**Participant flow**

**Did not go on to randomise (n=69, 46.3%)**

* Patient ineligible (n=50) (not mutually exclusive)
  + Ineligible due to pain score in diary (n=31)
  + Ineligible due to stool consistency in diary (n=8)
  + Patient does not meet the Rome IV criteria for IBS-D (n=3)
  + Alternative diagnosis not excluded (n=6)
  + Ineligible due to QTc interval (n=1)
  + Pulse, BP, FBC, LFTs outside normal range (n=2)
  + Started on SSRIs shortly after registration (n=2)
  + Patient unwilling to stop a prohibited medicine (n=1)
* Patient no longer interested (n=6)
* Unable to get hold of patient (n=3)
* Other (n=7)
* Missing (n=3)

**Not eligible at screening (n= 1251, 79.1%) (not mutually exclusive)**

*Inclusion*

* No informed consent (n=115)
* Not considered fit for participation (n=10)
* Rome IV criteria not met (n=882)
* Not aged ≥ 18 years old (n=3)
* Alternative diagnosis not excluded: *Microscopic colitis (n=47), Bile Acid Diarrhoea (n=138), Lactose Malabsorption (n=20)*
* Coeliac disease (n=18)
* Not agreed to contraception (n=1)

*Exclusion*

* Internal resection (n=9)
* Other known organic GI disease (n=14)
* Unable to stop anti-diarrhoeal drugs (n=3)
* Previous Ondansetron use (n=28)
* Pulse, BP, FBC, LFT out of normal range (n=3)
* Pregnant or breastfeeding (n=5)
* SSRI or tricyclic use (n=3)
* Taking Apomorphine or Tramadol (n=2)
* Taking meds likely to alter bowl habit (n=3)
* QTC interval outside of normal range (n=6)
* Unable to stop taking meds that alter bowel habit (n=4)

**Missing (n=36, 2.3%)**

**Formally Screened**

**(n=1582)**

Withdrawn due to pregnancy (n=1)

**PLACEBO**

(n=43, 53.8% of randomised)

Discontinued treatment (n=1)

Available (n=42)

**Available for analysis** (n=43)

Available (n=41)

**Available for analysis** (n=42)

Available (n=33)

**Available for analysis (n=34)**

Discontinued treatment (n=1)

**Withdrawn from trial follow-up** and discontinued treatment (n=3)

**FOLLOW-UP AT 16 WEEKS**

**ONDANSETRON**

(n=37, 46.3% of randomised)

**Eligible   
(n=295, 18.6% of screened)**

**Consented**

**(n=173, 58.6% of eligible)**

**Registered**

**(n=149, 86.1% of consented)**

**RANDOMISED**

**(n= 80, 53.7% of registered)­**

**Did not go on to consent (n= 122, 41.4% of eligible)**

* Consent refused (n=54)
* Unable to make contact with patient to proceed (n=24)
* Other (n=42)
* Reason missing (n=2)

**Did not go on to be registered (n=24, 13.9% of consented)**

* No longer eligible (n=12): *Microscopic colitis not excluded (n=1), Bile Acid Diarrhoea not excluded (n=4), Unable to stop anti-diarrhoeal drugs (n=2), QTC interval outside of normal range (n=1),* *Pulse, BP, FBC, LFT out of normal range (n=7), SSRI or tricyclic antidepressant use (n=1)*
* Other (n=10)
* Reason missing (n=2)

**BASELINE**

**TREATMENT FOR**

**12 WEEKS**

Available (n=33)

**Baseline characteristics**

|  |  |  |  |
| --- | --- | --- | --- |
| **Baseline characteristics** | **Treatment arm** | | |
| **Ondansetron (n=37)** | **Placebo (n=43)** | **Total (n=80)** |
| Mean **age** (years) (SD) | 45.0 (15.7) | 43.0 (16.3) | 43.9 (16.0) |
| **Sex** N (%) |  |  |  |
| Male | 16 (43.2%) | 17 (39.5%) | 33 (41.3%) |
| Female | 21 (56.8%) | 26 (60.5%) | 47 (58.8%) |
| **Ethnicity** N (%) |  |  |  |
| White | 34 (91.9%) | 41 (95.3%) | 75 (93.8%) |
| Black | 2 (5.4%) | 1 (2.3%) | 3 (3.8%) |
| Asian | 1 (2.7%) | 1 (2.3%) | 2 (2.5%) |
| **Current smoker** N (%) Missing | 4 (10.8%) 1 | 5 (11.6%) 0 | 9 (11.3%) 1 |
|  |  |  |  |
| **Pre-treatment diary** |  |  |  |
| Mean **pain score** (SD) | 61.4 (19.7) | 55.2 (16.7) | 58.0 (18.3) |
| Mean **days per week with loose stool** (SD) | 5.9 (1.3) | 5.4 (1.2) | 5.6 (1.3) |
| Mean **urgency score** (SD) Missing | 67.5 (19.6) 0 | 60.4 (17.8) 1 | 63.8 (18.9) 1 |
| **Mechanistic test undertaken N (%)** |  |  |  |
| Barostat | 8 (21.6%) | 10 (23.3%) | 18 (22.5%) |
| Colonic manometry | 7 (18.9%) | 6 (14.0%) | 13 (16.3%) |
| Both mechanistic tests undertaken | 6 (16.2%) | 6 (14.0%) | 12 (15.0%) |
| **PHQ-12 score** |  |  |  |
| **Male N** | 16 | 17 | 33 |
| Mean (SD) Missing | 7.5 (4.63) 0 | 7.5 (3.54) 0 | 7.5 (4.04) 0 |
| **Female N** | 21 | 26 | 47 |
| Mean (SD) Missing | 10.3 (4.32) 0 | 9.6 (4.63) 0 | 9.9 (4.46) 0 |

Pain and urgency scores: range 0 to 100

PHQ-12 score: range 0 to 22 in men, 0-24 in women, high score indicates high somatisation

**Outcome Measures**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary endpoint analysis** | **Responders** | | | | **Model output** | | |
| **Ondansetron (n=37)** | | **Placebo (n=43)** | |
| N (%) | (95% CI) | N (%) | (95% CI) | p-value | Odds ratio (OR) (95% CI) | N |
| **Primary endpoint responder** | 15 (40.5%) | (24.7%, 56.4%) | 12 (27.9%) | (14.5%, 41.3%) | 0.187 | 1.93 (0.73, 5.11) | 76 |
| **Components of primary endpoint:** | | | | | | | |
| **Pain intensity reduction** | 17 (46.0%) | (29.9%, 62.0%) | 16 (37.2%) | (22.8%, 51.7%) | 0.322 | 1.61 (0.63, 4.12) | 76 |
| **Stool consistency reduction** | 25 (67.6%) | (52.5%, 82.7%) | 22 (51.2%) | (36.2%, 66.1%) | 0.073 | 2.45 (0.92, 6.52) | 77 |

Primary model was adjusted for: the treatment group, the minimisation variables (receipt of manometry, receipt of barostat, age, and gender).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Secondary endpoint analyses – outputs from linear regression models** | | | | | | |
| **Analysis (linear models)** | **Adjusted mean ondansetron** | **Adjusted mean placebo** | **Difference in adjusted means (SE)** | **p-value** | **95% CI of difference in means** | **N analysed** |
| **Weeks 9-12 only (treatment period)** |  |  |  |  |  |  |
| **Stool frequency (n stools/ day)** | 2.5 | 2.5 | 0.1 (0.28) | 0.817 | (-0.5, 0.6) | 73 |
| **Stool consistency (BSFS) (1-7)** | 4.4 | 5.0 | -0.5 (0.25) | 0.042 | (-1.0, 0.02) | 71 |
| **Stool urgency (0-100)** | 35.6 | 42.5 | -6.8 (5.7) | 0.236 | (-18.2, 4.6) | 70 |
| **SFLDQ (0-32)** | 6.1 | 9.3 | -3.2 (1.43) | 0.028 | (-6.1, -0.4) | 66 |
| **IBS-SSS questionnaire score (0-500)** | 228 | 254.5 | -26.5 (32.51) | 0.418 | (-91.5, 38.5) | 69 |
| **Abdominal pain score (0-100)** | 40.0 | 41.6 | -1.6 (4.67) | 0.741 | (-10.9, 7.8) | 73 |
| **HADS anxiety score (0-21)** | 8.4 | 9.4 | -1 (0.84) | 0.246 | (-2.7, 0.7) | 72 |
| **HADS depression score (0-21)** | 5.3 | 6.1 | -0.8 (0.85) | 0.366 | (-2.5, 0.9) | 72 |
| **IBS-QOL questionnaire score (0-100)** | 61.9 | 53.1 | 8.8 (4.96) | 0.082 | (-1.1, 18.7) | 72 |
| **Weeks 1-12 (treatment period) – additional summaries** |  |  |  |  |  |  |
| **Stool frequency (n stools/ day)** | 2.5 | 2.8 | -0.3 (0.25) | 0.204 | (-0.8, 0.2) | 76 |
| **Stool consistency (BSFS) (1-7)** | 4.4 | 5.0 | -0.7 (0.19) | 0.001 | (-1.0, -0.3) | 75 |
| **Stool urgency (0-100)** | 38.4 | 44.9 | -6.5 (4.8) | 0.179 | (-16.1, 3.1) | 76 |
| **Abdominal pain score (0-100)** | 41.6 | 43.5 | -1.8 (3.88) | 0.639 | (-9.6, 5.9 ) | 77 |
| **Weeks 13-16 (post-treatment period)** |  |  |  |  |  |  |
| **Stool frequency (n stools/ day)** | 2.6 | 2.9 | -0.2 (0.3) | 0.425 | (-0.9, 0.4) | 70 |
| **Stool consistency (BSFS) (1-7)** | 5.1 | 5.2 | -0.1 (0.23) | 0.617 | (-0.6, 0.3) | 67 |
| **Stool urgency (0-100)** | 43.9 | 53.3 | -9.3 (4.94) | 0.064 | (-19.2, 0.6) | 63 |
| **Abdominal pain score (0-100)** | 44.4 | 46.0 | -1.5 (4.55) | 0.739 | (-10.6, 7.6) | 66 |

Standard error (SE)

BSFS (Bristol Stool Form Scale) range from 1 to 7

Urgency of defaecation from 0 to 100; 0 = no urgency, 100= worst imaginable urgency

Short-form Leeds Dyspepsia Questionnaire range 0 to 32; 0 is the best possible score and 32 is the worst

IBS symptom severity score range 0 to 500, where 0 is the best score and 500 the worst

Abdominal pain score scale from 0 to 100, 0 = no pain, 100 = worst imaginable pain

HADS score 0 to 21; 0 indicates no anxiety or no depression, 21 indicates high anxiety or high depression.

IBS-QOL range 0-100; 100 indicates good IBS-specific quality of life

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Secondary endpoint analyses - outputs from logistic regression models** | | | | | | | |
| **Analysis (logistic regression models)** | **Number of participants (proportions)** | | | | **Model output** | | |
| **Ondansetron (n=37)** | | **Placebo (n=43)** | |
| N (%) Missing | (95% CI) | N (%) Missing | (95% CI) | **p value** | **OR (95% CI)** | N |
| **Satisfactory relief of IBS symptoms (Yes) weeks 1-12** | 15 (40.5%) 12 | (24.7%, 56.4%) | 17 (39.5%) 9 | (24.9%, 54.1%) | 0.533 | 1.43 ( 0.46, 4.41) | 58 |
| **Avoided loperamide use (0 days used) weeks 1-12** |  |  |  |  |  |  |  |
| **Weeks 1-12 treatment** | 23 (62.2%) 7 | (46.5%, 77.8%) | 22 (51.2%) 8 | (36.2%, 66.1%) | 0.196 | 0.43 ( 0.12, 1.55) | 61 |
| **Weeks 9-12 only** | 23 (62.2%) 8 | (46.5%, 77.8%) | 23 (56.5%) 13 | (38.6%, 68.4%) | 0.362 | 0.48 ( 0.10, 2.31) | 56 |
| **Weeks 13-16 post-treatment** | 21 (56.8%) 8 | (40.1%, 72.7%) | 23 (56.5%) 13 | (38.6%, 68.4%) | 0.628 | 1.39 ( 0.36, 5.35) | 56 |

**Adverse events**

There were no SAEs, SARs, or SUSAR’s reported.

| **AE reporting at different time points** | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment period** | | | | | | **Post-treatment period** | | |
|  | **6 weeks** | | | **12 weeks** | | | **16 weeks** | | |
| **Experienced N (%) Missing** | **Ondansetron (n=37)** | **Placebo (n=43)** | **Total (n=80)** | **Ondansetron (n=37)** | **Placebo (n=43)** | **Total (n=80)** | **Ondansetron (n=37)** | **Placebo (n=43)** | **Total (n=80)** |
| **Constipation (Yes)** | 17 (45.9%) 2 | 11 (25.6%) 3 | 28 (35.0%) 5 | 12 (32.4%) 3 | 10 (23.3%) 3 | 22 (27.5%) 6 | 5 (13.5%) 3 | 8 (18.6%) 2 | 13 (16.3%) 5 |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 11 (64.7%) | 6 (54.5%) | 17 (60.7%) | 9 (75.0%) | 8 (80.0%) | 17 (77.3%) | 4 (80.0%) | 3 (37.5%) | 7 (53.8%) |
| Moderate | 5 (29.4%) | 5 (45.5%) | 10 (35.7%) | 2 (16.7%) | 2 (20.0%) | 4 (18.2%) | 0 (0.0%) | 5 (62.5%) | 5 (38.5%) |
| Severe | 1 (5.9%) | 0 (0.0%) | 1 (3.6%) | 1 (8.3%) | 0 (0.0%) | 1 (4.5%) | 1 (20.0%) | 0 (0.0%) | 1 (7.7%) |
| **Abdominal pain/ bloating** | 26 (70.3%) 2 | 36 (83.7%) 2 | 62 (77.5%) 4 | 23 (62.2%) 3 | 33 (76.7%) 3 | 56 (70.0%) 6 | 25 (67.6%) 3 | 36 (83.7%) 2 | 61 (76.3%) 5 |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 10 (38.5%) | 20 (55.6%) | 30 (48.4%) | 12 (52.2%) | 14 (42.4%) | 26 (46.4%) | 7 (28.0%) | 9 (25.0%) | 16 (26.2%) |
| Moderate | 10 (38.5%) | 11 (30.6%) | 21 (33.9%) | 7 (30.4%) | 15 (45.5%) | 22 (39.3%) | 11 (44.0%) | 17 (47.2%) | 28 (45.9%) |
| Severe | 6 (23.1%) | 5 (13.9%) | 11 (17.7%) | 4 (17.4%) | 4 (12.1%) | 8 (14.3%) | 7 (28.0%) | 10 (27.8%) | 17 (27.9%) |
| **Headache** |  |  |  |  |  |  |  |  |  |
| Yes | 17 (45.9%) | 8 (18.6%) | 25 (31.3%) | 11 (29.7%) | 14 (32.6%) | 25 (31.3%) | 10 (27.0%) | 12 (27.9%) | 22 (27.5%) |
| Missing | 2 (5.4%) | 4 (9.3%) | 6 (7.5%) | 3 (8.1%) | 3 (7.0%) | 6 (7.5%) | 3 (8.1%) | 2 (4.7%) | 5 (6.3%) |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 11 (64.7%) | 7 (87.5%) | 18 (72.0%) | 3 (27.3%) | 8 (57.1%) | 11 (44.0%) | 6 (60.0%) | 8 (66.7%) | 14 (63.6%) |
| Moderate | 5 (29.4%) | 1 (12.5%) | 6 (24.0%) | 5 (45.5%) | 5 (35.7%) | 10 (40.0%) | 3 (30.0%) | 4 (33.3%) | 7 (31.8%) |
| Severe | 1 (5.9%) | 0 (0.0%) | 1 (4.0%) | 3 (27.3%) | 1 (7.1%) | 4 (16.0%) | 1 (10.0%) | 0 (0.0%) | 1 (4.5%) |
| **Nausea** |  |  |  |  |  |  |  |  |  |
| Yes | 6 (16.2%) | 12 (27.9%) | 18 (22.5%) | 10 (27.0%) | 18 (41.9%) | 28 (35.0%) | 9 (24.3%) | 12 (27.9%) | 21 (26.3%) |
| Missing | 2 (5.4%) | 4 (9.3%) | 6 (7.5%) | 3 (8.1%) | 2 (4.7%) | 5 (6.3%) | 3 (8.1%) | 2 (4.7%) | 5 (6.3%) |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 4 (66.7%) | 9 (75.0%) | 13 (72.2%) | 7 (70.0%) | 10 (55.6%) | 17 (60.7%) | 3 (33.3%) | 6 (50.0%) | 9 (42.9%) |
| Moderate | 2 (33.3%) | 3 (25.0%) | 5 (27.8%) | 1 (10.0%) | 5 (27.8%) | 6 (21.4%) | 4 (44.4%) | 3 (25.0%) | 7 (33.3%) |
| Severe |  |  |  | 2 (20.0%) | 3 (16.7%) | 5 (17.9%) | 2 (22.2%) | 3 (25.0%) | 5 (23.8%) |
| **Vomiting** |  |  |  |  |  |  |  |  |  |
| Yes | 3 (8.1%) | 3 (7.0%) | 6 (7.5%) | 1 (2.7%) | 4 (9.3%) | 5 (6.3%) | 4 (10.8%) | 6 (14.0%) | 10 (12.5%) |
| Missing | 2 (5.4%) | 4 (9.3%) | 6 (7.5%) | 3 (8.1%) | 3 (7.0%) | 6 (7.5%) | 3 (8.1%) | 2 (4.7%) | 5 (6.3%) |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 2 (66.7%) | 2 (66.7%) | 4 (66.7%) |  |  |  | 0 (0.0%) | 1 (16.7%) | 1 (10.0%) |
| Moderate | 1 (33.3%) | 1 (33.3%) | 2 (33.3%) | 0 (0.0%) | 4 (100.0%) | 4 (80.0%) | 3 (75.0%) | 2 (33.3%) | 5 (50.0%) |
| Severe |  |  |  | 1 (100.0%) | 0 (0.0%) | 1 (20.0%) | 1 (25.0%) | 3 (50.0%) | 4 (40.0%) |
| **Rectal bleeding** |  |  |  |  |  |  |  |  |  |
| Yes | 3 (8.1%) | 7 (16.3%) | 10 (12.5%) | 4 (10.8%) | 6 (14.0%) | 10 (12.5%) | 4 (10.8%) | 6 (14.0%) | 10 (12.5%) |
| Missing | 2 (5.4%) | 2 (4.7%) | 4 (5.0%) | 3 (8.1%) | 3 (7.0%) | 6 (7.5%) | 3 (8.1%) | 2 (4.7%) | 5 (6.3%) |
| **If yes: Severity** |  |  |  |  |  |  |  |  |  |
| Mild | 3 (100.0%) | 6 (85.7%) | 9 (90.0%) | 4 (100.0%) | 6 (100.0%) | 10 (100.0%) | 4 (100.0%) | 6 (100.0%) | 10 (100.0%) |
| Moderate |  |  |  |  |  |  |  |  |  |
| Severe | 0 (0.0%) | 1 (14.3%) | 1 (10.0%) |  |  |  |  |  |  |