real-world clinical decision-making where neurologists must consider the entire treatment continuum.

- Synthesis approach depended on clinical and methodological homogeneity. Meta-analysis used random-effects models for consistent outcomes. For rare events (PML), only studies reporting events were pooled. Person-year data were analyzed separately. Heterogeneity was quantified using I^2.

- Narrative synthesis followed SWiM guidelines for outcomes with extreme heterogeneity. For ARR, meta-analysis was precluded due to EID definition variability (5-8 weeks), inconsistent reporting (7 calculation methods), and missing data. Median ARR difference was 0.00 (range -0.05 to +0.01).

For the post-cessation relapse analysis, we used a random-effects model and assessed heterogeneity using the I^2 statistic. To investigate sources of heterogeneity, we conducted pre-specified subgroup analyses based on cessation reason (planned vs. unplanned) and post-cessation management strategy (immediate switch vs. washout period). However, limited data reporting prevented formal subgroup analyses. The primary analysis included all studies, with sensitivity analysis excluding the outlier study (Weinstock-Guttman 2016) to assess robustness of findings. Supplementary Figure 3 shows the forest plot using Freeman-Tukey double arcsine transformation, and Supplementary Figure 4 presents the sensitivity analysis excluding the outlier study.

Sensitivity analyses assessed robustness. Evidence certainty was evaluated using GRADE (24) (Supplementary Table 4).

2.10 Software

We used Zotero, Rayyan QCRI (17) , R statistical software (25) with the meta package (26), and LibreOffice/Word for data management and analysis.

3. Results

3.1 Study Selection

Our systematic search identified 1,338 records (1,316 from databases and 22 from trial registries), with an additional 12 records found through citation searching. After removing 297 duplicates, 1,041 titles and abstracts were screened, of which 818 were excluded for irrelevance.
We assessed 223 full texts from databases/registries and 12 from citation searches. Nine full texts (7 database/register, 2 citation) could not be retrieved. Of the remaining 225 reports, 199 (189 database/register and 10 citation) were excluded due to ineligible study design, population, intervention, or outcomes.

Ultimately, 28 studies met inclusion criteria (27 from databases/registries, 1 from citation searching). The PRISMA 2020 flow diagram (18) (Figure 1) summarizes the selection process, and excluded studies are listed in Supplementary Table 2.

3.2 Study Characteristics

The 28 included studies (2015–2025) comprised 3 randomized controlled trials (RCTs), 24 observational studies (prospective and retrospective cohorts), and 1 case series. Across all studies, the total sample size was 45,803 participants, although one large registry (Ryerson et al. (27), n=35,521) heavily influenced this number. Studies were conducted in single-country or multinational settings across Europe, North America, the Middle East, and Australia.

Two primary comparisons were addressed:

- Standard-Interval Dosing (SID) vs Extended-Interval Dosing (EID) of natalizumab, with EID definitions ranging from >5 weeks to 8-week intervals.
- Outcomes after cessation of natalizumab.

The key reported outcomes were annualized relapse rate (ARR), disability progression (EDSS worsening), NEDA-3, PML incidence, and post-cessation outcomes (relapse rates, time to relapse, and EDSS worsening). Funding was disclosed for 22 trials, with 10 getting industrial support, largely from Biogen. Table 2 summarizes the detailed study characteristics, including the baseline demographics.

TABLE 2. Characteristics of Included Studies

Study (Author, Year)	Country	Study Design	Sample Size (Total N)	Comparison Groups	Funding Source / Conflict of Interest	Notes
Al-Mahdawi et al., 2023 (28)	Iraq	Observational Retrospective Cohort	150	SID (Q4W), EID (definition NR)	NR	Multicenter, aggregated data only (contributed to narrative

						context)
Bernardes et al., 2024 (29)	Portugal	Retrospective observational cohort	76	SID (Q4W) vs EID (Q6W)	No specific grant; COI disclosed	Single-center, within-patient comparison
Butzkueven et al., 2020 (TOP) (30)	Multinational	Prospective Observational Registry	472 (matched)	SID (Q4W), EID (Q6W)	Supported by Biogen; multiple author COIs	Multicenter registry, propensity score-matched
Clerico et al., 2019 (31)	Italy	Retrospective Cohort	360	SID (<5 weeks), EID (≥5 weeks)	NR	Multicenter (14 centers)
Coerver et al., 2021 (32)	Netherlands	Retrospective Observational Cohort	147	Cessation	ZonMW grant, Stichting MS Research grant	Monocentric, outcomes after cessation
De Mercanti et al., 2021 (33)	Italy	Retrospective cohort	316	SID (<5w) vs EID (≥5w)	Not reported	Multicenter (14 centers)
Foley et al., 2022 (NOVA) (34)	Multinational (11 countries)	RCT (open-label, rater-blinded)	499	SID (Q4W) vs EID (Q6W)	Biogen	Multicenter (89 centers), phase 3b trial
Fuentes-Rumí et al., 2020 (35)	Spain	Retrospective observational	50	Cessation	No specific grant; COI disclosed	Single-center, IVMP washout strategies
Gardinetti et al., 2017 (36)	Italy	Retrospective Cohort	30	Cessation	All authors have nothing to declare	Multicenter (2 centers), outcomes after cessation

González-Suarez et al., 2017 (37)	Spain	Case series	4	Cessation → switch to FTY	"No conflict of interest"	Multicenter (3 hospitals), rebound cases
Haas et al., 2016 (38)	Germany	Prospective cohort	57	SID (Q4W) vs Cessation (Wash-out)	German Ministry for Education and Research; "none declared"	Single-center, immunology focus
Jakimovski et al., 2023 (39)	USA (NY)	Retrospective cohort	242	Continue SID (Q4W) vs Discontinue (Cessation)	Biogen; Multiple authors disclosed industry funding	Consortium data, includes SPMS/PPMS patients
Jeantin et al., 2023 (40)	France	Retrospective Self-Controlled Cohort	57	SID (Q4W), EID (Q6W)	No funding; some author COIs	Monocentric, within-patient comparison
Kaufman et al., 2015 (RESTORE) (41)	Multinational	RCT	175	Continue Natalizumab (Q4W), Placebo (Cessation)	Funded by Biogen Idec Inc.	Multicenter, partially placebo-controlled
Kister et al., 2018 (42)	Multinational (20 centers)	Retrospective registry cohort	4842	Cessation (vs other DMTs)	MSBase Foundation; "No targeted funding"	MSBase registry, natalizumab cessation arm n=308
Mustonen et al., 2020 (43)	Finland	Retrospective cohort	89 (85 RRMS)	Cessation	Hospital grants; personal grants; honoraria/consultancies	Multicenter (4 hospitals), RRMS n=85 for PICO
Papeix et al.,	France	Prospective	715	Cessation	No targeted funding;	Nationwide

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2016 (44)		observational registry			COI disclosed	multicenter (TYSEDMUS registry)
Pelle et al., 2023 (45)	France	Retrospective cohort	303	SID (Q4W) vs EID (5-6w)	Caen University Hospital; additional funding from Biogen	Multicenter (5 centers)
Riancho et al., 2021 (46)	Spain	Retrospective Cohort	39	SID (Q4W), EID (Q8W)	Supported by IDIVAL; various author COIs	Monocentric, within-patient comparison (sequential arms)
Ruggieri et al., 2019 (47)	Italy	Retrospective Cohort	775	SID (≤35 days), EID (>35 and ≤56 days)	NR	Multicenter, includes a mixed-dose arm
Ryerson et al., 2015 (48)	USA	Registry Analysis	1998	SID (Q4W ±2d), EID (4w3d-8w5d)	No grant or industry support	Multicenter registry
Ryerson et al., 2019 (27)	USA	Retrospective Cohort (TOUCH)	35,521	SID (various def.), EID (various def.)	Funded by Biogen	TOUCH registry, analysis of PML risk only
Ruggieri et al., 2024 (49)	Italy	Retrospective Cohort	745	SID (Q4W), EID (>5-7 weeks), EID (>7-8 weeks)	Multiple author COIs with pharma companies	Multicenter (8 centers), arm Ns not reported
Toorop et al., 2024 (50)	Netherlands	Retrospective Cohort	82	SID (Q4W), EID (≥5 weeks)	Dutch MS Research Foundation, Brain Foundation Netherlands,	Monocentric

					Innovation Fund Healthcare insurers	
van Kempen et al., 2020 (51)	Netherlands	Prospective single-arm trial	61	EID (Personalized)	Brain Foundation Netherlands; Various COI disclosures	Multicenter, no SID comparator arm
Vidal-Jordana et al., 2015 (16)	Spain	Prospective observational cohort	47	Cessation	No specific grant	Single-center, outcomes after cessation
Weinstock-Guttman et al., 2016 (52)	USA	RCT (single-blinded MRI)	50	Cessation: Tapered (EID-like) vs Immediate	Biogen Idec (Investigator-initiated)	Single-center, phase IV, includes relapsing-SPMS
Yamout et al., 2018 (53)	Lebanon, Iran	Retrospective Review of Registry Cohorts	85	SID (Q4W), EID (≥5 weeks)	None	Multicenter (2 centers), within-patient comparison

Footnote: SID = standard-interval dosing; EID = extended-interval dosing; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; NR = not reported.

3.3 Risk of Bias

RCTs (n=3) were evaluated using ROB 2 (19): two were judged low risk, while one raised some concerns due to deviations from intended interventions.

Non-randomized studies (n=24) were assessed with ROBINS-I (20): 20 had moderate risk and 4 serious risk.

Confounding and missing data were the most common sources of bias. A case series (37) assessed with the JBI checklist was deemed moderate risk. Full risk-of-bias judgments are provided in Supplementary Table 1 and Supplementary Figures 1A and 1B.

3.4 Synthesis of Results

Feasibility of quantitative meta-analysis was pre-assessed for each outcome based on clinical and methodological homogeneity (Supplementary Table 3).

3.4.1 SID vs EID: Efficacy Outcomes (ARR)

Key Finding: No consistent difference in ARR was found between SID and EID.

Thirteen studies reported ARR, of which 9 provided data suitable for descriptive synthesis. ARR values were consistently low across both dosing strategies (range: 0.0006–0.14).

Based on the direction of point estimates, four studies reported a lower ARR with EID, three reported a lower ARR with SID, and two reported identical or nearly identical ARR between groups.

- The median ARR difference (EID – SID) was 0.000 (range –0.050 to +0.014).
- Pelle 2023 (45) was the only study allowing formal IRR estimation (IRR 0.44; 95% CI 0.16–1.26), showing no significant difference.

Table 3A summarizes ARR outcomes. Meta-analysis was not possible due to heterogeneity in EID definitions, outcome reporting, and lack of raw event data.

Summary: Both SID and EID maintained very low relapse activity. No strategy consistently outperformed the other.

Table 3A: ARR Comparison: Study Characteristics and Reported Outcomes

Study	EID Definition	Reported ARR (Quote/Direct Summary)	Notes
Bernardes 2024 (29)	6 weeks	ARR, median (IQR) 0 (0) [SID] vs 0 (0) [EID]; Relapses: 1 [SID] vs 4 [EID], p=0.083	Within-patient comparison; PY not reported
Clerico 2019 (31)	≥5 weeks	Adjusted ARR: 0.060 (95% CI 0.033–0.087) [SID] vs 0.039 (95% CI 0.017–0.063) [EID]	Adjusted mean; no raw counts/PY
De Mercanti 2021 (33)	≥5 weeks	ARR 0.075 (SD 0.20) [SID] vs 0.089 (SD 0.22) [EID]	Pre-intervention period
Foley 2022 (34)	6 weeks	Unadjusted ARR 0.0171 [SID] vs 0.0228 [EID];	Relapse counts not reported

		Adjusted ARR: 0.00010 (95% CI 0.00004–0.00024) [SID] vs 0.00013 (95% CI 0.00006–0.00027) [EID]	
Jeantin 2023 (40)	6 weeks (42 ± 7 days)	ARR 0.0 [SID] vs 0.0 [EID]	Zero relapses; PY calculable
Pelle 2023 (45)	5–6 weeks (≤9 infusions/12mo)	ARR 0.083 (SD 0.300) [SID] vs 0.041 (SD 0.231) [EID]; Relapses: 12/156 [SID] vs 5/147 [EID]	Only study with counts & PY suitable for IRR
Riancho 2021 (46)	8 weeks	ARR 0.025 (SD 0.026) [SID] vs 0.020 (SD 0.060) [EID]; p=0.72	Mean ARR only
Ryerson 2015 (48)	4.5–8.5 weeks	ARR 0.14 [SID] vs 0.09 [EID]	Mean ARR only
Yamout 2018 (53)	≥5 weeks	ARR 0.0006 ± 0.003 [SID] vs 0.001 ± 0.006 [EID]; p=0.42	Mean ARR and SD

Footnotes: SID = standard-interval dosing; EID = extended-interval dosing; ARR = annualized relapse rate; SD = standard deviation; PY = person-years; CI = confidence interval.

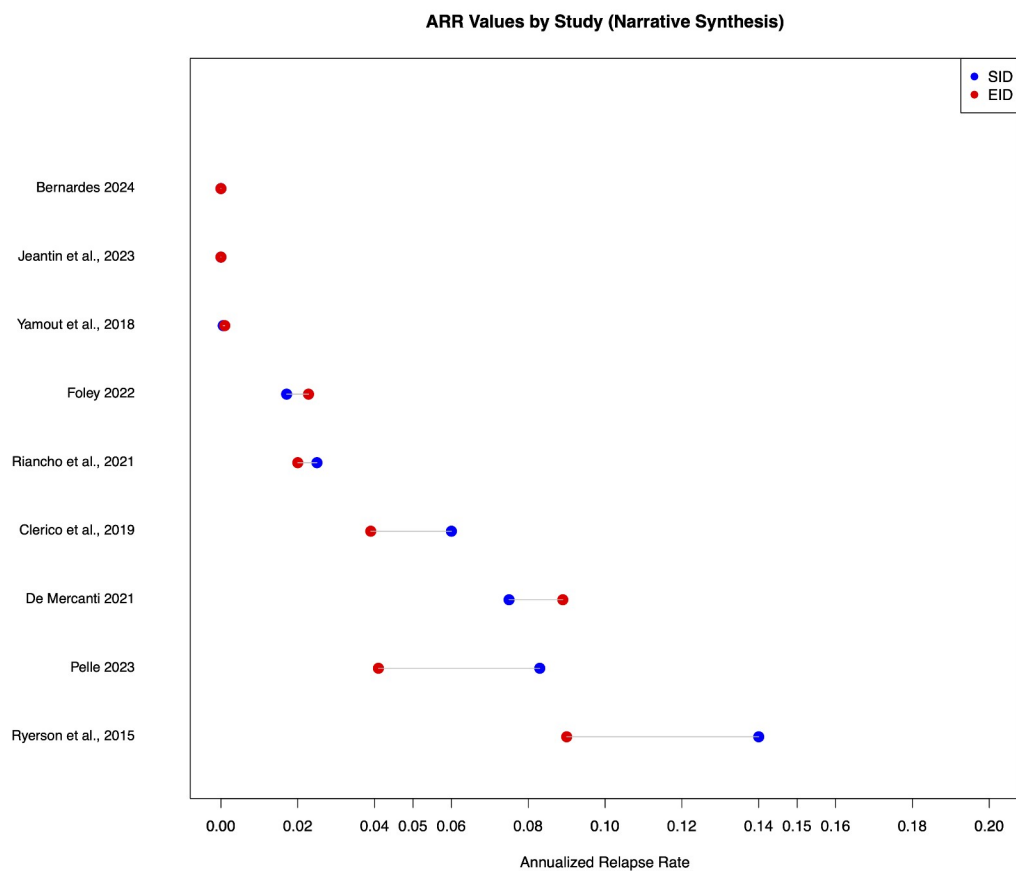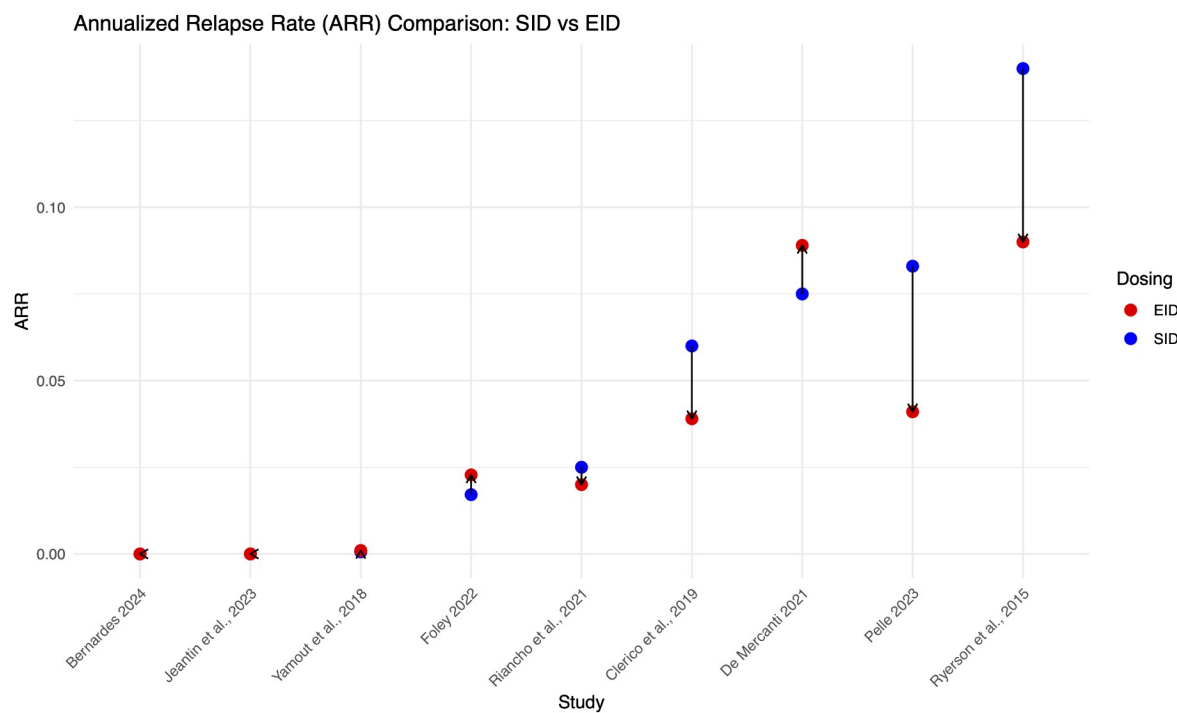Figure 2. Annualized Relapse Rate (ARR) Values by Study (Narrative Synthesis).

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Figure 3. Annualized Relapse Rate (ARR) Comparison: SID vs EID across the included studies.

3.4.2 SID vs EID: Disability Progression and NEDA-3

Heterogeneous outcome definitions and insufficient reporting prevented quantitative synthesis.

- Foley 2022 (34): 24 weeks of reported disability worsening: 92% [SID] vs 90% [EID] progression-free;
- NEDA-3: 163/242 [SID] vs 173/247 [EID].
- Pelle 2023 (45): Unconfirmed EDSS worsening: 13% [SID] versus 11% [EID].
- Riancho 2021 (46): Partial NEDA-3 reporting for EID only.

Table 3B presents study-specific outcomes.

Summary: Disability progression and NEDA-3 failure rates were modest across all regimens. The evidence shows equivalency, although comparability was constrained by inconsistent outcome criteria.

Table 3B: Disability Progression and NEDA-3 Outcomes

Outcome	PICO Comparison	Study	Outcome Definition	Reported Results	Notes
EDSS Worsening	SID vs EID	Foley 2022 (34)	24-week confirmed disability worsening	% free of progression: 92% [SID] vs 90% [EID]	Raw event counts not reported.
	SID vs EID	Pelle 2023 (45)	Worsening of EDSS score (not confirmed)	20 (13%) [SID] vs 14 (11%) [EID]	Definition is not confirmed disability progression.
	After Cessation	Vidal-Jordana 2015 (16)	Increase of ≥1.5 or ≥1.0 point at 6-12mo	Eight patients (19%) presented significant clinical worsening	Single arm reporting only.
NEDA-3	SID vs EID	Foley 2022 (34)	No relapses, no new MRI lesions, no 24-wk	NEDA-3: 163/242 [SID] vs 173/247 [EID]	Only study reporting complete NEDA-3 data.

				CDW	
SID vs EID	Riancho et al., 2021 (46)	Not fully defined	Proportions for EID arm only (94%, 73%, 70%)	Incomplete reporting; no SID data.	

Footnote: EDSS = Expanded Disability Status Scale; CDW = Confirmed Disability Worsening; NEDA-3 = No Evidence of Disease Activity (relapses, MRI activity, and CDW)

3.4.3 SID vs EID: Safety Outcome (PML)

Nine studies reported on PML. Given PML typically requires >24 months of natalizumab exposure, we conducted a duration-stratified analysis. The meta-analysis of risk ratio (RR) included three studies reporting PML events, with five additional studies reporting zero events in both arms presented in Table 3C for context (not included in pooled RR calculation).

- **Long-term exposure (>24 months)**: Ryerson et al. 2019 (27) (n=35,521) demonstrated a 94% PML risk reduction with EID (adjusted HR 0.06, 95% CI 0.01–0.44) in JCV-positive patients. Ryerson et al. 2015 (48) person-year analysis (IRR 0.11, 95% CI 0.01–1.99) supported this trend in JCV-positive patients.

- **Short-term exposure (<24 months)**: Butzkueven et al. 2020 (30) (median ~2 years) reported two PML cases in each group (SID n=210, EID n=236). Foley et al. 2022 (34) (72 weeks) reported one asymptomatic PML case in EID (n=247) and none in SID (n=242).

Primary pooled analysis (3 studies with events): RR 0.70 (95% CI 0.20–2.54), I^2=0%. Sensitivity analysis (excluding Foley 2022 asymptomatic case): RR 0.54 (95% CI 0.13–2.17). The pooled estimate was attenuated by inclusion of short-term studies with limited events and variable follow-up. Table 3C summarizes study-level PML data with exposure duration.

Summary: While the pooled analysis showed no significant difference in PML risk (RR 0.70), long-term data from Ryerson et al. (27) demonstrated substantial PML risk reduction with EID (94%) in patients with prolonged exposure. This discrepancy highlights limitations in meta-analyzing rare events without accounting for treatment duration. Ongoing monitoring remains warranted, particularly for long-term users.
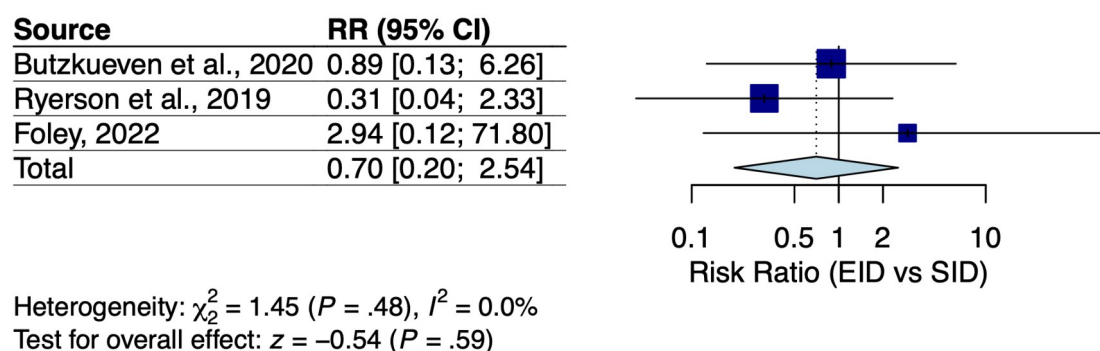
Table 3C: PML Incidence in Patients on Standard-Interval Dosing (SID) vs. Extended-Interval Dosing (EID)

STUDY (FIRST AUTHOR, YEAR)	DOSING GROUP	POPULATION AT RISK	PML EVENTS (N)	EXPOSURE DURATION	KEY NOTES
Studies Included in RR Meta-analysis					
Butzkueven et al., 2020	SID	210 (N)	2	Median ~2 years	Safety population

STUDY (FIRST AUTHOR, YEAR)	DOSING GROUP	POPULATION AT RISK	PML EVENTS (N)	EXPOSURE DURATION	KEY NOTES
(30)					
	EID	236 (N)	2	Median ~2 years	Safety population
Ryerson et al., 2019 (27)	SID	4801 (N)*	19	>24 months	Adjusted JCV+ patients
	EID	810 (N)	1	>24 months	Adjusted JCV+ patients
Foley et al., 2022 (34)	SID	242 (N)	0	72 weeks (~1.4 years)	Clinical & MRI monitoring
	EID	247 (N)	1 (asymptomatic)	72 weeks (~1.4 years)	Asymptomatic case via MRI
Study with Person-Year Data					
Ryerson et al., 2015 (48)	SID	1052 PY**	4	JCV+ PY	JCV+ person-years
	EID	1090 PY**	0	JCV+ PY	JCV+ person-years
Studies with Zero PML Events					
Clerico et al., 2019 (31)	SID	216 (N)	0	Up to 2 years	Follow-up up to 2 years
	EID	144 (N)	0	Up to 2 years	Follow-up up to 2 years
Pelle et al., 2023 (45)	SID	156 (N)	0	12 months	12 months follow-up
	EID	147 (N)	0	12 months	12 months follow-up
Riancho et al., 2021 (46)	SID	39 (N)	0	Mean 51.1 months	Mean follow-up
	EID	39 (N)	0	Mean 76.7 months	Mean follow-up
Toorop et al., 2024 (50)	SID	50 (N)	0	Not specified	Follow-up not specified
	EID	32 (N)	0	Not specified	Follow-up not specified
Yamout et al., 2018 (53)	SID	85 (N)	0	Not specified	Follow-up not specified
	EID	85 (N)	0	Not specified	Follow-up not specified

Footnote: EID: Extended-Interval Dosing; JCV: JC virus; MRI: Magnetic Resonance Imaging; N: Number of patients; PML: Progressive Multifocal Leukoencephalopathy; PY: Person-Years; SID: Standard-Interval Dosing

Figure 4: Forest Plot of PML Risk: SID vs EID (Main Analysis).

Heterogeneity: $\chi_2^2 = 1.45$ ($P = .48$), $I^2 = 0.0\%$
Test for overall effect: $z = -0.54$ ($P = .59$)

Supplementary Figure 2: Forest Plot of PML Risk is also provided which includes Sensitivity Analysis Excluding Foley et al. 2022 (34).

3.4.4 Outcomes After Cessation: Relapse and Disability

Key Finding: Natalizumab cessation carries a substantial 41.7% relapse risk within 12 months.

The most clinically significant finding of this review was the high relapse risk following natalizumab cessation. Four studies (n=857) reported the proportion of patients relapsing within 12 months post-cessation. This was meta-analyzed as a pooled proportion (absolute risk).

- Pooled proportion of patients relapsing: 41.7% (95% CI 30.8–53.6%) using a random-effects model (inverse variance with logit transformation).

- Heterogeneity: I^2=66.1% (τ^2=0.165, p=0.0315), largely due to Weinstock-Guttman 2016 (59.3%). Sensitivity analysis excluding this outlier reduced heterogeneity (I^2=44.6%) and yielded a pooled estimate of 36.9% (95% CI 33.7–40.3%).

EDSS worsening rates post-cessation were highly variable (19–100%). Excluding the outlier study (González-Suarez et al., 2017 (37), n=4), the weighted mean was 23.1%. Median time to first relapse ranged from 3-9.8 months, clustering within the first 6 months. This substantial relapse risk represents the most urgent clinical implication of our findings, directly impacting patient management and treatment planning.

A sensitivity analysis excluding Weinstock-Guttman 2016 showed minimal change in the pooled estimate *36.9% (95% CI 33.7–40.3%)* suggesting robustness of findings despite the heterogeneity introduced by this study's unique design. This study's protocol-mandated cessation and specific tapering approach may not reflect real-world clinical practice, justifying its treatment as an outlier in the sensitivity analysis.

Forest plots for the primary analysis, Freeman-Tukey transformation, and sensitivity analysis are presented in Figure 5, Supplementary Figure 3, and Supplementary Figure 4, respectively.

Table 3D summarizes cessation outcomes.

Summary: Natalizumab cessation carries a high and clinically significant relapse risk (41.7% at 12 months), with most relapses occurring early. This finding underscores the critical need for proactive management when discontinuing treatment.

Table 3D: Outcomes After Natalizumab Cessation

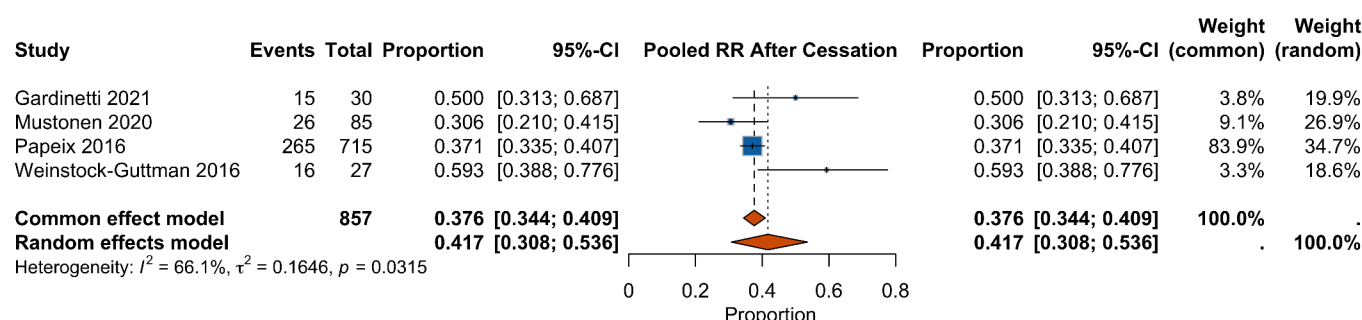| Study	N (Stop)	Primary Reason for Cessation	Follow-up post-cessation	% Relapse (12mo)	Median Time to First Relapse (months)	% with EDSS Worsening	Post-Cessation Strategy
Coerver et al., 2021 (32)	147	JCV positivity/PML risk (64.6%)	2 years	NR	6.1–9.8 (by group)	18.0–22.7%	No DMT, First-line, or Second-line DMT
Gardinetti et al., 2017 (36)	30	Planned interruption	Mean 18.8 mo	50%	6.0 (mean)	NR	Various (not specified)
Mustonen et al., 2020 (43)	85	Various	≥12 months	30.3%	3.0 (rebound subgroup)	NR	Switch to another DMT
Papeix et al., 2016 (44)	715	Various	Median 1.1 yr	37.1%	4.9	23%	Restart NTZ or switch to another DMT
Weinstock-Guttman et al., 2016 (52)	27	Study protocol	12 months	59.3%	NR	NR	Switch to another DMT

Additional descriptive studies

González-Suarez et al., 2017 (37)	4	PML risk	NR	NR	3.5 (mean)	100%	Switch to fingolimod
Vidal-Jordana et al., 2015 (16)	47	PML risk, other	Mean 23.9 mo	NR	NR	19%	Switch to another DMT

Footnote: NR = Not Reported; DMT = disease-modifying therapy; NTZ = natalizumab.

Pooled relapse risk: 41.7% (95% CI 30.8–53.6%) using random-effects model. Sensitivity analysis excluding Weinstock-Guttman 2016: 36.9% (95% CI 33.7–40.3%).

Figure 5. Forest plot of the proportion of patients relapsing within 12 months after natalizumab cessation (random-effects model).

Study	Events	Total	Proportion	95%-CI	Pooled RR After Cessation	Proportion	95%-CI	Weight (common)	Weight (random)
Gardinetti 2021	15	30	0.500	[0.313; 0.687]		0.500	[0.313; 0.687]	3.8%	19.9%
Mustonen 2020	26	85	0.306	[0.210; 0.415]		0.306	[0.210; 0.415]	9.1%	26.9%
Papeix 2016	265	715	0.371	[0.335; 0.407]		0.371	[0.335; 0.407]	83.9%	34.7%
Weinstock-Guttman 2016	16	27	0.593	[0.388; 0.776]		0.593	[0.388; 0.776]	3.3%	18.6%
Common effect model		857	**0.376**	**[0.344; 0.409]**		**0.376**	**[0.344; 0.409]**	**100.0%**	.
Random effects model			**0.417**	**[0.308; 0.536]**		**0.417**	**[0.308; 0.536]**	.	**100.0%**

Heterogeneity: $I^2 = 66.1\%$, $\tau^2 = 0.1646$, $p = 0.0315$

Forest plot of pooled relapse rate after natalizumab cessation using inverse variance method with logit transformation (random-effects model). Squares represent individual study proportions with size proportional to sample weight. Horizontal lines represent 95% confidence intervals. Diamond represents pooled estimate (41.7%, 95% CI 30.8–53.6%).

Supplementary Figure 3. Forest plot using Freeman-Tukey double arcsine transformation for relapse risk after natalizumab cessation. Squares represent individual study proportions with size proportional to sample weight. Horizontal lines represent 95% confidence intervals. Diamond represents pooled estimate (41.6%, 95% CI 30.3–53.3%).

Supplementary Figure 4. Sensitivity analysis forest plot excluding Weinstock-Guttman 2016. Squares represent individual study proportions with size proportional to sample weight. Horizontal lines represent 95% confidence intervals. Diamond represents pooled estimate (36.9%, 95% CI 33.7–40.3%).

Figure 6. Timeline of median/mean time to first relapse across studies reporting this outcome.
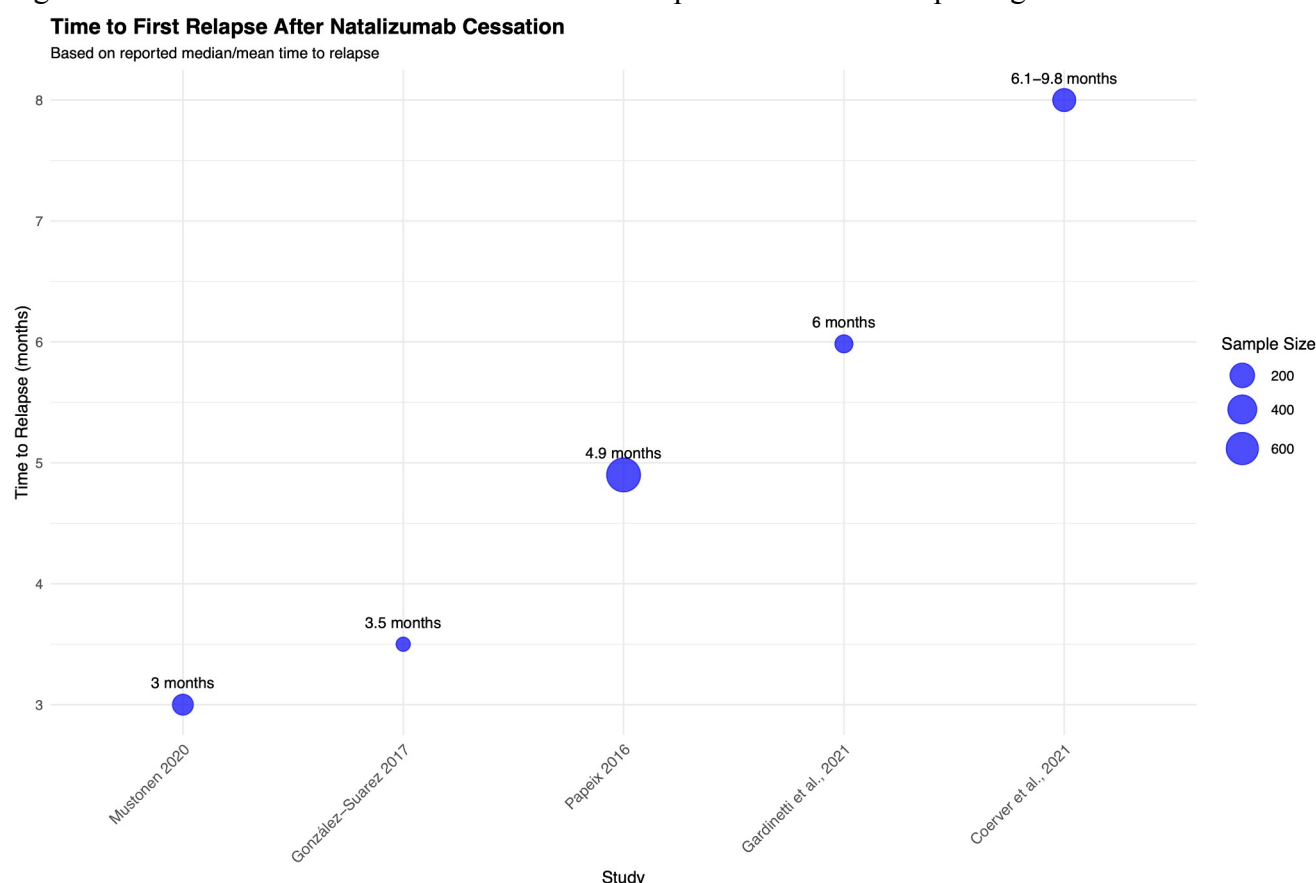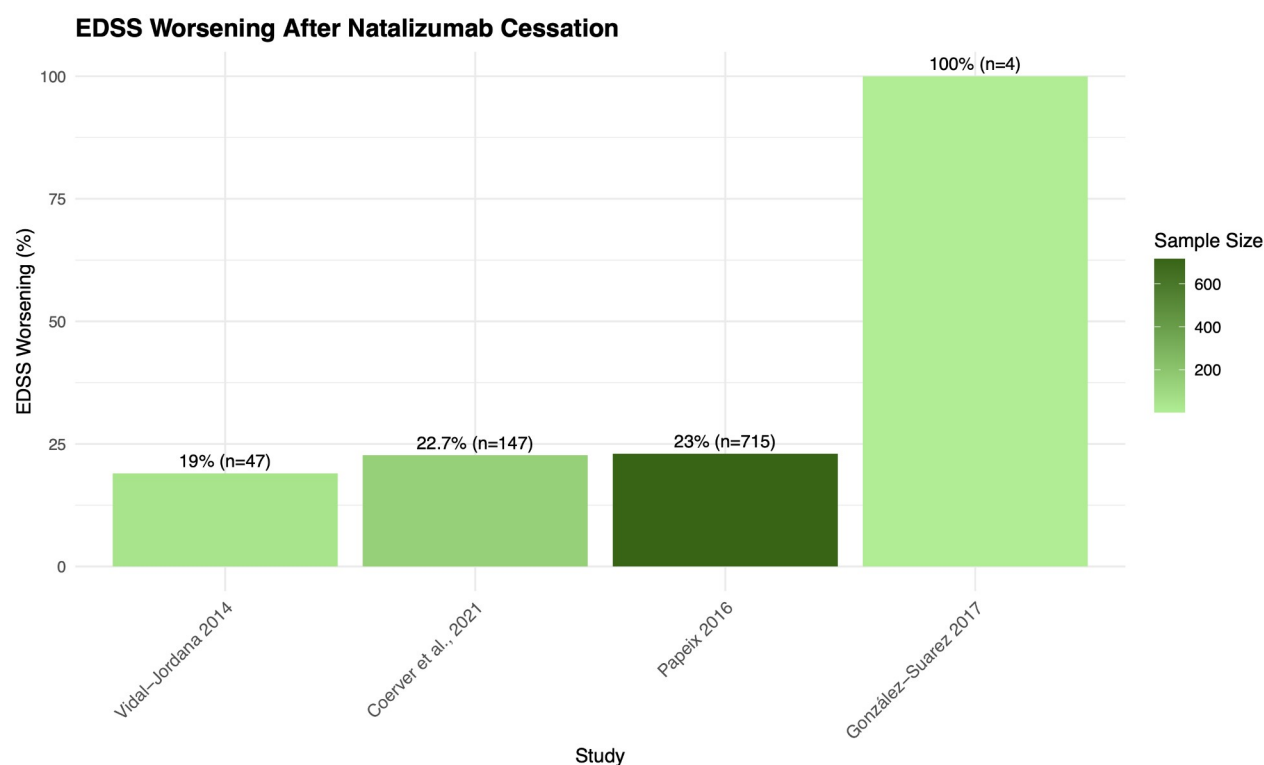

Figure 7. Bar plot of EDSS worsening rates after cessation. The weighted mean excluding the outlier (37) was 23.1%.

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3.5 Sensitivity Analyses

Leave-one-out analyses confirmed robustness of pooled results for both PML risk and cessation relapse rates. Exclusion of single studies influenced heterogeneity but not overall interpretation (Supplementary Table 5).

3.6 Reporting Bias

Publication bias could not be formally assessed as each outcome had fewer than 10 studies, insufficient for funnel plots or statistical testing.

3.7 Certainty of Evidence (GRADE)

Certainty of evidence for each outcome is summarized in Supplementary Table 4:

- SID vs EID:
- o ARR = Low (risk of bias, inconsistency)
- o Disability progression = Very Low (bias, inconsistency, indirectness)
- o PML = Low (bias, imprecision)
- o NEDA-3 = Low (bias, imprecision, single study)

- Outcomes After Cessation:
- o Relapse = Low (bias, heterogeneity)
- o EDSS worsening = Very Low (bias, inconsistency, imprecision)

The evidence for PML risk reduction was upgraded from Very Low to Low for long-term users (>24mo) based on the large effect size (94% reduction) in Ryerson 2019.

4. Discussion.

4.1. Interpreting the Results

This systematic review provides a systematic analysis of natalizumab treatment outcomes, with the most critical finding being the substantial risk of disease reactivation following treatment cessation. Our analysis of 28 trials (45,803 participants) revealed that 41.7% (95% CI 30.8-53.6%) of patients experience relapse within 12 months of discontinuing natalizumab. This estimate was robust in sensitivity analysis excluding an outlier study (36.9%; 95% CI 33.7-40.3%). The substantial heterogeneity (I^2=66.1%) was largely driven by one study (Weinstock-Guttman 2016), as shown in the sensitivity analysis (Supplementary Figure 4). This high relapse risk, with most events occurring within the first 6 months, represents the most urgent clinical implication for neurologists managing natalizumab treatment.

Regarding dosing strategies, our analysis found no consistent difference in efficacy between SID and EID. Across nine trials, the median ARR difference was zero (range -0.050 to +0.014), indicating comparable relapse

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control between approaches. Similarly, when disability progression and NEDA-3 results were reported, they were modest and similar between groups, despite the limitations of different classifications.

The safety outcomes, specifically PML incidence, were meta-analyzed across the three studies that recorded occurrences. The pooled risk ratio was 0.70 (95% confidence interval: 0.20–2.54). While our point estimate shows that EID may reduce PML risk, the confidence intervals are broad and can account for both clinically significant benefit and harm. The frequency of PML episodes, combined with the observational nature of the evidence, limits the certainty of this discovery. Our pooled PML estimate (RR 0.70) conflicts with Ryerson 2019's 94% reduction because meta-analysis of rare events is highly sensitive to study duration. When restricted to long-term users (>24mo), EID shows significant PML risk reduction, supporting its use in JCV+ patients with prolonged exposure.

The most critical finding, however, is the substantial relapse risk following cessation. This underscores that the most challenging aspect of natalizumab management is not optimizing dose intervals, but safely navigating treatment discontinuation. Neurologists must prioritize proactive transition planning when natalizumab must be stopped.

4.2. Treatment Continuum Framework

Our findings support examining natalizumab as a treatment continuum because: (1) dosing strategy selection affects future cessation planning, (2) treatment duration impacts both PML risk and post-cessation relapse risk, and (3) neurologists must consider both aspects simultaneously for optimal patient management. This approach aligns with clinical practice where treatment decisions cannot be made in isolation.

4.3. Limitations of the Evidence

Key limitations include: (1) Predominance of observational studies (24/28) with potential for confounding despite adjustment attempts; (2) Substantial heterogeneity in EID definitions (5-8 weeks) and outcome reporting precluding meta-analysis for efficacy outcomes; (3) Imprecise PML estimates due to rare events, with one large registry (Ryerson 2019, n=35,521) dominating findings; (4) Limited data on optimal post-cessation management strategies, with most studies reporting observational outcomes rather than comparative effectiveness of different approaches; (5) Potential residual confounding despite statistical adjustments in observational studies

Regarding our comprehensive scope examining both dosing and withdrawal outcomes, we recognize that some may question whether these topics belong in a single review. However, we believe this integrated approach is justified because: (1) clinical decisions about dosing intervals cannot be made without considering future withdrawal implications, (2) withdrawal outcomes depend on prior dosing history, and (3) neurologists managing natalizumab treatment must consider the entire treatment continuum. This comprehensive perspective reflects real-world clinical practice and provides the integrated evidence needed for clinical decision-making.

4.4. Limitations of the Review Procedure

Our review followed PRISMA 2020 guidelines (15). However, several limitations should be acknowledged. The search was restricted to studies published after 2015 and in English, which may have induced bias. Due to heterogeneity in outcome definitions, narrative synthesis was required for the majority of efficacy outcomes. Finally, the small number of papers for each outcome limited our formal assessment of publication bias. The inclusion of one study from late 2014 (published in 2015) is a minor change from the protocol, stated for transparency.

4.5 Implications for Practice and Research

Clinical implications: Based on our findings, we propose specific clinical recommendations for natalizumab management:

- For treatment initiation: Consider the entire treatment continuum from the outset, including eventual discontinuation planning.

- For ongoing treatment: For stable patients considering EID, discuss that while efficacy may be maintained, the primary challenge remains managing discontinuation regardless of dosing strategy.

- For discontinuation planning: Initiate alternative therapy promptly after the final natalizumab dose, given the 41.7% relapse risk within 12 months and median time to relapse of 3-6 months.

- For patient counseling: Explicitly inform patients that more than one-third experience relapse after stopping natalizumab, with most events occurring in the first 6 months.

- For high-risk patients: Those with higher relapse risk prior to natalizumab or longer treatment duration may require particularly close monitoring and prompt transition to alternative therapies.

Future research should prioritize: (1) Randomized trials comparing different post-cessation management strategies, including optimal timing for initiating alternative therapies and comparative effectiveness of bridging treatments; (2) Prospective studies identifying risk factors for post-cessation relapse to develop prediction models for clinical use; (3) Studies evaluating cost-effectiveness of different monitoring and transition strategies; (4) Research on patient-reported outcomes and preferences regarding natalizumab discontinuation planning; (5) Long-term follow-up studies assessing disability outcomes beyond 12 months post-cessation. Additionally, large prospective registries with adjusted confounders are needed to better define risk factors for post-cessation relapse and identify patients at highest risk. While further research on EID safety is warranted, the most urgent knowledge gap remains understanding how to safely manage the dangerous period following natalizumab discontinuation.

5. Conclusion.

This review demonstrates a substantial 41.7% relapse risk within 12 months of natalizumab cessation (random-effects model), with sensitivity analysis confirming a 36.9% risk after excluding an outlier study, necessitating prompt therapy transition. While EID may maintain efficacy comparable to SID, neurologists must adopt a

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treatment continuum approach that integrates dosing decisions with cessation planning. Future research should prioritize optimal transition protocols to mitigate this high-risk period.

Clinical Practice Summary: The key finding of 41.7% relapse risk within 12 months of natalizumab cessation (95% CI 30.8–53.6%) necessitates prompt therapy transition. Sensitivity analysis showed a 36.9% relapse risk (95% CI 33.7–40.3%) when excluding an outlier study. This has immediate clinical implications. Neurologists should recognize the first 6 months post-cessation as the high-risk period, with median time to relapse occurring at 3-6 months, necessitating initiation of alternative therapy within 1-2 months of the final natalizumab dose. Patient counseling must explicitly inform that more than one-third of patients experience relapse after stopping, with close clinical and MRI monitoring recommended during the first 6 months. Regarding dosing strategies, while EID may maintain efficacy comparable to SID for stable patients, the dosing interval does not substantially affect post-cessation relapse risk, and treatment decisions should consider the entire continuum from initiation through discontinuation.

6. Statements and Declarations

6.1 Competing Interests
The authors declare that they have no competing interests.

6.2 Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

6.3 Ethics Approval and Consent to Participate
Not applicable. This study is a systematic review and meta-analysis of previously published data.

6.4 Consent for Publication
Not applicable.

6.5 Availability of Data and Materials
All data analyzed in this study were obtained from previously published sources. No new datasets were generated or analyzed during the current study.

6.6 Author Contributions

V.S. conceptualized and designed the study, developed the protocol (PROSPERO registration), conducted the literature search with assistance from M.B., performed title/abstract screening and full-text assessment as first reviewer with M.B. as second reviewer, extracted data, conducted formal analyses including meta-analyses and GRADE assessment, interpreted the results, drafted the entire manuscript including all sections, figures, and tables, created all visualizations (forest plots and PRISMA flowchart), and provided project administration, supervision, and correspondence as the corresponding author. M.B. assisted with literature search, served as second reviewer for title/abstract screening and full-text assessment, performed risk of bias assessment for all included studies using Cochrane RoB 2.0 for RCTs and ROBINS-I for observational studies, served as third-party adjudicator for conflicts during screening and data extraction, and provided critical review of the final manuscript. D.J. assisted with data extraction as secondary reviewer, served as third-party conflict resolver

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during study selection, and provided critical review of the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for the work's integrity and accuracy.

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