



CASE REPORT

Ataluren/ivacaftor combination therapy: Two N-of-1 trials in cystic fibrosis patients with nonsense mutations

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Abstract

Premature termination codons (PTCs) in cystic fibrosis transmembrane conductance regulator (CFTR) produce nonfunctional protein. No approved therapies exist for PTC mutations, including W1282X. We hypothesized that ivacaftor, combined with readthrough therapy, may benefit W1282X patients. Two N-of-1 clinical trials were conducted with ataluren and ivacaftor in various combinations. No meaningful clinical benefit was observed in either patient with ivacaftor alone or ataluren/ivacaftor combination. However, isolated improvements of uncertain significance were noted by a nasal potential difference (NPD) and FEV₁% with ivacaftor in Patient-1 and with ataluren/ivacaftor combination by NPD and body mass index in Patient-2. Drug regimen composed of readthrough agents and potentiators warrant further development for W1282X and other CFTR nonsense mutations.

KEYWORDS

ataluren, G542X, ivacaftor, N-of-1 trial design, personalized medicine, PTC mutations, translational readthrough, W1282X

Approximately 11% of cystic fibrosis (CF) patients have premature termination codons (PTC), causing truncated cystic fibrosis transmembrane conductance regulator (CFTR) protein with minimal to no function.¹ One emerging pharmacologic strategy is using readthrough agents to promote PTC suppression. Aminoglycosides induce PTC readthrough; however, toxicity limits the feasibility of long-term administration. The investigational readthrough agent ataluren did not show sufficient clinical efficacy.² Improvements in CFTR-PTC mutations were achieved using CFTR modulators in vitro and this approach is specifically applicable to W1282X CFTR, since it exhibits partial function even in the truncated state.³ The combination of the

CFTR potentiator ivacaftor to readthrough agents has shown to enhance W1282X CFTR activity compared with the readthrough agent alone.⁴ On the basis of this, we hypothesized that ivacaftor may increase efficacy of a readthrough agent, ataluren by activating the W1282X readthrough product. Here we present two open-label, single center, N-of-1 clinical studies in patients with nonsense mutations to evaluate the efficacy of ataluren/ivacaftor combination therapy. Of note, the two N-of-1 studies have different protocols due to the specific medications that the respective patients were already taking and the limited availability of those agents beyond our control posed to the study design.

Patient-1 is a 31-year-old Caucasian female (W1282X/G542X) with mild CF-lung disease. The N-of-1 trial took place over eight scheduled visits from March 2017 through July 2017 (Figure 1A). Patient-1 was already taking ataluren, so the purpose of their trial was to assess if ataluren/ivacaftor dual therapy or ivacaftor monotherapy provided clinical benefit than ataluren alone. From visit-1 through visit-6, Patient-1 remained on ataluren (10, 10, and 20 mg/kg three times daily) and received two 2-week on/off cycles of ivacaftor (150 mg twice daily), followed by ivacaftor monotherapy for 4 weeks; the off ataluren period was necessitated by discontinuation of the trial offering ataluren, which was terminated at 10 weeks. Clinical efficacy of ataluren monotherapy was assessed at visits 1, 2, 4, and 7 ($N=4$; Figure 1A, black arrow), ataluren/ivacaftor combination therapy was measured at visits 3, 5 ($N=2$, red arrow) and ivacaftor monotherapy was evaluated at visit 8 ($N=1$, blue arrow). No changes in sweat chloride (SC) levels were noted either during ataluren (93.9 mmol/L) and ivacaftor (98 mmol/L) monotherapy or ataluren/ivacaftor combination therapy (98.5 mmol/L) (Figure 1B,C). By contrast, CFTR-mediated chloride activity within airways was monitored using NPD measurements⁴ and revealed a modest benefit with ivacaftor. NPD (ΔCl^- -free plus isoproterenol) was -3.7 mV for ataluren monotherapy and -9.4 mV for ivacaftor monotherapy (Figure 1D-F). Mean FEV_1 was 90.7% of predicted for ataluren monotherapy, 95% for ivacaftor monotherapy, and 95% for ataluren/ivacaftor combination therapy (Figure 1G,H). Of interest, Patient-1 had two pulmonary exacerbations during the N-of-1 trial, both occurred following cessation of ivacaftor. Body mass index (BMI) was not affected; 23.4 kg/m^2 during ataluren monotherapy, 23.4 kg/m^2 after ataluren/ivacaftor combination therapy, and 23.6 kg/m^2 for ivacaftor monotherapy (Figure 1I,J). Respiratory symptoms by CF Questionnaire-Revised improved during ivacaftor treatment periods (Figure 1K). Overall, we did not observe a consistent effect that suggested clinically meaningful improvements given the inconsistency between $\text{FEV}_1\%$ and CFTR function by NPD as compared with sweat chloride observed with ivacaftor.

Patient-2 is a 32-year-old non-Hispanic, Caucasian female CF patient (W1282X/W1282X) with severe CF lung disease and in whom ivacaftor monotherapy was found to be potentially effective in a prior study.⁵ Since Patient-2 was already taking ivacaftor, the purpose of their trial was to assess if ataluren/ivacaftor dual therapy provided clinical benefit than ivacaftor alone. The N-of-1 occurred over seven scheduled visits from May 2016 through May 2017 (Figure 2A). For the first month, Patient-2 received ivacaftor monotherapy (150 mg twice daily), and for the remainder of the trial, she took ataluren/ivacaftor combination therapy (10,10,20 mg/kg three times daily). Clinical efficacy of ivacaftor monotherapy was assessed at visits 1 and 2 ($N=2$; Figure 2A, blue arrow); ataluren/ivacaftor combination therapy was measured at visits 3 to 7 ($N=3-5$; Figure 1A, red arrow). During ivacaftor treatment, mean SC was 102.3 mmol/L. Following ataluren/ivacaftor combination therapy, there was no improvement in SC (105.8 mmol/L) (Figure 2B,C) compared with ivacaftor monotherapy. NPD measurements demonstrated that ataluren/ivacaftor combination therapy improved total chloride conductance than ivacaftor alone (PD -6.7 mV for ivacaftor and -22.5 mV for ataluren/ivacaftor

combination; Figure 2D-F). Mean FEV_1 was 35.0% predicted for ivacaftor monotherapy and 35.2% for ataluren/ivacaftor combination therapy (Figure 2G,H). Ataluren/ivacaftor combination therapy showed marginal effect on BMI (19.8 kg/m^2) compared with ivacaftor monotherapy (19.3 kg/m^2 ; Figure 2I,J). CFQ-R score was greater with ivacaftor monotherapy (88.9) compared with ataluren/ivacaftor combination therapy (78.9; Figure 2K). Overall, ataluren/ivacaftor combination therapy altered BMI and CFTR function by NPD in the patient homozygous for W1282X CFTR, but did not improve sweat chloride, FEV_1 or respiratory symptoms. We interpret this as unlikely to be clinically meaningful, noting the possibility that as outcome measures become more sensitive, or that longer time domains, small effects can emerge.

Messenger RNA (mRNA) isolated from human nasal epithelial (HNE) cells from each CF patient subjected to real-time polymerase chain reaction (RT-PCR) showed reduced CFTR mRNA expression compared to non-CF donors (3.6% and 10% of non-CF in Patient-1 and -2, respectively; data not shown) suggesting low levels of CFTR expression in both patients, likely due to nonsense-mediated decay. This may responsible for the lack of meaningful and consistent effect.

Using two individualized N-of-1 trials impacted by limitation in drug availability, we demonstrate preliminary evidence that ivacaftor provides potential for augmentation of translational readthrough, noting findings were small and mixed. One possibility is that ivacaftor is needed to observe clinical benefit of ataluren or other weakly active readthrough agents. Patient-1 had isolated improvements in FEV_1 , NPD, and respiratory symptoms (CF questionnaire) with ataluren/ivacaftor combination therapy over ivacaftor monotherapy and the addition of ataluren to ivacaftor regimen slightly improved BMI and NPD in Patient-2. While we did not conduct inferential testing since measurements were conducted within single patients, and thus not independent, changes were sufficient in magnitude to raise the possibility that effects were related to drug. In contrast, SC levels did not substantially change in either treatment. Further research is needed to understand this discrepancy, but could be related to the sensitivity of sweat glands to nonsense mutation repair or the magnitude of CFTR benefit. In total, the N-of-1 trials presented here do not provide convincing evidence that ataluren/ivacaftor combination therapy is an efficacious therapy for patients with PTC mutations due to a lack of consistent effects in these two patients, partially driven by reduced mRNA levels; thus drug development for patients with nonsense mutations must continue.

W1282X is a mutation that exhibits function even in truncated state, making it potentially amenable to potentiator treatment. While results recapitulated beneficial effects of ivacaftor for W1282X,⁵ results with ataluren were mixed, potentially due to low activity of ataluren and diminished mRNA levels, a known negative covariate for readthrough efficacy.⁶ Novel corrector-potentiator combination regimens could benefit patients with the W1282X-CFTR mutation. Note that N-of-1 trial designs have limitations with potential carryover effects of short treatment blocks impacting measurements of treatment efficacy. Furthermore, appropriate statistical models for such trials remain

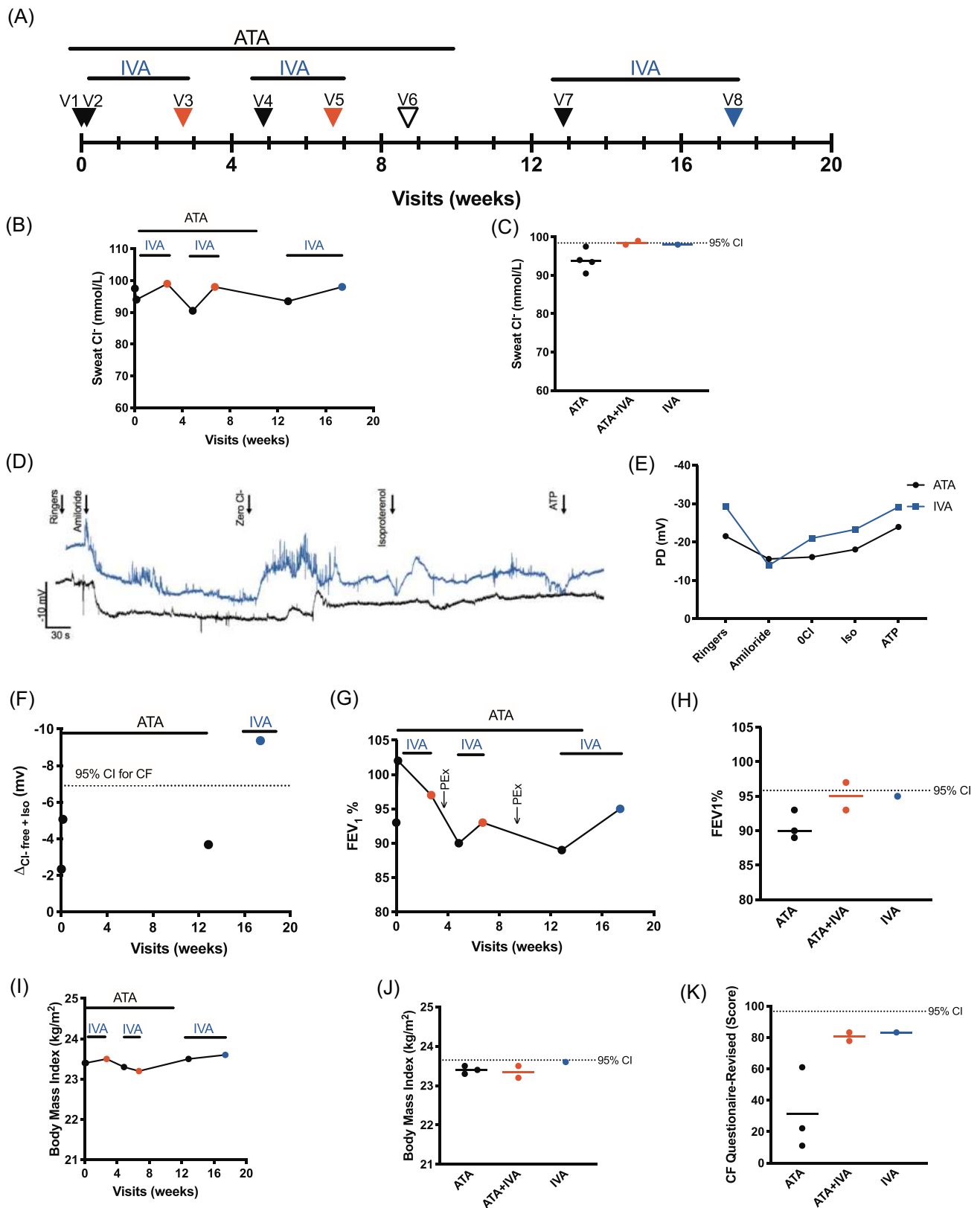


FIGURE 1 Effect of ataluren/ivacaftor combination therapy in cystic fibrosis (CF) patient with W1282X/G542X mutation (Patient-1).

Combination therapy is denoted with red, ataluren (ATA) monotherapy denoted by black, and ivacaftor (IVA) monotherapy denoted by blue. A, Timeline of visits and treatment with ATA and IVA for Patient-1. B, Sweat chloride longitudinally. C, Sweat chloride by therapy. D, Representative tracings of nasal potential difference (NPD) for ATA and IVA monotherapies. E, Averaged NPD values for ATA and IVA. F, Mean chloride conductance PD (Δ Cl⁻-free plus isoproterenol) longitudinally. G, FEV₁ longitudinally. Note the pulmonary exacerbations (PEX) following cessation of combination therapy. H, FEV₁ by therapy. I, Body mass index longitudinally. J, Body mass index by therapy. K, CF Questionnaire-Revised Score by therapy [Color figure can be viewed at wileyonlinelibrary.com]

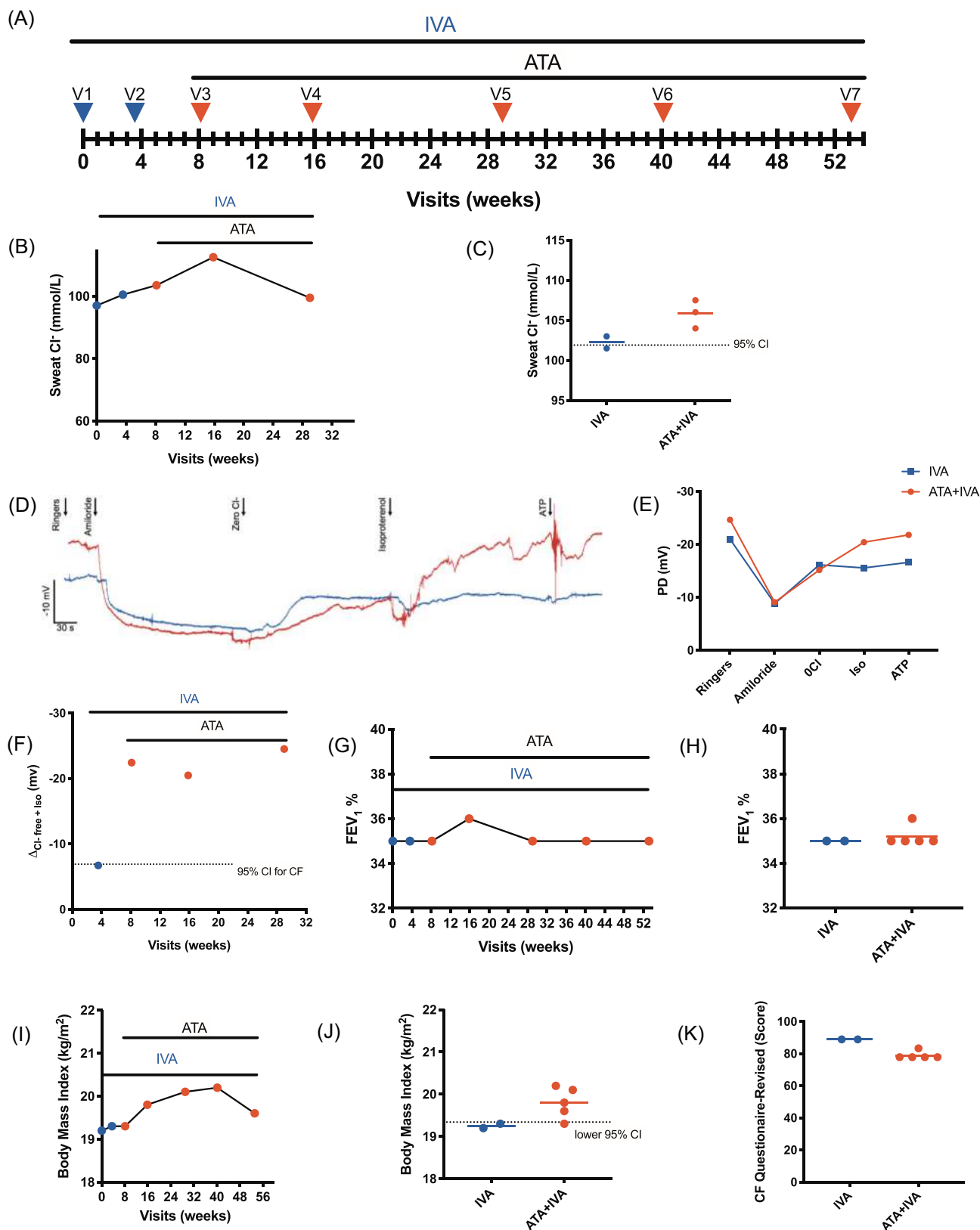


FIGURE 2 Effect of ataluren/ivacaftor combination therapy in cystic fibrosis (CF) patient with W1282X/W1282X mutation (Patient-2). Combination therapy is denoted with red and ivacaftor (IVA) monotherapy denoted by blue. A, Timeline of visits and treatment with ATA and IVA for Patient-1. B, Sweat chloride longitudinally. C, Sweat chloride by therapy. D, Representative tracings of nasal potential difference (NPD) for IVA monotherapy and ATA/IVA combination therapy. E, Averaged NPD values for ATA and IVA. F, Mean chloride conductance PD (Δ Cl-free plus isoproterenol) longitudinally. G, FEV₁% longitudinally. H, FEV₁% by therapy. I, Body mass index longitudinally. J, Body mass index by therapy. K, CF Questionnaire-Revised Score by therapy [Color figure can be viewed at wileyonlinelibrary.com]

controversial since multiple complementary outcome measures increase type-1 error, especially when findings are not consistent across all outcomes, as in this case. Future N-of-1 trials may require blinding to improve validity. Nevertheless, N-of-1 studies could accelerate the assessment of rationally selected multi-drug therapy regimens for rare mutation groups.

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CONFLICT OF INTERESTS

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AUTHOR CONTRIBUTIONS

SMR conceived of the experiments; JEPL, VM, HYH, JS, NP, and SMR conducted the research; JEPL, VM, and SMR analyzed the data; JEPL,

VM, and SMR wrote the manuscript; VM, HYH, and SMR supervised the project. All authors had an opportunity to edit the manuscript and approved of its submission.

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