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SARS-CoV-2 mRNA-based vaccines in the Aicardi Goutières Syndrome

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Aicardi Goutières Syndrome (AGS) is an autoinflammatory disorder resulting in sustained interferon activation through defects in nucleic acid modification and sensing pathways. Thus, mRNA-based vaccination used against SARS-CoV-2, raise disease-specific safety concerns. To assess interferon signaling, we tested mRNA SARS-CoV-2 vaccines in AGS whole blood samples. Interferon activation is measured through quantitation of interferon signaling gene (ISG) expression and is increased in AGS patients. There was no increase in ISG scores from baseline following treatment with the nucleoside modified mRNA formulation compared to an increase with unmodified.

A patient-family survey reported that the vaccines were well tolerated. These findings suggest that COVID vaccination using nucleoside-modified forms of mRNA vaccines are unlikely to directly stimulate ISG expression in response to mRNA internalization in AGS tissues. With continued community spread, we recommend vaccination using nucleoside-modified mRNA vaccines in this rare disease group in individuals for whom vaccines were previously well tolerated.

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1. Introduction

Vaccination represents a safe and effective approach to prevent and control serious, and sometimes deadly, infectious diseases [1]. The ongoing coronavirus disease 2019 (COVID-19) pandemic has required rapid development of vaccines to prevent disease, reduce mortality, and curb the ongoing crisis. Approved vaccines have been shown to be largely safe and well tolerated with demonstrated efficacy in both randomized clinical trials and clinical practice [2]. More recently, as mRNA vaccines have been approved for the pediatric population, individuals with rare disease remain a marginalized group in vaccination efforts due to limited safety data.

Rare genetic disorders affecting the production of interferon (IFN) are known as type I interferonopathies [3]. The type I interferonopathies include Aicardi Goutières Syndrome (AGS), a neurologic disease with early infantile-onset and clinical features caused by systemic inflammation [4]. The systemic autoinflammatory state in these patients is believed to result from poor discrimination between endogenous and exogenous nucleic acids, leading to constitutive upregulation of the IFN pathway [5]. Upregulation of IFN pathways can be assessed by measuring the levels of interferon signaling gene (ISGs) expression from whole blood [6,7]. ISG signaling in blood samples has been used in AGS clinical trials to understand therapeutic target response [8,9].

In viral infections, pathogen-derived nucleic acid (NAS) recognition by cell membrane, endosomal, and intracellular sensors leads to the production of type I IFN and subsequent ISG expression. IFN signaling upon SARS-CoV-2 infection is critical for disease control [10]. IFN production results from endosomal recognition by toll-like receptor

Abbreviations: COVID-19, coronavirus disease 2019; IFN, interferon; ISG, interferon stimulatory gene; AGS, Aicardi Goutières Syndrome; LNP, lipid nanoparticle.

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(TLR)-3 and TLR-7, and components of the inflammasome including melanoma differentiation-associated protein 5 (MDA-5), retinoic acid-inducible gene I (RIG-I), Nucleotide Binding Oligomerization Domain Containing 2 (NOD2), and protein kinase R (PKR) among others coming together upon binding of single- or double-stranded RNA fragments [11]. IFN inhibits viral replication while assisting in stimulating the adaptive immune response.

mRNA-based vaccinations are a development in the clinical arsenal against SARS-CoV-2 infection [12,13] and other infectious diseases [14]. Translation of mRNA vaccines and other mRNA-based therapeutic modalities in clinical trials was enabled by the discovery that modified nucleosides, and proper purification, improved translation and reduced the innate immune activating potential of in vitro transcribed mRNA, with the parallel development of efficient non-viral delivery systems [15–17]. mRNA delivery has been enhanced by the addition of lipid nanoparticles (LNPs) as a capsule for the modified mRNA [15]. BNT162b (Comirnaty, Pfizer–BioNTech) [18] and mRNA-1273 (Spiekve, Moderna) hereafter referred to by their commercial names [19], are nucleoside modified mRNA vaccines encapsulated into LNPs (mRNA-LNPs). mRNA-based vaccines are thought to provide a safer and more effective form of immunity compared to other vaccine types [20]. Studies from the development of nucleoside-modified mRNA demonstrated a lack of induction of proinflammatory cytokines and type 1 IFNs in dendritic cells treated in vitro or after in vivo treatment [21,22].

AGS is a disorder of disrupted nucleic acid metabolism that may be treated with immunosuppressive medications [8,23], making unvaccinated affected individuals potentially at risk for severe SARS-CoV-2 infection. Although chronically raised IFN might be protective against severe infection [24], many patients also suffer from severe neurologic compromise, which may also increase risks for severe disease associated with infection. It is currently unclear whether mRNA-based vaccination may have the potential for detrimental innate immune activation and high levels of inflammation or whether the use of mRNA-LNP based platforms may mitigate this upregulation. To evaluate the immunostimulatory potential of these vaccines in AGS individuals, we formulated nucleoside modified mRNA into LNPs and assessed their impact on peripheral blood monocyte cells in whole blood collected from individuals affected with AGS versus controls. We measure ISGs, a measure of IFN activation used in clinical trials, to assess the IFN response in vitro after addition of mRNA-LNPs.

2. Materials and methods

2.1. Cohort identification for sample collection

Disease and control samples were collected under a leukodystrophy associated biorepository protocol, the Myelin Disorders Biorepository and Natural History Study, approved at Children’s Hospital of Philadelphia (IRB 14-011236). Individuals were identified as having either AGS (Disease Cohort) based on molecular identification of one of the AGS-related genes (TREX1 [n = 1], RNASEH2B [n = 3], SAMHD1 [n = 3], ADAR1 [n = 3], or IFIH1 [n = 1]; no patients variants in RNASEH2A, RNASEH2C, RN7U-1 and LSM11 were identified) or as having a non-AGS related leukodystrophy or their unaffected parents (Control Cohort) (Table S1). Absence or presence of treatment with the Janus Kinase inhibitor, baricitinib, was noted. In the Disease and Control Cohorts, samples were drawn during a venipuncture immediately into EDTA containing tubes and aliquoted into 1 mL aliquots. Whole, unprocessed blood was incubated with mRNA-LNP vaccines, and associated controls as detailed below.

2.2. Cohort identification for patient reported outcomes

In a distinct cohort from the above, the Aicardi Goutières Syndrome Americas Association (AGSAA) queried patients enrolled in an IRB approved patient registry (Genetic Alliance) with a standard series of questions around vaccine and COVID-19 vaccine experience. Patients or their care givers entered responses to the questionnaire. Individuals were identified as having molecular identification of one of the AGS-related genes (TREX1 [n = 1], RNASEH2B [n = 7], RNASEH2C [n = 1], SAMHD1 [n = 7], ADAR1 [n = 2], IFIH1 [n = 3], RN7U-1 [n = 2]); no patients variants in RNASEH2A and LSM11 were identified). Genotype data was unknown or unavailable for 9 respondents. The severity of the infection was not included on the questionnaires.

2.3. Production of the mRNA encoding the di-proline stabilized SARS-CoV-2 spike and the firefly luciferase protein

Codon optimized SARS-CoV-2 di-proline modified spike sequence (S2P), and firefly Luciferase (fLuc) sequence were cloned into an mRNA production plasmid (optimized 3’ and 5’ UTR and containing a 101 polyA tail), in vitro transcribed in the presence of UTP (unmodified) or in the presence of N1-methylpsuedouridine modified nucleoside (N1-mψ, modified), co-transcriptionally capped using the CleanCap™ technology (TriLink) and cellulose purified to remove dsRNA [25]. Purified mRNA was ethanol precipitated, washed, re-suspended in nuclease-free water, and subjected to quality control (electrophoresis, dot blot, endotoxin content and transfection into human DCs). mRNA sequence used in this study is identical to the one used in the Pfizer/BioNTech vaccines except for the 3’ and 5’ UTRs. mRNA was stored at −20 °C until use.

2.4. Production of empty LNPs and mRNA-LNP vaccines

mRNA loaded LNPs, or vaccines, were formulated using a total lipid concentration of 40 mM as previously described [14]. The ethanolic lipid mixture comprising ionic cationic lipid, phosphatidylcholine, cholesterol and polyethylene glycol-lipid was rapidly mixed with an aqueous solution containing cellulose-purified UTP and N1-mψ in vitro transcribed mRNAs to generate the unmodified mRNA-LNP vaccine and the nucleoside modified mRNA-LNP resembling the clinical vaccine Comirnaty, respectively [26]. The LNP formulation used in this study is proprietary to Acuitas Therapeutics; the proprietary lipid and LNP composition are described in US patent US10,221,127. Empty particles were prepared as described above without the addition of mRNA in the aqueous phase. RNA-loaded and empty particles were characterized and subsequently stored at −80 °C at an RNA concentration of 1 μg/μL (in the case of loaded particles) and total lipid concentration of 30 μg/μL (both loaded and empty particles). The mean hydrodynamic diameter of mRNA-LNPs was ~80 nm with a polydispersity index of 0.02–0.06 and an encapsulation efficiency of ~95%. Two or three batches from each mRNA-LNP formulations were used in these studies.

2.5. In vitro mRNA expression to demonstrate the bioactivity of mRNA-LNP vaccines

ExpI293F cells were transiently transfected with 3 μg mRNA encoding the nucleoside modified S2P using Lipofectamine messenger Max™ or mRNA-LNP. Transfected cells were harvested 48 h post-transfection, washed once, resuspended in FACS buffer (1 x PBS, 2% FBS, 0.05% sodium azide) at 1 x 10^6 cell/mL, and stained with 10 μg/mL D001 antibody (Sino Biological) in U-bottom 96-well plates for 30 min at 4 °C. Cells were washed once and incubated with a PE labelled goat anti-human IgG secondary antibody (Thermo Fisher Scientific 12–4998-92) at a final concentration of 2.5 μg/mL of 30 min at 4 °C. Dead cells were stained with amine reactive Live/Dead fixable aqua dead cell stain kit for 5 min, washed, and resuspended in 200 μL 1% paraformaldehyde containing FACS buffer. Flow cytometric data was acquired using an LSRII and data analyzed using Flowjo 10.2.
2.6. Generation of human DC from blood detection of IFN-α, and spike protein following transfection with mRNA-LNPs

Blood derived monocytes were isolated at the University of Pennsylvania Human Immunology Core (HIC) and differentiated into monocyte derived dendritic cells (MoDCs) using 50 ng/ml human IL-4 and 100 ng/ml GM-CSF in R10 media. Monocytes were differentiated for 3 days, and MoDCs counted, and seeded at 1 million cells/well. mRNA-LNP were added to each well at 0.3 μg/well, and the supernatant collected 24 h post transfection to measure IFN-α using the Human IFN-α Pan specific ELISA kit (MabTech); MoDCs were lysed using RIPA buffer supplemented with protease inhibitors, clarified centrifugation at 10,000g for 1 min, and total protein content was measured using the microBCA kit (Pierce). 10 μg total protein was denatured at 65 °C in the presence of 1 X Laemmli buffer and loaded on a 14% SDS-PAGE gel. Electrophoresis was performed for 1 h at 100 V, and the gel transferred to a Hybond PVDF membrane using the i-blot system (Thermo Fisher scientific). Membranes were blocked in 5% non-fat milk for 1 h, washed twice with TBS-T, incubated with rabbit anti-SARS-2-S at 1:2000 (Sino Biological, 80,519-T62) in 1% non-fat dry milk for 1 h, washed twice in TBS-T and incubated in the presence of a goat HRP conjugated anti-rabbit secondary IgG antibody (1:10000; Abcam 6721). Membranes were revealed using the Amersham ECL prime reagent (GE healthcare), and imaged on an Amersham A6000 system. Membranes were stripped and re-blotted in the presence of an anti-GAPDH antibody as a loading control.

2.7. Internalization of mRNA-LNPs encoding the firefly luciferase protein

Whole blood samples containing 10% RPMI-1640 medium were incubated at 37 °C for 6 h with nucleoside modified Luc mRNA-LNP. Blood samples were collected and PBMCs were purified using HISTOPAQUE-1077 (Sigma) followed by PBS wash. PBMCs were lysed in 50 μL of RIPA buffer and assessed using the Promega One Glo Luciferase assay.

2.8. Interferon signaling gene (ISG) expression from whole blood samples

One mL of whole blood collected into EDTA blood tube (BD) was mixed with 10% RPMI-1640 and plated 1.1 mL per well in a 96-well plate. mRNA-LNP vaccines, empty LPNs (eLPN), and naked mRNA was added to each blood containing well, and the plate was incubated for 6 h in a 5% CO2 incubator at 37 °C. At the end of the incubation, samples were mixed with 3 mL of PAXgene RNA stabilization reagents (PreAnalytix) to stabilize RNA and further incubated at room temperature for up to 24 h to allow complete RBC lysis. RNA stabilized blood samples were mixed with 3 mL of PAXgene RNA stabilization reagents (PreAnalytix) to stabilize RNA and further incubated at room temperature for up to 24 h to allow complete RBC lysis. RNA stabilized blood samples were mixed with 3 mL of PAXgene RNA stabilization reagents (PreAnalytix) to stabilize RNA and further incubated at room temperature for up to 24 h to allow complete RBC lysis.

To calculate the ISG scores, the normalized RNA counts were used calculate z-scores in Stata 17 (Collage Station, Texas) as previously published [7]. The median of the z- scores of six ISGs based on the gene list provided by Rice et al. (IFI27 + IFI44 + IFI44L + ISG15 + RSAD2 + SIGLEC1) [4-6] was then used to generate an ISG score, as previously used in AGS related studies [8,27].

2.9. Statistical analysis

We treated the ISG scores for each vaccine type as a cluster of measurements on each subject. Our goal was to evaluate the effect of treatment on ISG scores and to compare the effect between AGS and non-AGS subjects. We first evaluated the impact of treatment on change from no treatment in all subjects (Table S2) by fitting quasi-least squares (QLS) regression models [28,29] with the following variables: an indicator variable for each of the 4 vaccine types (with untreated as the reference category); an indicator variable for control (versus AGS); and baseline ISG (untreated). To account for the correlation among the multiple ISG scores per subject, we fit the exchangeable correlation structure with the robust sandwich covariance estimator of covariance of the regression parameter. After fitting the model, we used the lincom procedure in Stata 17 to estimate the difference in ISG scores (with 95% confidence intervals [CI] and p-value for test that the difference is zero). We did not fit the unstructured correlation matrix for this analysis because application of the QLS matrix for QLS requires an equal number of measurements per subject; 13 subjects had 5 vaccine types, while 9 subjects had 7 vaccine types. Generalized estimating equations (GEE) allows for an unequal number of measurements per subject for the unstructured matrix, but GEE failed to converge for this structure.

We next evaluated the impact of treatment on change from no treatment (Table S3) while also allowing the change from no treatment to vary between AGS and non-AGS samples. We accomplished this by fitting QLS regression models on change from no treatment with the following variables: an indicator variable for each of the 4 vaccine types (with untreated as the reference category); an indicator variable for control (versus AGS); baseline ISG (no treatment); and a control by treatment type interaction term. Naked unmodified and naked modified could not be included in this analysis because there was only one AGS sample for these vaccine types. Due to the limited sample size, we included the interaction terms one at a time, in separate models for each treatment type. To account for the correlation among the multiple ISG scores per subject, we fit the exchangeable and unstructured correlation matrices. We evaluated the fit of the exchangeable and robust structures using several criteria and selected the structure with the best fit. We then fit the model for the best fitting correlation structure. After fitting the model, we used the lincom procedure in Stata 17 to estimate the difference in ISG scores (with 95% confidence intervals [CI] and p-value for test that the difference is zero) between treatment types.

We next compared normalized counts for each gene between vaccine types and eLPN in control and AGS patients (Fig. S1) by fitting QLS models for each gene with an exchangeable correlation structure and robust sandwich covariance matrix. The models included indicator variables for control (versus AGS), untreated baseline, and each of the 4 vaccine types (with eLPN was the reference category).
3. Results

3.1. Validation of internalization, immunogenicity and expression of SARS-CoV-2 di-proline modified spike sequence (S2P) in human dendritic cells in culture and Expi293F cells

We validated the internalization, immunogenicity and expression of SARS-CoV-2 di-proline modified spike sequence (S2P) in two cell lines. mRNA-LNP encoding the di-proline modified spike protein were transfected into human blood derived dendritic cells and commercially available high density human embryonic kidney cell line (Expi293F cells) at a dose of 0.3 μg/million cells. Western blot analysis demonstrated that the spike protein was efficiently translated 24 h post transfection into human blood derived DCs with an expected band around 165 kDa (Fig. 1A). The specificity of the spike protein was demonstrated by the lack of expression in the empty LNP (eLNP) control. Analysis of the supernatant demonstrated that nucleoside modified mRNA-LNP is not immunogenic with no detectable levels of IFN-α. In contrast, and as expected, uridine containing unmodified mRNA-LNPs induced high levels of IFN-α (data not shown). Flow cytometry analysis of cells transfected with the mRNA-LNP vaccine showed that expression of the di-proline modified spike protein could be detected at high levels on the cell surface (Fig. 1B and C). Detection of the protein using western blot or flow cytometry in whole blood was difficult to achieve. To demonstrate that these LNPs are able to enter cells and express their cargo following their addition into blood, we used a luciferase reporter mRNA for improved sensitivity. Blood form AGS patients and control subjects was transfected with either Luc mRNA-LNP or eLNP for 6 h, and PBMCs were purified, lysed and subjected to the luciferase assay. Luciferase expression was detected at least 1000 fold higher than control (or background) (Fig. 1D) indicating that mRNA-LNPs are able to transfect target cells in blood.

3.2. Interferon Stimulatory Gene (ISG) scores in AGS and control subjects with and without SARS-CoV-2 mRNA vaccine treatment

ISG scores are known to be increased when the IFN signaling pathway is stimulated, as in AGS. We used ISG scores to assess the baseline and the IFN response in vitro after addition of mRNA-LNPs. Most AGS patients (8/11) demonstrate an elevated ISG score (>1.96) at baseline compared to controls (n = 11, average ISG scores: -0.05 ± 0.48) regardless of treatment with baricitinib or not (Table S1). After treatment with the unmodified mRNA-LNP vaccine (5 and 30 μg/mL), an increase was seen in ISG scores in both control and AGS patient cells, and combined average (p < 0.0005, Fig. 2, Table S2 and S3). However, after the nucleoside modified mRNA-LNP vaccine is applied (30 μg/mL) there is no increase in ISG scores in control (Fig. 2, Table S3) and a significant decrease in ISG scores in AGS cells (p < 0.0005, Fig. 2, Table S3). Empty LNP (eLNP) alone did not change ISG expression (Fig. 2, Table S2 and S3). It should be noted that unmodified mRNA-LNP increases specific IFN signaling genes (CXCL10, IFIT1, IFIT2, ISG15, MX, RASS2) in a dose dependent manner (Fig. S1) but no such effect is seen using modified mRNA-LNP. The nucleoside modified mRNA-LNP vaccine does not increase these signature genes. We found statistically significant changes in expression of IFIT2 (p = 0.021), ISG15 (p < 0.005), OASL (p < 0.005), and SOCS1 (p = 0.05) when comparing treatment with nucleoside modified mRNA-LNP and eLNP (Fig. S1). We did not find any statistically significant changes in expression of other RNA sensing genes (MDA5, IFIH1, OAS1, OAS2, OAS3, PKR/EIF2AK2, RIG-I/DDX58) between baseline and treatment across any condition (Fig. S1). Of note, AGS versus non-AGS individuals were not significantly different across all vaccine types with respect to ISG, except for the nucleoside modified mRNA-LNP vaccine, in which ISG expression was significantly decreased (p = 0.0024, Table S3).

3.3. Patient reported experience with COVID-19 infection and vaccination

A total of 35 individuals reported experience with COVID-19 infection and vaccination (Fig. 3). 12 individuals experienced a patient reported COVID-19 infection; of these individuals 3 indicated that subsequent to infection they had long term impact to their health. Of the 12 individuals who experienced a known COVID-19 infection, 8 were not vaccinated, 2 were vaccinated and 2 had an unknown vaccination status. 16 individuals have not experienced a known COVID-19 infection. Of these 16 individuals, 8 individuals received a COVID-19 vaccination course. Among all 12 vaccinated individuals, mRNA vaccines were used in 10 individuals; 2 individuals who were 18 or older received Johnson & Johnson vaccination. Of the vaccinated individuals, 9 individuals had no side effects to vaccination, and 3 individuals had expected side effects (fever, muscle pains, headache and fatigue). No individual had unexpected side effects to vaccination.
4. Discussion

AGS and related heritable interferonopathies have unregulated IFN production, which can worsen in the context of infection or other immune triggers. [9] In addition, several AGS related genes are involved in the detection (sensing) and the regulation of endogenous nucleic acids [30–34]. This has led to concern that nucleic acid based vaccines such as the FDA approved Comirnaty [18] and Spikevax [19] mRNA-based vaccines could result in further dysregulation of IFN signaling and has led to hesitancy around vaccine adoption in this rare disease population.

Our experiments do not suggest an increase in IFN signaling, as measured by the calculation of an ISG score, after internalization of the clinical S2P in an LNP (Table S1, Fig. 1). Although unmodified mRNA vaccines increase ISGs in both control and AGS samples, the internalization of the nucleoside modified mRNA vaccine representing the clinical vaccine significantly decreases ISGs relative to untreated samples in AGS samples (Table S3, \( p < 0.0005 \)). It should be noted that the SARS-CoV-2 spike protein itself has been shown in vitro to result in decreased production of type I IFN, [35] but that SARS-CoV-2 overall has a complex interaction with IFNs, likely associated with tissue specificity and disease severity [11]. It is promising that the expression of RNA sensing genes is upregulated only in the presence of unmodified mRNA vaccine, as expected. However, it remains unclear if the decreased production in vitro of ISGs by cultured cells after treatment with the nucleoside modified mRNA vaccine may not represent the complexity of vaccine response at the organism level in AGS affected individuals.

The elevated ISGs seen on in vitro treatment of AGS patient cells with unmodified mRNA vaccines is of particular concern. The HERALD phase

Fig. 2. Measurement of interferon signaling gene expression scores after incubation with vaccine formulations. Interferon signaling gene (ISG) scores were assessed for all patients and controls following in vitro vaccine treatment. Most AGS patients (8/11) demonstrate an elevated ISG score (>1.96) at baseline compared to controls (n = 11) regardless of treatment with baricitinib or not (Table S1). The empty LNP (eLNP) did not affect ISG scores in control and AGS patient combined Average (Table S2, \( p = 0.142 \)) while they were elevated upon treatment with both doses of the unmodified mRNA-LNP vaccine (5 and 30 μg/mL, \( p < 0.0005 \) for both doses, Table S2) in both control and AGS patient cells. There was no increase in ISG scores following treatment with the nucleoside modified mRNA-LNP in control, but a decrease in AGS cells (Table S3, \( p < 0.0005 \)).

Fig. 3. COVID Infection by Vaccination Status in Patient Reported Survey. Surveys were conducted by the AGSAA of their membership. 35 members reported vaccination status and 30 members reported infection status.
2b/3 clinical trial using a chemically unmodified mRNA vaccine, named CVnCoV, demonstrated only 53% efficacy against SARS-CoV-2 compared to the 95% and 94% efficacy of the Comirnaty [18] and Spikevax [19] vaccines, respectively [36]. Importantly, in this trial adverse events was more frequently reported in CVnCoV recipient population than in a placebo group in healthy participants despite a reduced dose (12 μg) used to immunize [36]. We observed elevated ISGs and elevation in RNA sensing genes upon administration of an unmodified mRNA-LNP vaccine in both AGS and control patient cells, demonstrating the immunogenicity of this formulation. We suggest that the use of mRNA-based vaccinations overall in AGS patients should be limited to nucleoside modified based formulations and the development of other unmodified mRNA-LNP vaccine formulations should take this into account.

It is important to note that the safety and effectiveness of COVID-19 vaccines in those with AGS has not been formally studied. Patient-reported surveys conducted in a small number of subjects suggest that vaccinations were well tolerated. It is unlikely that a formal clinical trial of vaccination will occur in a rare disease population, and it is not possible to control for ascertainment bias for the patient-reported survey. Because of limited information, our guidance is based on this limited in vitro evidence and patient reported data. Although a systemic autoinflammatory response to the spike protein is likely and may increase ISG signaling, it is likely that this will not be as great as the autoimmune response seen in the context of an actual infection. Thus, in the context of continued COVID-19 infections in the community, we are cautiously recommending that AGS affected individuals consider being vaccinated with one of the United States Food and Drug Administration approved nucleoside modified based formulations of mRNA vaccines, as appropriate for their ages in the general population, unless individuals have documented vaccine related adverse events.

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**Author contributions**

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**Data availability**

Data will be made available on request.

**Declaration of Competing Interest**

AV receives grant and in-kind support for translational research without personal compensation from Eli Lilly, Biogen, Takeda, Passage Bio, Homology, Illumina, Orchard therapeutics, and Ionis. In accordance with the University of Pennsylvania policies and procedures and our ethical obligations as researchers, we report that DW is named on patents that describe the use of nucleoside-modified mRNA as a platform to deliver therapeutic proteins and vaccines. DW and MGA are also named on patents describing the use of lipids nanoparticles, and lipid compositions for nucleic acid delivery and vaccination. We have disclosed those interests fully to the University of Pennsylvania, and we have in place an approved plan for managing any potential conflicts arising from licensing of our patents.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2022.10.001.

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