SARS-CoV-2 DNA Vaccine INO-4800 Induces Durable Immune Responses Capable of Being Boosted in a Phase 1 Open-Label Trial

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Running Title: SARS-CoV-2 DNA Vaccine Can be Boosted
Abstract Word Count: 199

Text Word Count: 3477

Meeting presentations:

In part at ID Week (September 2021), Virtual

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Abstract

**Background:** Additional SARS-CoV-2 vaccines that are safe and effective as both primary series and booster remain urgently needed to combat the COVID-19 pandemic. Here we describe the safety and durability of the immune response from two doses of a DNA vaccine (INO-4800) targeting the full-length Spike antigen and a subsequent homologous booster dose.

**Methods:** INO-4800 was evaluated in 120 healthy participants across three dose groups (0.5 mg, 1.0 mg and 2.0 mg), each stratified by age. INO-4800 was injected intradermally followed by electroporation at 0 and 4 weeks followed by an optional booster dose 6-10.5 months following the second dose.

**Results:** INO-4800 was well-tolerated, with no treatment-related serious adverse events reported. Most adverse events were mild in severity and did not increase in frequency with age and subsequent dosing. A durable antibody response was observed 6 months following the second dose; a homologous booster dose significantly increased immune responses. Cytokine producing T cells and activated CD8+T cells with lytic potential were detected in the 2.0 mg dose group.

**Conclusion:** INO-4800 was well-tolerated as a 2-dose series and as a homologous booster dose in all adults, including the elderly. These results support further development of INO-4800 as a primary series and as a booster.

**Keywords:** SARS-CoV-2; Clinical trial; DNA Vaccine; COVID-19; Immunogenicity; Booster

**Trial Registration:** ClinicalTrials.gov NCT04336410
Introduction

Despite aggressive vaccination campaigns, most of the world’s population remains unvaccinated and susceptible to COVID-19, the disease caused by SARS-CoV-2[1]. To date, over 3.8 million people have succumbed to COVID-19[2]. The urgent need remains for additional safe and effective vaccines against SARS-CoV-2 that are affordable, scalable and can be distributed to countries where the infrastructure may not be supportive of ultra-cold chain transport and storage.

The preliminary safety and immunogenicity of the COVID-19 DNA vaccine, INO-4800, in both Phase 1 and Phase 2 clinical studies were previously reported[3, 4]. INO-4800 targets the SARS-CoV-2 spike protein which exists as a trimer on the virus surface. Attachment to host cells is mediated by binding of the spike protein’s receptor binding domain (RBD) onto angiotensin converting enzyme 2 (ACE2) receptors of host cells[5]. Once attached, the viral transport through the host cell membrane is facilitated by the S2 region of spike protein[5]. Eliciting humoral and cellular responses against the spike protein can prevent the virus from gaining access to host cells, and this strategy has led to several impactful vaccine developments.

INO-4800 consists of an optimized plasmid DNA that is injected into the skin with enhanced delivery into the cells by in vivo electroporation[6]. This approach potentially offers several advantages, including induction of humoral and cellular immunity, favorable tolerability and thermal stability profiles and its ease of manufacture[7, 8].

The previous preliminary analysis[3] demonstrated that two 1.0 mg or 2.0 mg doses of INO-4800 administered one month apart were well-tolerated in 38 healthy participants 18-50 years of age and induced the production of neutralizing antibodies and/or T-cells targeting SARS-CoV-2 spike protein using a two-dose regimen. Here we describe the durability of that response at 6 months following the second dose, as well as the safety and immunogenicity of the 2-dose regimen in older and elderly participants including following a subsequent homologous booster dose.
Methods

Trial Design and Participants

The Phase 1, open-label, multi-center trial (NCT04336410) evaluated the safety, tolerability, and immunogenicity of INO-4800 injected intradermally (ID) followed by electroporation (EP). A total of 120 healthy participants without a known history of COVID-19 were assigned to receive a 0.5 mg, 1.0 mg, or 2.0 mg dose of INO-4800 in a 2-dose regimen (Weeks 0 and 4) and a subsequent optional booster dose no earlier than 8-weeks after dose 2. An equal number of participants were enrolled in each dose group (n=40) and further stratified by age groups [18-50 years of age; n=20, 51-64 years of age; n=10, and ≥65 years of age; n=10].

The trial was approved by the institutional review board of each clinical site and all participants provided written informed consent prior to enrollment. The trial was conducted under current Good Clinical Practices (GCP).

DNA Vaccine INO-4800

The DNA vaccine INO-4800 was previously described[3, 9]. Briefly, the vaccine expresses the full-length sequence of the SARS-CoV-2 spike glycoprotein derived from the original Wuhan strain based on an optimized synthetic sequence that was created using a proprietary algorithm. The final vaccine drug product, manufactured under Good Manufacturing Practices, was formulated at 10 mg/mL in saline sodium citrate buffer.

INO-4800 is injected ID immediately followed by EP using the CELLECTRA® 2000 device that generates a controlled electric field at the injection site to enhance the cellular uptake and expression of the DNA plasmid as previously described[10, 11]. The device delivers a total of four
electrical pulses per EP, each of 52 msec in duration, at current of 0.2 Amp and voltage of 40-200 per pulse.

Endpoints

Primary safety endpoints included incidence of adverse events (AEs) using the “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trial” including frequency and severity of injection site reactions. Primary immunological endpoints included the measurement of SARS-CoV-2 Spike glycoprotein antigen-specific binding antibodies as well as the measurement of antigen-specific cellular immune responses by IFN-γ, ELISPOT and flow cytometry assays. Endpoints reflected in this publication are inclusive of 6 months after second dose (non-boosted participants) and, when applicable, 2 weeks after booster dose.

Trial Procedures

Vaccine was administered in 0.1 ml ID injections in the deltoid region followed by EP at the injection site. At each dosing visit, either a single injection for 0.5 mg and 1.0 mg dose groups or two injections for 2.0 mg dose group were given, one in each deltoid region.

The initial forty participants 18-50 years were enrolled sequentially into 1.0 mg and 2.0 mg dose groups with a safety run-in period[3]. The trial design was expanded to include older participants in all dosing groups (including a 0.5 mg dose level). Upon favorable safety assessment review by an independent Data Safety Monitoring Board (DSMB) of Week 1 data for 0.5 mg dose group
participants aged 51-64 years and ≥ 65 years, enrollment of the corresponding age strata in the 1.0 mg and subsequently 2.0 mg dose groups was initiated.

Participants were assessed for safety (complete blood count, serum chemistry, and urinalysis), including local and systemic AEs, at serial time points [screening, Week 0 (Dose 1), next day phone call, and Weeks 1, 4 (Dose 2), 6, 8, 12, 28, 40 and 52. Blood immunology collections occurred at all clinic visits except Week 1. Upon completion of the Week 12 visit, participants who consented to and received the optional booster dose were transitioned to an extended schedule of events to include the booster dose (Dose 3) and subsequent visits for safety at 2, 12, 24, 36, and 48 weeks following the booster dose with blood immunology collections at all clinic visits except 36 weeks.

The DSMB reviewed laboratory and AE data for the participants up to 24 weeks after the second dose (non-boosted) and 2 weeks after booster dose.

Protocol Eligibility

Key inclusion criteria included: healthy adults aged at least 18 years; and Body Mass Index of 18-30 kg/m² at screening. Key exclusion criteria included: individuals in a current occupation with high risk of exposure to SARS-CoV-2; previous known exposure to SARS-CoV-2 or receipt of an investigational product for the prevention or treatment of COVID-19; autoimmune or immunosuppression as a result of underlying illness or treatment; hypersensitivity or severe allergic reactions to vaccines or drugs; and medical conditions that increased risk for severe COVID-19.
Immunogenicity Assessment Methods

Samples were collected at timepoints described above with screening and pre-dose 1 samples considered baseline. Peripheral blood mononuclear cells (PBMCs) were collected as previously described[3]. After isolation, PBMCs were stored in the vapor phase of a liquid nitrogen freezer until analysis, while serum samples were stored at -80°C. Eight participants were excluded from the immunogenicity analyses due to a seropositive response, as determined by a positive ELISA titer to the SARS-CoV-2 nucleoprotein, indicating SARS-CoV-2 infection.

**SARS-CoV-2 Pseudovirus Neutralization Assay:** Serum samples were measured using a pseudovirus neutralization assay as described previously[4]. Data was reported as ID$_{50}$, which is the reciprocal serum dilution resulting in 50% inhibition of infectivity by comparison to control wells with no serum samples added. Please see supplementary methods for additional information.

**SARS-CoV-2 Spike Enzyme-Linked Immunosorbent Assay (ELISA):** Binding antibodies to SARS-CoV-2 spike protein were measured by ELISA as described previously[4]. SARS-CoV-2 spike antibody concentrations were determined by interpolation from a dilution curve of SARS-CoV-2 convalescent plasma with an assigned concentration of 20,000 Units per mL. Further details are described in the supplementary methods.

**SARS-CoV-2 Spike ELISpot Assay Description:** The SARS-CoV-2 spike antigen-specific IFN-γ T-cell response was measured as described previously[3]. Values were reported as the mean spot-forming units per million PBMCs across three triplicate wells after background subtraction using DMSO-only negative control wells. Please see supplementary methods for additional information.

**INO-4800 SARS-CoV-2 Spike Flow Cytometry Assays:** PBMCs were also assessed in Intracellular Cytokine Staining (ICS) and Lytic Granule Loading (LGL) assays. The ICS assay was performed as previously described[3] and included cytokines IFNγ, TNFα, and IL-2. The LGL
assay was also performed as reported previously [12] following stimulation with overlapping peptides to the full-length spike protein to measure CD8+ T cell activation (CD38, CD69, CD137, Ki67) and capacity to produce lytic proteins (granzymes A and B, perforin and granulysin).

**Statistical Analysis**

No formal power analysis was applicable to this trial. Descriptive statistics were used to summarize the safety endpoints based on the safety population: proportions of participants with AEs, through 6 months following dose 2 (non-boosted participants) or 2 weeks following booster dose. The safety population included all participants who received at least one dose of INO-4800 and were grouped by age and the dose of INO-4800. Post-hoc within subject analyses of post-vaccination minus pre-vaccination paired differences in SARS-CoV-2 neutralization and ELISA spike responses (on the natural log-scale, with a paired t-test), ELISpot responses (with Wilcoxon signed-rank tests), and flow assay responses (with Wilcoxon signed-rank tests) were performed.
Results

Trial Population Demographics

Between 06 April 2020 and 07 July 2020, 154 participants were screened and 120 enrolled into the trial (Figure 1). The median age was 50.5 years (range 18 to 86 years). Participants were 57.5% female (69/120) and 42.5% male (51/120) (Table 1). Most participants were white (94.2%, 113/120).

Vaccine Safety and Tolerability

A total of 117 of 120 (97.5%) participants received both doses. One participant in the 2.0 mg group discontinued trial participation prior to receiving the second dose solely due to lack of transportation to the clinical site. Two participants in the 0.5 mg group did not receive the second dose due to exclusionary eligibility criteria (hypertension) having been determined following Dose 1; (Figure 1).

Ninety-nine of 120 (82.5%) participants consented to and received the booster dose, approximately 6 to 10.5 months following the second dose. Reasons for not receiving booster dose included receipt of another SARS-CoV-2 vaccine (available under Emergency Use Authorization), new medical condition precluding participation (having had COVID-19, pregnancy or hypertension), or loss to follow-up.

A total of 34 treatment-related local and systemic AEs were reported by 18 participants. Thirty-one AEs were Grade 1 (mild) in severity and comprised mostly injection site reactions. Three treatment-related Grade 2 (moderate) AEs were reported as lethargy, abdominal pain, and injection site pruritus. There were no febrile reactions reported. No participants discontinued due to AEs. No treatment-related SAEs were reported. There were no abnormal laboratory values that
were deemed treatment-related and clinically significant by the Investigators. There was no increase in the number of participants who experienced related AEs in the 2.0 mg group (12.5%, 5/40), compared to that in the 1.0 mg group (15%, 6/40) or the 0.5 mg group (17.5%, 7/40). In addition, there was no appreciable increase in the frequency of AEs with the second or booster doses when compared to the first dose (Figure 2). A decrease in frequency of treatment-related AEs in the older and elderly age cohorts was observed when compared to the younger age group (Supplementary Figure 3).

INO-4800 induces durable humoral immune responses capable of being boosted: The generation of antibodies against SARS-CoV-2 following vaccination with INO-4800 was measured from the sera. The functional ability of antibodies was assessed using a pseudovirus neutralization assay. All three dose groups induced neutralizing antibodies that peaked two weeks following the second dose (GMTs- 14.9, 19.1, 54.1 in the 0.5 mg, 1.0 mg and 2.0 mg dose groups, respectively) (Figure 3A, left panel, Supplementary Table 1). These increased responses were statistically significant over baseline in the 2.0 mg dose group for each time point through study week 28, approximately 6 months after dose 2 (Figure 3A, table). Following administration of a booster dose, statistically significant increases over pre-boost titers were observed in all dose groups (GMTs- 58.7, 76.1, 100 in the 0.5 mg, 1.0 mg and 2.0 mg dose groups, respectively; all P<0.001) (Figure 3A, right panel, Supplementary Table 1). The 2.0 mg dose group had a 12.8 (95%CI 6.3, 26.0) geometric fold rise (GMFR) over pre-boost titers, the highest of any dose group. Neutralization titers by participant age are shown in Supplementary Figure 1A; GMTs were numerically lower in the
older age groups but statistically significantly higher than baseline at week 6 in the 2.0 mg dose group.

Antibodies to the spike trimer protein were measured in a binding ELISA. All three dose groups induced binding antibodies that peaked four weeks following dose 2 (GMTs- 428.5, 595.9, 678.0 in the 0.5 mg, 1.0 mg and 2.0 mg dose groups, respectively) (Figure 3B, left panel, Supplementary Table 2). Increases over baseline were observed in all participants who received the 2.0 mg dose and GMTs were statistically significantly higher than baseline 6 months following dose 2 (GMTs- 250.1, 215.3, 407.2 in the 0.5 mg, 1.0 mg and 2.0 mg dose groups, respectively; all P<0.026). Following administration of a booster dose, statistically significant increases over pre-boost titers were observed in all dose groups (GMTs- 1963.8, 3685, 5953 in the 0.5 mg, 1.0 mg and 2.0 mg dose groups, respectively; all P<0.007) (Figure 3B, right panel, Supplementary Table 2). The 2.0 mg dose group had a 20.8 (95%CI 13.9, 31.2) GMFR over pre-boost titers which was the highest of any dose group. ELISA binding titers by participant age are shown in Supplementary Figure 1B.

INO-4800 induces cellular immune responses capable of being boosted

Interferon-gamma (IFNγ) Enzyme-linked immunospot (ELISpot) was performed on PBMCs. Increases in spot forming units (SFU) per million PBMCs over baseline are shown in Figure 4A, left panel. Magnitudes of IFNγ peaked at week 6 for the 0.5 mg and 2.0 mg dose groups (median 19.4 and 43.3, respectively) and at week 8 for the 1.0 mg dose group (median 17.8). Six months following dose 2, magnitudes remained high in the 2.0 mg dose group (median 19.6). Of note, magnitudes in the 1.0 mg and 2.0 mg dose groups were statistically significantly increased following the booster dose (P=0.018 and P=0.008, respectively) (Figure 4A, right panel). The 2.0 mg dose group had a difference in medians of 10 following the booster, resulting in the highest
post-boost increase of any dose group. ELISpot responses by participant age are shown in
Supplementary Figure 2A.

**INO-4800 induces cytokine producing T cells and activated CD8+ T cells with lytic potential**

Further exploration of the T cell response was performed on participants following 2 doses. The
contribution of SARS-CoV-2 specific CD4+ and CD8+ T cells was assessed by intracellular
cytokine staining (ICS), Figure 4B-C. The median frequency of CD4+ T cells producing IFNγ
increased following vaccination in all three dose groups of INO-4800, and the frequency of CD4+
T cells producing TNFα was statistically significantly increased in the 2.0 mg dose group
(P<0.001) (Figure 4B). The frequency of CD8+ T cells producing TNFα was statistically
significantly increased following vaccination in all three dose groups of INO-4800 (All P<0.041)
(Figure 4C). The 2.0 mg dose group had the highest difference in medians for CD8+ T cells
producing any response, IFNγ and TNFα (0.066, 0.026, and 0.011 respectively). ICS responses
by participant age are shown in Supplementary Figure 2B-C.

SARS-CoV-2 specific CD8+ T cells were also characterized on a subset of participants with
remaining sample following 3 doses by a lytic granule loading flow cytometry assay that included
T cell receptor activation induced markers, CD69 and CD137. The median frequency of
CD8+CD69+CD137+ cells increased following immunization with 2.0 mg of INO-4800, with a
difference in the medians of 0.072 (Figure 5A, left panel). Further characterization of these
activated cells, including the co-expression of proteins utilized in cytolytic killing (granzyme A,
granzyme B, perforin or granulysin) revealed a statistically significant increase in both the 1.0 mg
(P=0.008) and 2.0 mg (P=0.003) dose groups (Figure 5A middle and right panels). The 2.0 mg
dose group had a difference in medians of 0.085 in the CD69+CD137+ population co-expressing
perforin and granzymes A and B and 0.054 in the population co-expressing granulysin. CD8+ T
cells expressing the activation marker CD38 and proliferation marker Ki67 were also assessed
The frequency of SARS-CoV-2 specific CD38+CD8+ T cells statistically significantly increased following 2.0 mg of INO-4800 (P=0.016), with a difference in medians of 1.45 (Figure 5B, left panel). CD38+CD8+ T cells with lytic potential (Figure 5B middle and right panels) statistically significantly increased following 2.0 mg of INO-4800 (P<0.001). Following immunization with 2.0 mg of INO-4800, the mean frequency of activated CD8+ T cells expressing granzymes A and B and perforin was 1.7% with a difference in medians of 0.710 and those expressing granulysin was 1.8% with a difference in medians of 0.433 (Figure 5B middle and right panels). Statistically significant increases in the frequency of these CTL phenotypes were also observed in the 1.0 mg dose group (P≤0.012) (Figure 5B middle and right panels). The 2.0 mg dose group had the highest frequencies of CD8+ T cells expressing Ki67 with a difference in medians of 0.367 and Ki67 with cytolytic proteins: GrzA+GrzB+Prf+ and 0.230 (Gnly+). All three Ki67+ populations were statistically significantly increased in the 2.0 mg dose group (P<0.001; Figure 5C). The 2.0 mg dose group consistently showed the highest median responses across all phenotypes assessed when compared to the other two dose groups.

**Discussion**

This report provides results for the expansion of a Phase 1 trial on the safety, tolerability, and immunogenicity of INO-4800, a SARS-CoV-2 vaccine encoding the spike protein [3], including the durability of the immune response 6 months following dose 2 and immune responses 2 weeks following an optional booster dose.

INO-4800 appeared to be well-tolerated at all three dose levels, with no treatment-related serious adverse events reported. Most adverse events were mild in severity and did not increase in frequency with age and subsequent dosing. These safety and tolerability results are generally...
consistent with U.S. Phase 2 trial results evaluating the 1.0 mg and 2.0 mg doses of INO-4800 in approximately 400 subjects[4] and those studies conducted outside the U.S. by Inovio collaborators (International Vaccine Institute, Advaccine – manuscripts in preparation).

Induction of both humoral and cellular responses were observed across all three dose groups in the current trial, inclusive of binding and neutralizing antibodies and cytokine producing T cells as well as exhibiting lytic potential in response to SARS-CoV-2 spike antigen. Immunization with the 2.0 mg dose of INO-4800 resulted in the highest GMTs of neutralizing and binding antibodies as well as the highest magnitudes of IFNγ production to SARS-CoV-2 of any dose in all age groups tested, and the increase in antibody levels were statistically significant above baseline out to 6 months following dose 2. Importantly, increases in both humoral and cellular immune responses were statistically significant following the booster dose.

The contribution of the CD8+T cell response to vaccine efficacy has become increasingly recognized as they have been detected early after vaccination[13] and due to their role in controlling infection[14, 15]. Specifically, it has been established that CD8+T cells expressing cytokines such as IFNγ and TNFα as well as markers involved in activation status and proliferation such as CD38 and Ki67 contribute to limiting disease severity during SARS-CoV-2 infection[14]. Additional studies have identified the expression of CD69 and CD137 on SARS-CoV-2 specific CD8+T cells being associated with less severe disease[15]. This expanded Phase 1 trial demonstrates that immunization with INO-4800 induces SARS-CoV-2 specific CD8+ T cells exhibiting these specific characteristics, suggesting the induction of a vaccine induced cellular response that has potential to protect against severe COVID-19. As variants of concern continue to emerge, the generation of cross-reactive activated CD8+T cells with lytic potential are likely to play an important role in preventing severe disease. We have previously demonstrated that vaccination with INO-4800 induces T cells and neutralizing antibodies that are active against the parental SARS-CoV-2 strain as well as the Variants of Concern[16]. We acknowledge limitations
to this trial that include the relatively small study population and the limited number of PBMCs available for testing across more than one assay. This trial was not powered to formally compare immune responses between dose groups or age stratifications.

The immune responses observed in the current trial and in our larger Phase 2 trial [4] support advancing the 2.0 mg dose of INO-4800 to a Phase 3 efficacy evaluation. This dose has elicited the highest binding and neutralizing antibody titers, the highest T cell cytokine production from both CD4+ and CD8+ T cells and the highest expression of markers associated with attenuation of severe COVID-19 on CD8+ T cells (which could be critically important for vaccine efficacy in preventing hospitalization and death in the context of the circulating Delta variant).

Our demonstration in this trial that the immune responses, both antibody as well as T cell responses, elicited by a 2-dose primary series of INO-4800 could be further boosted with a third dose without safety or tolerability concerns positions INO-4800 as an important candidate for continued development as a stand-alone SARS-CoV-2 vaccine, as well as for continued examination in combination approaches. The potential ability to administer INO-4800 multiple times, with high tolerability, along with its ease of scalability and thermostability, contribute to its potential value in combatting the COVID-19 pandemic and addressing the persistence of SARS-CoV-2 as an ongoing endemic threat to global health.
Declaration of Interests

KAK, EB, JA, MG, DA, ACQ, NL, VA, MD, SW, ML, AS, MPM, PP, TM, TRFS, SJR, JL, MD, ASB, JES, JJK, KEB, LMH, JDB, MPM, Jr. report grants from Coalition for Epidemic Preparedness Innovations, during the conduct of the trial; other from Inovio Pharmaceuticals including salary and stock options, outside the submitted work. PT, ELR, MP, AJK, FIZ, DF, KL, JE, MA, and DBW report grants from Coalition for Epidemic Preparedness Innovations, during the conduct of the trial. D.B.W. participates in industry collaborations and has received renumeration for individual services. In the interest of disclosure, D.B.W. reports the following paid associations with commercial partners: Pfizer (Advisory Board), Geneos (Advisory, SRA), Advaccine (Advisory) Astrazeneca (Advisory, Speaker), Inovio (BOD, SRA, Stock ownership), Sanofi (Advisory Board), BBI (Advisory Board, SRA). All other authors declare no potential conflicts of interest.

Funding

This work is funded by the Coalition for Epidemic Preparedness Innovations (CEPI) and Inovio Pharmaceuticals, Inc.

Acknowledgments

The investigators and sponsor express their gratitude for the contribution of all the trial participants and the invaluable advice of the international Data Safety Monitoring Board. We also acknowledge the broader support from the various teams within Inovio: [Ning Jiang, MD, PhD; Greta Kcomt Del Rio, BS; Alysia Ryan, BS; Dennis Van De Goor, MS; Kelly Morales, BS; Jacob Walton, BS; Srujan Vadlamudi, BS; David Valenta, PhD; EJ Brandreth, MBA; Dan Jordan, BS; Robert J. Juba Jr, MS; Stephen Kemmerrer, BSME, MBA, PE; Timothy Herring, MPH; Susan Duff, BS]; the Wistar Institute: Dr. Ziyang Xu and Edgar Tello Ruiz; National Infections Service,
Public Health England: Naomi Coombes, PhD; Mike Elmore, PhD and the Alliance for Multispecialty Research, Kansas City, MO and Lexington, KY.
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Table 1: Participant Demographics

| Variable            | Statistic | 0.5 mg (N=40) | 1mg (N=40) | 2mg (N=40) | Total (N=120) |
|---------------------|-----------|---------------|------------|------------|---------------|
| **Sex**             | n (%)     | 18 (45.0)     | 17 (42.5)  | 16 (40.0)  | 51 (42.5)     |
| Male                | n (%)     | 22 (55.0)     | 23 (57.5)  | 24 (60.0)  | 69 (45.0)     |
| **Race**            | n (%)     | 40 (100)      | 38 (95.0)  | 35 (87.5)  | 113 (94.2)    |
| White               | n (%)     | 0             | 1 (2.5)    | 1 (2.5)    | 2 (1.7)       |
| Black or African American | n (%)   | 0             | 1 (2.5)    | 4 (10.0)   | 5 (4.2)       |
| **Ethnicity**       | n (%)     | 3 (7.5)       | 0          | 0          | 3 (2.5)       |
| Hispanic or Latino  | n (%)     | 35 (87.5)     | 40 (100)   | 40 (100)   | 40 (100.0)    |
| Not Hispanic or Latino | n (%) | 2 (5.0)       | 0          | 0          | 0             |
| Not Reported        | n (%)     | 40            | 40         | 40         | 120           |
| Age (years)         | Mean (SD) | 50.7 (15.30)  | 49.2 (16.75)| 50.7 (17.90)| 50.2 (16.56)  |
|                     | Median    | 52.5          | 51.0       | 50.5       | 50.5          |
|                     | Min, Max  | 23, 76        | 18, 73     | 19, 86     | 18, 86        |
Figure 1: Consort Flow Diagram
Figure 2- Related systemic and local adverse events. Post First Dose, N=120 (N=40 in each dose group), Post Second Dose, N=117 (N=38 in the 0.5 mg dose group, N=40 in the 1.0 mg dose group and N=39 in the 2.0 mg dose group, and Post Third Dose, N=99 (N=33 in the 0.5 mg dose group, N=31 in the 1.0 mg dose group and N=35 in the 2.0 mg dose group).
significance versus baseline. The dose groups are represented by orange triangles (0.5 mg), blue circles (1.0 mg) and green squares (2.0 mg).
the minimum to maximum values. The mean is denoted with a “+” sign. Wilcoxon signed-rank was used to assess significance versus baseline. The dose groups are represented by triangles (0.5 mg), circles (1.0 mg) and squares (2.0 mg).
Figure 5 - INO-4800 induces spike specific activated CD8+T cells with lytic potential. A lytic granule loading flow cytometry assay was used to characterize the expression of the activation markers CD69 and CD137 (A), CD38 (B), and the proliferation marker Ki67 (C) from samples collected at baseline or post-dose 2. The expression of proteins found in lytic granules: granzymes A (GrzA) and B (GrzB), perforin (Prf) and granulysin (Gnly) were assessed together with activation/proliferation subset. The graphs include n=4 participants in the 0.5 mg dose group and n=10 participants in the 1.0 mg dose group and n=13 in the 2.0 mg dose group. Open symbols represent individual participants, the box extends from the 25th to the 75th percentile, line inside the box is the median, and the whiskers extend from the minimum to maximum values. The mean...
is denoted with a “+” sign. Wilcoxon signed-rank was used to assess significance versus baseline.

The dose groups are represented by orange triangles (0.5 mg), blue circles (1.0 mg) and green squares (2.0 mg).