

Statistical Report V3 — FINAL

Neu-BoNT-A 50 U versus 100 U cervical dystonia randomized two-period crossover trial

This final V3 report uses the prespecified trial design as the analysis framework. Fifty participants were randomized 1:1: 25 to Neu-BoNT-A 100 U followed by 50 U and 25 to 50 U followed by 100 U. For statistical reporting, the two treatment phases are referred to as Period 1 and Period 2, consistent with the study design. Internal operational visit labels are not used in this report.

1. Analysis framework and conventions

- Randomized cohort: N=50; Sequence A, 100 U→50 U (n=25); Sequence B, 50 U→100 U (n=25).
- Treatment periods are designated Period 1 and Period 2, with crossover at Week 12.
- TWSTRS change is Week 12 minus pretreatment baseline; negative values indicate improvement.
- Treatment contrast is 100 U minus 50 U; negative values favor 100 U.
- No missing TWSTRS observations were imputed.
- The principal between-dose analysis is a linear mixed-effects crossover model with treatment, period, and randomized sequence as fixed effects and participant as a random effect.
- A Gaussian GEE with robust standard errors and an unstructured within-participant correlation is used as a robustness analysis because the fitted random-intercept variance is on/near the boundary.
- A first-period-only analysis is retained as a carryover-oriented sensitivity analysis.

2. Evaluable TWSTRS data

Outcome	Period-level observations	Participants with ≥1 evaluable value	Complete pairs
TWSTRS total	87	45	42
Severity	87	45	42
Disability	87	45	42
Pain	88	45	43

The randomized trial denominator remains N=50. The smaller numbers above reflect availability of calculable outcome changes for the relevant analysis, not a change in the randomized cohort. The principal crossover model uses all available evaluable period-level observations; complete-pair analyses are supportive sensitivity analyses.

3. Complete-pair TWSTRS sensitivity analysis

Outcome	Paired N	Mean change 50 U	Mean change 100 U	100 U–50 U	95% CI	P	Cohen dz
TWSTRS total	42	+0.09	–1.93	–2.02	–7.41 to +3.38	0.454	–0.12
Severity	42	–0.52	–0.14	+0.38	–4.54 to +5.30	0.876	+0.02
Disability	42	+0.07	–0.50	–0.57	–2.53 to +1.39	0.559	–0.09
Pain	43	+0.53	–1.26	–1.78	–3.13 to –0.44	0.0104	–0.41

Among participants with evaluable pain-change data under both regimens, the complete-pair pain analysis included 43 participants and favored 100 U. This analysis is supportive; the crossover model below is the principal reviewer-requested inference.

4. Principal crossover mixed-effects analysis

Model: change ~ treatment (100 U vs 50 U) + period (2 vs 1) + randomized sequence (100→50 vs 50→100) + participant random intercept, fitted by maximum likelihood.

Outcome	Obs	Adjusted 100 U–50 U	95% CI	P treatment	Period P	Sequence P
TWSTRS total	87	–1.76	–5.99 to +2.47	0.414	0.594	0.936
Severity	87	+0.58	–3.05 to +4.21	0.753	0.659	0.549
Disability	87	–0.49	–2.12 to +1.14	0.554	0.283	0.483
Pain	88	–1.83	–2.93 to –0.73	0.0011	0.300	0.560

TWSTRS total, severity, and disability remained nonsignificant. TWSTRS pain favored 100 U after adjustment for period and randomized sequence (adjusted difference, –1.83 points; 95% CI, –2.93 to –0.73; P=0.0011). No statistically significant period or randomized-sequence effect was detected for any TWSTRS outcome.

5. GEE robustness analysis

The participant random-intercept variance was estimated on/near the boundary. A Gaussian GEE with robust standard errors and an unstructured within-participant working correlation was therefore used to assess robustness.

Outcome	GEE 100 U–50 U	Robust 95% CI	Robust P
TWSTRS total	–1.69	–6.93 to +3.55	0.527
Severity	+0.66	–4.23 to +5.56	0.791
Disability	–0.48	–2.16 to +1.20	0.573
Pain	–1.83	–3.04 to –0.62	0.0030

The pain finding remained statistically significant under the alternative covariance approach; the other TWSTRS outcomes remained nonsignificant.

6. First-period-only sensitivity and carryover interpretation

Outcome	Period-1 N	100 U–50 U	95% CI	P
TWSTRS total	44	–1.94	–7.61 to +3.73	0.494
Severity	44	–0.53	–5.68 to +4.63	0.838
Disability	44	+0.09	–1.86 to +2.05	0.925
Pain	44	–1.50	–3.18 to +0.17	0.077

Period 1 precedes exposure to the alternate randomized study dose and therefore avoids between-period carryover from that alternate dose, but it loses the within-participant crossover advantage. The pain estimate remained directionally concordant but less precise. A nonsignificant randomized-sequence term is not interpreted as proof of absence of biological carryover; residual biological carryover cannot be completely excluded.

7. Multiplicity sensitivity analysis

Holm's step-down procedure was applied across all 24 efficacy comparisons. For the four TWSTRS outcomes, the updated crossover-model P values were used; for the remaining secondary outcomes, the nominal P values reported in Table 2 were retained.

Outcome	Nominal/updated P	Holm-adjusted P	Interpretation
TWSTRS total	0.414	1.000	Not significant
TWSTRS severity	0.753	1.000	Not significant
TWSTRS disability	0.554	1.000	Not significant
TWSTRS pain	0.0011	0.025	Significant
CDIP-58 psychosocial functioning	0.001	0.024	Significant
All other 19 efficacy comparisons	≥0.060	1.000	Not significant

Both positive secondary findings remained significant after multiplicity adjustment: TWSTRS pain, Holm-adjusted $P \approx 0.025$, and CDIP-58 psychosocial functioning, Holm-adjusted $P = 0.024$. No other efficacy comparison remained significant.

8. Integrated statistical interpretation

- The trial cohort remains $N=50$ with balanced 25:25 randomized sequence allocation.
- The overall TWSTRS total-score comparison is nonsignificant.
- TWSTRS pain consistently favors 100 U in the complete-pair, crossover mixed-effects, and robust GEE analyses.
- No significant period or randomized-sequence effect was detected.
- Residual biological carryover cannot be completely excluded.
- TWSTRS pain and CDIP-58 psychosocial functioning remain significant after Holm multiplicity adjustment.
- Pain remains a secondary endpoint, and no validated TWSTRS-pain-specific MCID anchor is available in the study materials.

9. Reviewer-facing wording for the crossover analysis

We thank the reviewer for this important recommendation. We verified the randomized treatment sequence according to the prespecified two-period crossover design ($N=50$; 25 participants assigned to 100 U followed by 50 U and 25 assigned to 50 U followed by 100 U) and reanalyzed the individual-level TWSTRS data using a linear mixed-effects crossover model including treatment, treatment period, and randomized sequence as fixed effects, with participant as a random effect. The adjusted between-dose difference remained nonsignificant for TWSTRS total (–1.76 points; 95% CI, –5.99 to 2.47; $P=0.414$), severity (+0.58; 95% CI, –3.05 to 4.21; $P=0.753$), and disability (–0.49; 95% CI, –2.12 to 1.14; $P=0.554$). TWSTRS pain favored 100 U (adjusted 100-U minus 50-U difference, –1.83 points; 95% CI, –2.93 to –0.73; $P=0.0011$). Neither period nor randomized sequence was statistically significant. Because the participant random-intercept variance was estimated at the boundary, we additionally performed a Gaussian GEE sensitivity analysis with robust standard errors and an unstructured within-participant

correlation; the pain result remained significant (−1.83 points; 95% CI, −3.04 to −0.62; $P=0.003$), whereas the other TWSTRS outcomes remained nonsignificant.

10. Manuscript-facing statistical wording

Methods — Between-dose TWSTRS comparisons were analyzed using a linear mixed-effects crossover model with treatment, period, and randomized treatment sequence as fixed effects and participant as a random effect. The randomized cohort comprised 50 participants, 25 in each sequence. Change was defined as Week 12 minus pretreatment baseline. Because the fitted random-intercept variance was at the boundary, a Gaussian GEE with robust standard errors and an unstructured within-participant correlation was used as a sensitivity analysis. A first-period-only analysis was additionally examined as a carryover-oriented sensitivity analysis. Holm's step-down procedure was applied across the 24 efficacy comparisons.

Results — In the crossover model, 100 U did not significantly differ from 50 U for TWSTRS total, severity, or disability. TWSTRS pain favored 100 U (adjusted difference, −1.83 points; 95% CI, −2.93 to −0.73; $P=0.0011$). Period and sequence effects were not significant. The pain result remained significant in the robust GEE analysis (−1.83 points; 95% CI, −3.04 to −0.62; $P=0.003$). TWSTRS pain and CDIP-58 psychosocial functioning remained significant after Holm adjustment (adjusted $P\approx 0.025$ and 0.024, respectively).