Preliminary evaluation of a novel coronavirus vaccine (CORVax) using electroporation of plasmid DNA encoding a stabilized prefusion SARS-CoV-2 spike protein alone or with transfection of plasmid IL-12

Shawn M Jensen[1], Christopher G. Twitty[2], Christopher Paustian[3], Madeleine Laws[3], Glenna McDonald[3], Keith Wegmann[1], Tarsem Moudgil[1], Michael Afentoulis[1], Mia Han[2], Kellie Malloy Foerter[2], David A. Canton[2], Jack Y. Lee[2], Bianca Nguyen[2], John Rodriguez[2], Kim Jaffe[2], Brian Piening[1], Carlo Bifulco[1], Daniel J. O'Connor[2], Walter Urba[1], Rom S. Leidner[1], Traci L. Hilton[3], Hong-Ming Hu[1,3], Bernard A. Fox[1,3]

[1]Earle A Chiles Research Institute, Robert W Franz Cancer Center, Providence Portland Medical Center, Portland, Oregon
[2]OncoSec Medical Incorporated, San Diego, California.
[3]UbiVac, Portland, Oregon

ABSTRACT

Background: SARS-CoV-2 (CoV2) has precipitated a global pandemic and the effectiveness of standard vaccine strategies to induce potent and persistent immunity to CoV2 is in question, particularly for the elderly. This problem is not dissimilar to what we have struggled with in our quest to induce immunity to cancer antigens, where vaccine-induced anti-cancer immune responses can be weak. Here, we describe a novel vaccine approach which leverages electroporation (EP) of a plasmid encoding a prefusion stabilized CoV2 spike protein (CORVax). As IL-12 has been shown to augment the efficacy of immunotherapy in aged mice[1], we have initiated studies to evaluate if plasmid IL-12 (TAVO™) can similarly augment anti-CoV2 immune responses in young mice and have planned studies in aged animals.

Methods: A prefusion stabilized CoV2 spike plasmid expression vector was constructed, a master cell bank generated and clinical-grade plasmid manufactured. C57BL/6 and BALB/c were vaccinated via intramuscular (IM) and/or intradermal (ID) injection followed immediately by EP of plasmids encoding the CoV2 spike protein with or without plasmid-encoded murine IL-12 on days 1 and 14 or 21. Mice were followed for >120 days to assess safety. Splenocytes and serum were harvested at different time points to interrogate virus-specific cellular responses as well anti-spike IgG1/IgG2 antibody titers. A surrogate viral neutralization test (sVNT) assessed serum blockade of soluble hACE2R binding to immobilized CoV2 spike.

Results: Preliminary data shows that EP of CORVax alone or combined with IL-12 was safe. EP of CORVax was able to elicit anti-Spike IgG antibodies (EC50 = 1/2112), as well as IgG antibodies targeting the receptor binding domain of the Spike protein (EC50 = 1/965) approximately 40 days after the booster vaccination. In 2 of 2 experiments, CORVax combined with IL-12 significantly (P<0.0001) increased the sVNT titers at 2 months, but this benefit was lost by 3 months.

CORVax vaccination

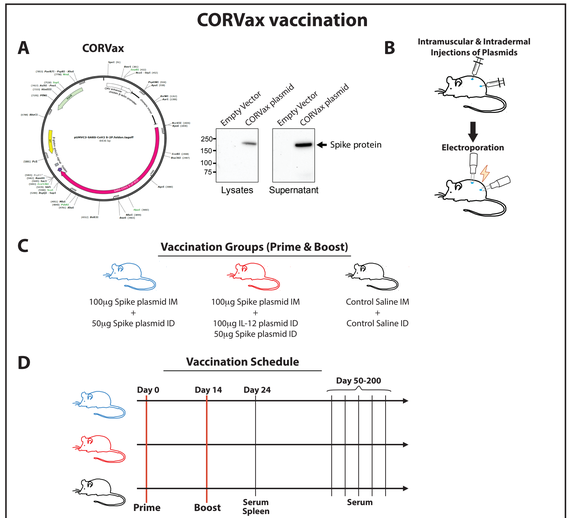

Figure 1. The CORVax vaccine includes a DNA plasmid encoding a prefusion stabilized SARS-CoV-2 Spike protein of ~160kD as seen in cell lysate and conditioned media from transfected HEK293 cells (**A**). Plasmids are injected intramuscularly (IM) and intradermally (ID) and subsequently electroporated, illustrated in (**B**). Mice in this study were vaccinated with 100µg IM & with 50µg ID with the CORVax vaccine (blue). In addition, a group of mice received 100µg IL-12 plasmid ID with CORVax (red). A third group of control mice received saline injections (black, **C**). All mice in all groups received electroporation of both IM and ID injection sites. (**C**). Fourteen days following vaccination, mice received a booster vaccination equal to their prime vaccination (**D**). A portion of mice were euthanized 10 days following the booster vaccine to assay for anti-Spike immune responses, while remaining mice were bled at regular intervals to examine the anti-Spike immune response over time.

IL-12 and other cytokines expressed following CORVax/IL-12 vaccination with no significant toxicity observed

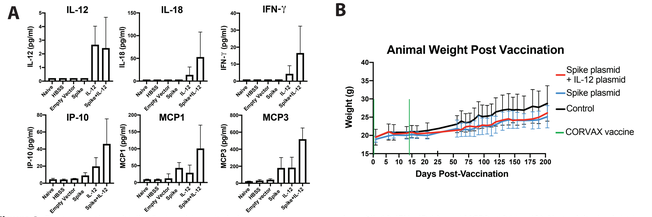

Figure 2. Electroporation with IL-12 plasmid results in increased serum expression of IL-12, IFNγ, IP-10, and MCP3 at 24 hrs (**A**). No toxicity (weight loss >10%, cachexia, labored breathing, hunched posture)was observed in mice that were vaccinated with CORVax with and without IL-12 (**B**).

CORVax vaccination elicits a T cell response against the Spike Protein

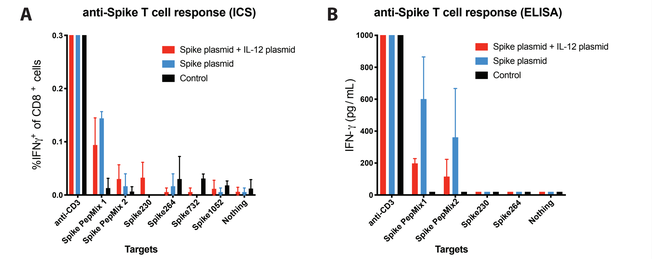

Figure 3. Splenocytes were collected from mice 10 days following the booster vaccination and assayed for reactivity to T cell epitopes from SARS-CoV2 Spike protein. Splenocytes were cultured in the presence of anti-CD3, 1µg/ml peptide, or nothing. Spike PepMix 1 & 2 are pools containing 15 amino acid peptides spanning SARS-CoV-2 Spike protein (Jpt Technologies). $Spike_{264}$, $Spike_{530}$, $Spike_{732}$, and $Spike_{1052}$ are 9mers determined to bind to H-2^d using NetMHCpan. Brefeldin A (5µg/ml) was added to wells at hour 4 for intracellular cytokine staining (**A**) or not included for cytokine secretion determined by ELISA (**B**). Data is representative of two independent experiments.

Anti-Spike serum antibody responses prior to 60 days

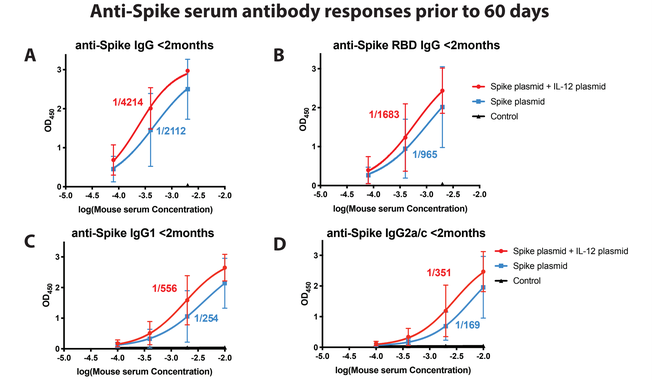

Figure 4. Serum was collected from mice 10 days or 38 days following booster vaccination and assayed for the presence of total IgG antibodies targeting SARS-CoV2 Spike protein (**A**) or SARS-CoV2 Spike receptor-binding domain (**B**). The anti-Spike antibody titrations for specific IgG subclasses is shown for IgG1 (**C**) and IgG2a/c (**D**). EC_{50} values are shown for each corresponding antibody titration curve. Data is 2 independent experiments; n=9 mice for Spike plasmid+IL12 plasmid, n=9 mice for Spike plasmid, and n=5 mice for Control.

Enhanced Spike neutralization in mice vaccinated with CORVax + IL12

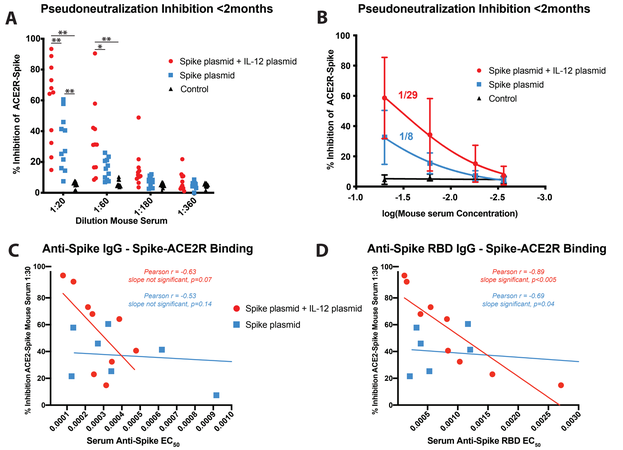

Figure 5. Serum was collected from mice 10 days or 38 days following booster vaccination and assayed for the ability to inhibit ACE2R binding to SARS-CoV2 Spike protein. Serum from mice vaccinated with Spike plasmid + IL-12 plasmid demonstrated statistically significant inhibition of binding at 1:20 and 1:60 dilution of serum, ** p<0.0001, * p<0.005 (**A**). It also exhibited a 3.5-fold lower IC_{50} compared to serum from mice vaccinated with Spike plasmid alone (**B**). There was a strong correlation of anti-Spike RBD IgG with %Inhibition ACE2R-Spike from mice that were vaccinated with Spike plasmid + IL-12 plasmid, Pearson r = -0.89 p<0.005 (compare **C** & **D**).

Anti-Spike antibodies present in serum 6 months post vaccination

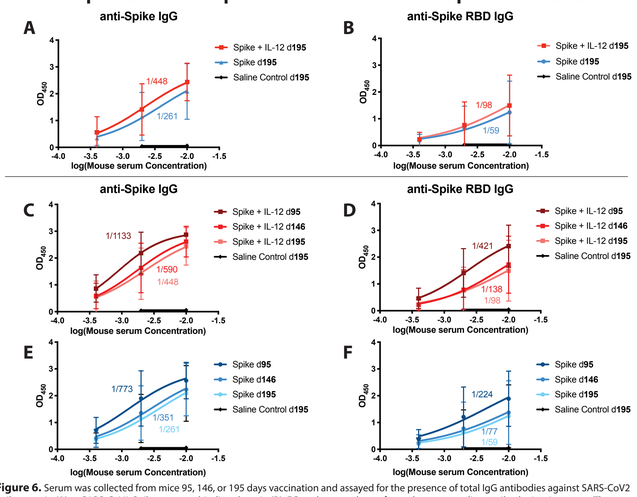

Figure 6. Serum was collected from mice 95, 146, or 195 days vaccination and assayed for the presence of total IgG antibodies against SARS-CoV2 Spike protein (**A**) or SARS-CoV2 Spike receptor-binding domain (**B**). EC_{50} values are shown for each corresponding antibody titration curve. The anti-Spike IgG antibody decay is shown for Spike plasmid + IL-12 plasmid vaccinated mice (**C**) and Spike plasmid vaccinated mice (**E**). The anti-Spike RBD IgG antibody decay is shown for Spike plasmid + IL-12 plasmid vaccinated mice (**D**) and Spike plasmid vaccinated mice (**F**). Data is 2 independent experiments; n=9 mice for Spike plasmid+IL12 plasmid, n=9 mice for Spike plasmid, and n=8 mice for Control.

Spike pseudoneutralization 6 months after vaccination

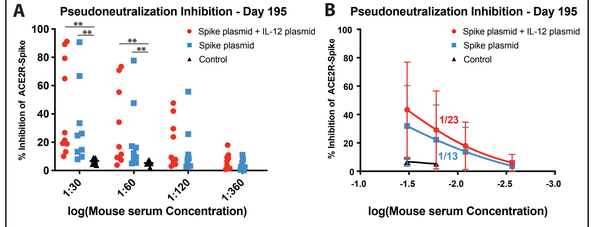

Figure 7. Serum was collected from mice 195 days following vaccination and assayed for the ability to inhibit ACE2R binding to SARS-CoV2 Spike protein. Serum from mice vaccinated with Spike plasmid + IL-12 plasmid or Spike plasmid alone demonstrated statistically significant inhibition of binding at 1:30 and 1:60 dilution of serum compared to control vaccinated mice (**A**). The IC_{50} for each of the serum pseudoneutralization titrations is shown in (**B**). ** p<0.0001, * p<0.005.

CORVax12 - a phase I trial of SARS-CoV-2 spike (S) protein plasmid DNA vaccine (CORVax) +/- pIL-12 (tavokinogene telseplasmid) in healthy volunteers, with immunodynamic biomarker monitoring of coordinated cellular/humoral response.

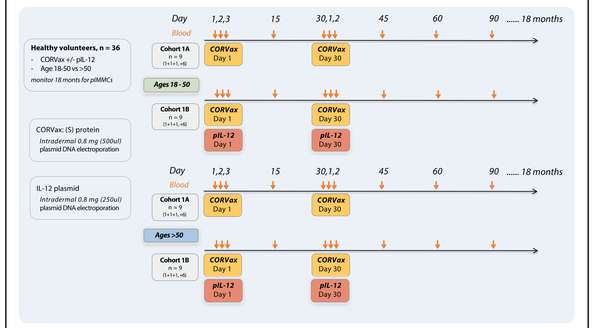

Figure 8. This is a Phase 1, open-label study to evaluate the safety profile of CORVax +/- pIL-12, (electroporated SARS-CoV-2 spike (S) protein plasmid DNA vaccine with or without the combination of electroporated IL-12p70 plasmid), given as prime & boost doses, four weeks apart, in healthy volunteers, divided into age groups of 18-50 versus > 50 years old. IL-12p70 plasmid DNA electroporation (tavokinogene telseplasmid) has been extensively studied in over 209 subjects across 11 trials including later stage human cancer trials with more than 1000 administrations. The IGEA CLINIPORATOR® system is approved for clinical use in Europe but remains investigational in this study.

Conclusions: Early preclinical data shows that EP of CORVax can induce IgG responses to CoV2 Spike and the receptor binding domain (RBD) as well as apparent viral neutralizing activity. The addition of IL-12, at least transiently, increased sVNT titer. We plan to investigate alternate vaccine boosting strategies while extending these studies into aged animals and initiate a clinical trial in the near future.

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[1]*Ruby CE, Weinberg AD. OX40-Enhanced Tumor Rejection and Effector T Cell Differentiation Decreases with Age. J Immunol 2009;182:1481–9. https://doi.org/10.4049/jimmunol.182.3.1481.*