**Supplementary materials**

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| **Table 1:** Intensity Grading for Solicited Local Adverse Events |
| --- |
| **AE** | **Grade** | **Definition** |
| Pain at injection site | 0 | Absent |
| 1 | Does not interfere with activity |
| 2 | Interferes with activity and/or repeated use of non-narcotic pain reliever > 24 hours |
| 3 | Prevents daily activity and/or repeated use of narcotic pain reliever |
| Redness | 0 | ≤ 2·5 cm |
| 1 | 2·5 – 5 cm |
| 2 | 5·1 – 10 cm |
| 3 | >10 cm |
| Swelling | 0 | ≤ 2·5 cm |
| 1 | 2·5 – 5 cm and does not interfere with activity |
| 2 | 5·1 – 10 cm or interferes with activity |
| 3 | > 10 cm or prevents daily activity |
| Itching | 0 | Absent |
| 1 | Mild, no interference with normal activity  |
| 2 | Moderate, some interference with normal activity |
| 3 | Significant, prevents normal activity |

| **Table 2:** Intensity Grading for Solicited Systemic Adverse Events |
| --- |
| **Adverse Event** | **Grade** | **Definition** |
| Fever | 0 | <38°C |
| 1 | ≥38 – 38·4°C |
| 2 | ≥38·5 – 38·9°C |
| 3 | ≥39°C |
| Headache | 0 | Absent |
| 1 | Mild, no interference with normal activity |
| 2 | Moderate, some interference with normal activity and/or repeated use of non-narcotic pain reliever >24 hours |
| 3 | Significant; any use of narcotic pain reliever and/or prevents daily activity |
| Fatigue | 0 | Absent |
| 1 | Mild, no interference with normal activity  |
| 2 | Moderate, some interference with normal activity |
| 3 | Significant, prevents normal activity |
| Chills  | 0 | Absent |
| 1 | Mild, no interference with normal activity |
| 2 | Moderate, some interference with normal activity |
| 3 | Significant, prevents normal activity |
| Myalgia | 0 | Absent |
| 1 | Mild, no interference with normal activity  |
| 2 | Moderate, some interference with normal activity |
| 3 | Significant, prevents normal activity |
| Arthralgia | 0 | Absent |
| 1 | Mild, no interference with normal activity  |
| 2 | Moderate, some interference with normal activity |
| 3 | Significant, prevents normal activity |
| Nausea/ Vomiting | 0 | Absent  |
| 1 | Mild, no interference with activity and/or 1 – 2 episodes/ 24 hours |
| 2 | Moderate, some interference with activity and/or >2 episodes/ 24 hours |
| 3 | Significant, prevents daily activity, requires outpatient i.v. hydration |
| Diarrhoea | 0 | Absent |
| 1 | 2 – 3 loose stools or <400 g/24 hours |
| 2 | 4 – 5 stools or 400 – 800 g/24 hours |
| 3 | 6 or more watery stools or >800 g/24 hours or requires outpatient i.v. hydration |
| i.v.= Intravenous |

**Immunogenicity assessments**

**Anti-SARS-CoV-2-specific IgG levels were measured by ELISA**. Briefly, plates were coated with 1µg/ml of SARS-CoV-2 Spike (Spike S1+S2 ECD-His Recombinant Protein, Sino Biological, Chesterbrook, PA, USA) or Spike RBD (Spike RBD-His Recombinant Protein, Sino Biological) recombinant protein. Blocking was performed in 5% milk. Coated plates were incubated with heat-inactivated (56°C for 30 min) human serum in a 1:2-fold serial dilution (starting at 1:100). Antigen-specific IgG detection was performed with goat anti-human IgG-HRP conjugate and tetramethyl benzidine (TMB) substrate (Bethyl Laboratories, Montgomery, Texas, USA) at OD 450 nm.

**SARS-CoV-2 virus neutralization titers were determined using a microneutralization assay with Cytopathic Effect (CPE)-read out**. In brief, heat-inactivated (56°C for 30 min) human serum was serially diluted 1:2 (starting at 1:10) and incubated with 102.7 TCID50/ml tissue culture infective dose of wild type SARS-CoV-2 virus strain 2019-nCov/Italy-INMI1 at 37⁰C 5% CO2 for 1 hour. Afterwards, semi-confluent Vero E6 cells (ATCC) were incubated with the virus-serum mixtures at 37°C 5% CO2 for 3 days. Cells were assessed for virus-induced CPE by light microscopy. The neutralization titer (MN50) was the reciprocal of the highest serum dilution that protected more than the 50% of cells from CPE and reported as geometric mean titer (GMT) of duplicates.

|  |
| --- |
| **Table 3.** Solicited local AEs with severity after one and two doses according to baseline serostatus for SARS-CoV-2 N antigen, n (%) |
|  |  | **SARS-CoV-2 seronegatives** | **SARS-CoV-2 seropositives** |
|  | **Severity** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** |
|  | N = | 38 | 40 | 40 | 38 | 24 | 24 | 8 | 8 | 6 | 6 | 4 | 8 |
| **After first dose** |
| **Pain** | **Any** | **16 (42)** | **30 (75)** | **28 (70)** | **30 (79)** | **21 (88)** | **2 (8)** | **8 (100)** | **6 (75)** | **4 (67)** | **4 (67)** | **4 (100)** | **1 (13)** |
| Mild | 14 (37) | 28 (70) | 23 (58) | 21 (55) | 15 (63) | 2 (8) | 8 (100) | 6 (75) | 4 (67) | 3 (50) | 3 (75) | 1 (13) |
| Moderate | 2 (5) | 1 (3) | 4 (10) | 8 (21) | 6 (25) | 0 | 0 | 0 | 0 | 1 (17) | 1 (25) | 0 |
| Severe | 0 | 1 (3) | 1 (3) | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Redness** | **Any** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **Swelling** | **Any** | **0** | **3 (8)** | **0** | **0** | **1 (4)** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Mild | 0 | 3 (8) | 0 | 0 | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Itching** | **Any** | **0** | **0** | **3 (8)** | **1 (3)** | **1 (4)** | **1 (4)** | **0** | **0** | **0** | **0** | **0** | **0** |
| Mild | 0 | 0 | 3 (8) | 1 (3) | 1 (4) | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 |
| **After second dose** |
|  | **N=** | **36** | **37** | **37** | **35** | **23** | **22** | **8** | **7** | **6** | **6** | **3** | **8** |
| **Pain** | **Any** | **19 (53)** | **29 (78)** | **26 (70)** | **28 (80)** | **19 (83)** | **1 (5)** | **5 (63)** | **5 (71)** | **2 (33)** | **6 (100)** | **3 (100)** | **0** |
| Mild | 19 (53) |  27 (73) | 21 (57) | 23 (66) | 15 (65) | 1 (5) | 5 (63) | 5 (71) | 2 (33) | 6 (100) | 2 (67) | 0 |
| Moderate | 0 | 2 (5) | 5 (14) | 5 (14) | 4 (17) | 0 | 0 | 0 | 0 | 0 | 1 (33) | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Redness** | **Any** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **Swelling** | **Any** | **0** | **1 (3)** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Mild | 0 | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Itching** | **Any** | **0** | **1 (3)** | **1 (3)** | **2 (6)** | **1 (4)** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Mild | 0 | 1 (3) | 1 (3) | 2 (6) | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

|  |
| --- |
| **Table 4a.** Solicited systemic AEs with severity after a first dose according to baseline serostatusfor SARS-CoV-2 N antigen, n (%) |
|  |  | **SARS-CoV-2 seronegatives** | **SARS-CoV-2 seropositives** |
|  | **Severity** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** |
|  | N = | 38 | 40 | 40 | 38 | 24 | 24 | 8 | 8 | 6 | 6 | 4 | 8 |
| **Fever** | **Any** | **2 (5)** | **6 (15)** | **5 (13)** | **11 (29)** | **9 (38)** | **0** | **0** | **2 (25)** | **1 (17)** | **3 (50)** | **3 (75)** | **0** |
| Mild | 1 (3) | 3 (8) | 4 (10) | 8 (21) | 5 (21) | 0 | 0 | 1 (13) | 0 | 3 (50) | 1 (25) | 0 |
| Moderate | 1 (3) | 1 (3) | 1 (3) | 2 (5) | 3 (13) | 0 | 0 | 1 (13) | 1 (17) | 0 | 2 (50) | 0 |
| Severe | 0 | 2 (5) | 0 | 1 (3) | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Headache** | **Any** | **15 (39)** | **21 (53)** | **22 (55)** | **28 (74)**  | **21 (88)** | **8 (33)** | **3 (38)** | **5 (63)** | **2 (33)** | **3 (50)** | **4 (100)** | **1 (13)** |
| Mild | 12 (32) | 13 (33) | 11 (28) | 12 (32) | 6 (25) | 6 (25) | 3 (38) | 5 (63) | 2 (33) | 2 (33) | 1 (25) | 1 (13) |
| Moderate | 1 (3) | 7 (18) | 10 (25) | 15 (40) | 15 (63) | 2 (8) | 0 | 0 | 0 | 1 (17) | 3 (75) | 0 |
| Severe | 2 (5) | 1 (3) | 1 (3) | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Fatigue** | **Any** | **13 (34)** | **24 (60)** | **31 (78)** | **28 (74)**  | **21 (88)** | **10 (42)** | **5 (63)** | **5 (63)** | **3 (50)** | **3 (50)** | **4 (100)** | **3 (38)** |
| Mild | 11 (29) | 14 (35) | 18 (45) | 14 (37) | 9 (38) | 8 (33) | 4 (50) | 3 (38) | 1 (17) | 2 (33) | 1 (25) | 3 (38) |
| Moderate | 0 | 5 (13) | 11 (28) | 11 (29) | 10 (42) | 2 (8) | 1 (13) | 2 (25) | 2 (33) | 1 (17) | 3 (75) | 0 |
| Severe | 2 (5) | 5 (13) | 2 (5) | 3 (8) | 2 (8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Chills** | **Any** | **3 (8)** | **8 (20)** | **9 (23)** | **10 (26)** | **13 (54)** | **0** | **0** | **3 (38)** | **1 (17)** | **3 (50)** | **2 (50)** | **0** |
| Mild | 1 (3) | 6 (15) | 5 (13) | 7 (18) | 7 (29) | 0 | 0 | 2 (25) | 0 | 3 (50) | 1 (25) | 0 |
| Moderate | 2 (5) | 0 | 4 (10) | 2 (5) | 6 (25) | 0 | 0 | 1 (13) | 1 (17) | 0 | 1 (25) | 0 |
| Severe | 0 | 2 (5) | 0 | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Myalgia** | **Any** | **6 (16)** | **14 (35)** | **19 (48)** | **18 (45)** | **16 (67)** | **2 (8)** | **2 (25)** | **4 (50)** | **2 (33)** | **4 (67)** | **3 (75)** | **3 (38)** |
| Mild | 4 (11) |  8 (20) | 13 (33) | 9 (24) | 10 (42) | 2 (8) | 2 (25) | 3 (38) | 2 (33) | 3 (50) | 2 (50) | 3 (38) |
| Moderate | 2 (5) | 5 (13) | 6 (15) | 6 (16) | 6 (25) | 0 | 0 | 1 (13) | 0 | 1 (17) | 1 (25) | 0 |
| Severe | 0 | 1 (3) | 0 | 3 (8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Arthralgia** | **Any** | **5 (13)** | **8 (20)** | **9 (23)** | **11 (29)** | **11 (46)** | **0** | **0** | **2 (25)** | **0** | **0** | **0** | **0** |
| Mild | 5 (13) | 6 (15) | 6 (15) | 5 (13) | 9 (38) | 0 | 0 | 2 (25) | 0 | 0 | 0 | 0 |
| Moderate | 0 | 2 (5) | 3 (8) | 5 (13) | 2 (8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 0 | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Nausea/ Vomiting** | **Any** | **2 (5)** | **4 (10)** | **2 (5)** | **6 (16)** | **8 (33)** | **1 (4)** | **1 (13)** | **1 (13)** | **0** | **0** | **0** | **0** |
| Mild | 1 (3) | 4 (10) | 2 (5) | 6 (16) | 6 (25) | 1 (4) | 1 (13) | 1 (13) | 0 | 0 | 0 | 0 |
| Moderate | 1 (3) | 0 | 0 | 0 | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 0 | 0 | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Diarrhoea** | **Any** | **2 (5)** | **5 (13)** | **8 (20)** | **3 (8)** | **3 (13)** | **0** | **1 (13)** | **1 (13)** | **0** | **0** | **1 (25)** | **0** |
| Mild | 2 (5) | 5 (13) | 7 (18) | 3 (8) | 3 13) | 0 | 1 (13) | 1 (13) | 0 | 0 | 1 (25) | 0 |
| Moderate | 0 | 0 | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

|  |
| --- |
| **Table 4b.** Solicited systemic AEs with severity after a second dose according to baseline serostatus, n (%) |
|  |  | **SARS-CoV-2 seronegatives** | **SARS-CoV-2 seropositives** |
|  | **Severity** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** | **2 μg** | **4 μg** | **6 μg** | **8 μg** | **12 μg**  | **Placebo** |
|  | N = | 36 | 37 | 37 | 35 | 23 | 22 | 8 | 7 | 6 | 6 | 3 | 8 |
| **Fever** | **Any** | **1 (3)** | **3 (8)** | **10 (27)** | **12 (34)** | **12 (52)** | **0** | **0** | **0** | **1 (17)** | **0** | **2 (67)** | **0** |
| Mild | 1 (3) | 2 (5) | 6 (16) | 6 (17) | 5 (22) | 0 | 0 | 0 | 1 (17) | 0 | 0) | 0 |
| Moderate | 0 | 0 | 3 (8) | 3 (9) | 4 (17) | 0 | 0 | 0 | 0 | 0 | 2 (67) | 0 |
| Severe | 0 | 1 (3) | 1 (3) | 3 (9) | 3 (13) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Headache** | **Any** | **8 (22)** | **22 (59)** | **24 (65)** | **31 (89)**  | **19 (83)** | **6 (27)** | **3 (38)** | **4 (57)** | **4 (67)** | **5 (83)** | **3 (100)** | **1 (13)** |
| Mild | 6 (17) | 13 (35) | 12 (32) | 16 (46) | 4 (17) | 5 (23) | 2 (25) | 3(43) | 3 (50) | 5 (83) | 1 (33) | 1 (13) |
| Moderate | 2 (6) | 10 (11) | 8 (22) | 10 (29) | 12 (52) | 1 (5) | 1 (13) | 0 | 1 (17) | 0 | 2 (67) | 0 |
| Severe | 0 | 10 (11) | 4 (11) | 5 (14) | 3 (13) | 0 | 0 | 1 (14) | 0 | 0 | 0 | 0 |
| **Fatigue** | **Any** | **9 (25)** | **21 (57)** | **24 (65)** | **31 (89)**  | **21 (88)** | **10 (42)** | **4 (50)** | **4 (63)** | **3 (50)** | **3 (50)** | **3 (100)** | **2 (25)** |
| Mild | 6 (17) | 13 (35) | 18 (45) | 16 (46) | 9 (38) | 8 (33) | 3 (38) | 3 (43) | 3 (50) | 3 (50) | 1 (33) | 2 (25) |
| Moderate | 3 (8) | 4 (11) | 11 (28) | 10 (29) | 10 (42) | 2 (8) | 1 (13) | 0 | 0 | 0 | 2 (67) | 0 |
| Severe | 0 | 4 (11) | 2 (5) | 5 (14) | 2 (8) | 0 | 0 | 1 (14) | 0 | 0 | 0 | 0 |
| **Chills** | **Any** | **3 (8)** | **7 (19)** | **13 (35)** | **13 (37)** | **19 (83)** | **0** | **1 (13)** | **0** | **2 (33)** | **2 (33)** | **2 (67)** | **0** |
| Mild | 2 (6) | 3 (8) | 7 (19) | 6 (17) | 5 (22) | 0 | 1 (13) | 0 | 2 (33) | 2 (33) | 0 | 0 |
| Moderate | 0 | 3 (8) | 5 (14) | 4 (11) | 10 (44) | 0 | 0 | 0 | 0 | 0 | 2 (67) | 0 |
| Severe | 1 (3) | 1 (3) | 1 (3) | 3 (9) | 4 (17) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Myalgia** | **Any** | **5 (14)** | **14 (38)** | **20 (54)** | **16 (46)** | **16 (70)** | **1 (5)** | **2 (25)** | **1 (14)** | **3 (50)** | **4 (67)** | **3 (100)** | **0** |
| Mild | 4 (11) |  8 (22) | 12 (32) | 7 (20) | 8 (35) | 1 (5) | 2 (25) | 1 (14) | 3 (50) | 2 (33) | 3 (100) | 0 |
| Moderate | 1 (3) | 4 (11) | 7 (19) | 7 (20) | 7 (30) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 2 (5) | 1 (3) | 2 (6) | 1 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Arthralgia** | **Any** | **1 (3)** | **5 (14)** | **12 (32)** | **12 (34)** | **10 (43)** | **0** | **0** | **3 (43)** | **1 (17)** | **0** | **1 (33)** | **0** |
| Mild | 1 (3) | 3 (8) | 6 (16) | 9 (26) | 6 (26) | 0 | 0 | 3 (43) | 1 (17) | 0 | 1 (33) | 0 |
| Moderate | 0 | 1 (3) | 4 (11) | 2 (6) | 2 (9) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 1 (3) | 2 (5) | 1 (3) | 2 (9) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Nausea/ Vomiting** | **Any** | **1 (3)** | **5 (14)** | **6 (16)** | **8 (23)** | **5 (22)** | **1 (5)** | **0** | **1 (14)** | **0** | **0** | **1 (33)** | **0** |
| Mild | 1 (3) | 3 (8) | 4 (11) | 8 (23) | 4 (17) | 1 (5) | 0 | 1 (14) | 0 | 0 | 0 | 0 |
| Moderate | 0 | 2 (5) | 1 (3) | 0 | 1 (4) | 0 | 0 | 0 | 0 | 0 | 1 (33) | 0 |
| Severe | 0 | 0 | 1 (3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Diarrhoea** | **Any** | **0** | **2 (5)** | **5 (14)** | **1 (3)** | **2 (9)** | **2 (9)** | **0** | **0** | **0** | **0** | **0** | **3 (38)** |
| Mild | 0 | 2 (5) | 3 (8) | 3 (3) | 2 (9) | 2 (9) | 0 | 0 | 0 | 0 | 0 | 3 (38) |
| Moderate | 0 | 0 | 2 (5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Figure 1.** Boosting of baseline antibody levels in individual seropositive participants vaccinated with either 2 μg (upper panel) or 4 μg (lower panel) of CVnCoV on days 1 and 29. RBD binding antibodies and SARS-CoV-2 neutralising MN50 titers were analysed at multiple time points. Lines show individual participants in both dose groups.

