A Randomized, Double-Blind, Placebo-Controlled, Single-Administration, Dose-Escalation Study of Entolimod on Immunosenescence in Healthy Geriatric Subjects Receiving Influenza Vaccination

NCT04176133

18JUN2020

A Randomized, Double-Blind, Placebo-Controlled, Single-Administration, Dose-Escalation Study of Entolimod on Immunosenescence in Healthy Geriatric Subjects Receiving Influenza Vaccination

Principal Investigator and Regulatory Sponsor:
Robert Pignolo, M.D., Ph.D.
Mayo Clinic
200 First Street SW
Rochester, MN 55905
[redacted]

Funding Sponsor:
Genome Protection, Inc (GPI)
[redacted]
Buffalo, NY 14203, USA
[redacted]

Study Product: Entolimod (CBLB502)

Protocol Number: (IRBe) 19-004847

IND Number: IND 145282

Initial version: 14Aug2019, Version **2.0**
Revised version: 18JUN2020, Version **3.0**

Table of Contents

LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse Event/Adverse Experience
ALC	Absolute Lymphocyte Count
ALT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
AST	Serum aspartate aminotransferase
AUC	Area Under the Curve
CFR	Code of Federal Regulations
C_{max}	Maximum plasma concentration
CMV	Cytomegalovirus
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRTU	Clinical Trial Research Unit
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
DLTs	Dose Limiting Toxicities
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GBS	Guillain Barré syndrome
HIPAA	Health Insurance Portability and Accountability Act
HAI	Hemagglutination inhibition (assay)
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IgG	Immunoglobulin G
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
kD	Kilodalton
NHP	Non-human primates
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
RNA	Ribonucleic acid
SAE	Serious Adverse Event/Serious Adverse Experience
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SAVE	Sub-hertz Analysis of ViscoElasticity
SOP	Standard Operating Procedure
$t_{½}$	Mean terminal elimination half-live
Td	Tetanus-diphtheria vaccine
TEAEs	Treatment-emergent adverse events

TLR5	toll-like receptor 5
TLRs	Tall-like receptors
Tmax	Time at which maximum plasma concentration is observed
TT	Tetanus toxoid
UPIRTSO	Unanticipated Problems Involving Risk to Subjects or Others
ULN	Upper Limit of Normal
URI	Upper respiratory infection

Study Summary

Title	A Randomized, Double-Blind, Placebo-Controlled, Single-Administration, Dose-Escalated Study of Entolimod on Immunosenescence in Healthy Geriatric Subjects Receiving Influenza Vaccination
Running Title	Entolimod (CBLB502)
Protocol Number	19-004847
Phase	Phase II
Methodology	Double-blind, randomized placebo-controlled 4-arm study (3 active doses and placebo)
Overall Study Duration	Approximately 1- 1 ½ years
Subject Participation Duration	12 months from study drug administration
Single or Multi-Site	Single-Site
Objectives	Primary efficacy objective is to measure changes of the anti- A/H1N1, anti-A/H3N2, and anti-B influenza virus strains serum circulating antibodies (as assessed using hemagglutination inhibition (HAI) assay) levels
Number of Subjects	100 Subjects
Diagnosis and Main Inclusion Criteria	Elderly (age ≥65 years) men and women who are medically stable and eligible to receive Fluzone High-Dose and have not received the flu vaccine within the past 90 days
Study Product, Dose, Route, Regimen	This study will involve a dose-escalated, single dose administration of Entolimod (CBLB502) or placebo. Subjects will be randomized to one of the 4 arms: • Dose Level 1: 1 μg • Dose Level 2: 3 μg • Dose Level 3: 10 μg • Placebo The route of administration will be intramuscular.
Duration of Administration	Single dose administration
Reference therapy	Placebo-controlled
Statistical Methodology	Linear mixed effects models compare influenza antibody titers measured repeatedly over time, with the primary outcome being the 4-week measurement.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Due to immunosenescence related to aging, vaccination within the geriatric population is not as efficacious as within the general population. Goodwin et al. reported that the influenza vaccine had a 17-53% clinical efficacy in in the elderly while on the other hand displayed 70-90% clinical efficacy in young adults [1]. This is as a result of vaccinations' inability to produce an adequate immune response in older individuals due to age-related changes in both the nonspecific (innate) and specific (adaptive) immune response. Weinburger et al. summarizes the changes in vaccine response with age as an increase in the threshold for induction of an antibody response to a vaccine. Older individuals often produce insufficient antibody titers required to booster critical vaccinations [2]. Henry et al. has shown that elderly adults have less *de novo* somatic hypermutations in immunoglobulin variable genes which leads to less adaptability in their antibody responses and less potent hemagglutinin epitopes [3]. This inadequate antibody response can be life threatening, particularly more so in the aging population with other comorbid diseases. Compromises to their ability to resist the influenza virus, the most common cause of pneumonia infections, has led to hospitalizations, lengthened hospital stays and an estimated 10,000 to 14,000 deaths in the United States during the 2015-2016 flu season in the over 65-year-old population [4].

Enhancing the immune response to various stimuli in the geriatric population is imperative due to the increase in life expectancy and the aging population. During 1975-2015, life expectancy at birth increased from 72.6 to 78.8 years in the U.S. (CDC, National Center for health statistics) and data obtained from the social security administration indicates that a man reaching age 65 today can expect to live, on average, until the age of 84.3 and a woman turning age 65 today can expect to live, on average, until the age of 86.6.

Multiple methods have attempted to improve vaccine-induced responses in the elderly, including: increasing the vaccine antigen dosage, using booster vaccines, changing the route of administration (e.g. intradermal), using vector-based vaccines and adjuvants. Entolimod, a novel recombinant protein, is a toll-like receptor 5 (TLR5) agonist developed by Cleveland Biolabs, Inc (CBLI) for reducing the risk of death following exposure to potentially lethal irradiation [5], and by Kadvax Technologies, Inc as an enhancer for anti-addiction therapies. Toll-like receptors (TLRs) recognize conserved pathogen associated molecular patterns and play an important role in initiating an innate immune response, which in turn is able to lead to the development of an adaptive immune response. Entolimod's engagement of TLR5 has been shown to result in the activation of NF-kB, which mobilizes an innate immune response that drives the expression of numerous genes, including inhibitors of apoptosis, scavengers of reactive oxygen species, modulators of key hematopoietic growth factors including G-CSF and IL-6, and a spectrum of protective or regenerative cytokines [5]. Systemic activation of TLR5 by entolimod provides a

pharmacological advantage over the activation of other TLRs. Unlike other TLRs, systemic activation of TLR5 by entolimod does not induce cytokines dysregulation '(i.e., "cytokine storm")' that can contribute to immunopathology. Rather, entolimod induces tissue-protective factors including anti-inflammatory cytokines (IL-10), antimicrobial factors (L-17, S100A8/S100A9) and antimicrobial small peptides [5].

Limitations of current and developing vaccines can be overcome by increasing the initial or boosted antibody titers thereby increasing the antibody titers above the threshold required for efficacy. A recently completed study of entolimod alongside the tetanus-diphtheria vaccine (Td) with alum (anti-TT and anti-diphtheria) (IND 017372), demonstrated that a single intermuscular (IM) injection of entolimod was able to significantly increase levels of anti-tetanus and anti-diphtheria antibodies while producing no significant adverse effects in healthy adults (ages 18-64). Two subjects also reported subjective feelings of increased immunity (e.g decreased seasonal allergy symptoms and decreased incidence of upper respiratory infection (URI) and an improvement in their general well-being. Data from this study has prompted further investigation into the effects of entolimod on antibody generation in the geriatric population. Pre-clinical data have shown that in male swiss mice, a single week-long treatment with entolimod is capable of having positive long-term effects on their physiological frailty index [7].

Based on the collective nonclinical and clinical data with entolimod, it is hypothesized that its administration in combination with the influenza vaccine will be safe within the geriatric population and result in increased circulating influenza-specific antibodies.

Coronavirus Disease 2019 (COVID-19) Background

COVID-19 infection-related mortality has a strong dependence on age. The risk of death in the elderly is so high that COVID19-induced illness can potentially be defined as aging-related disease. Hence, therapeutic development of COVID19 prophylaxis should focus primarily on the elderly population. High vulnerability to COVID19 reflects inefficient immune responses of older people to viral infections, a well-recognized phenomenon named immune senescence [8]. In fact, older people have impaired immunization capabilities requiring the use of increased doses of vaccines; furthermore, they commonly have activated latent viruses that stay suppressed in younger people, such as hepatitis B (HBV) or cytomegalovirus (CMV). Today, there are no approved drugs for reverting immune senescence and the high vulnerability of older people to viral infections, together with their reduced capability to be protected by vaccination, remain unmet medical needs.

Broad studies of entolimod's pharmacological properties and mechanism of action uncovered its efficacy as an antiaging drug, including its ability to significantly improve responses of elderly mice with immune senescence to different vaccines. These data, together with published results on an antiviral effect of TLR5 stimulation obtained by others, suggest that treatment of elderly humans who are at risk of COVID19 infection with entolimod can reduce their risk of infection, the severity of illness and even death by reverting their immune senescence and thereby improving resistance to infection. We intend to generate preliminary data on the use of entolimod, in treating people who are at high-risk of COVID 19 infection and development of severe illness.

1.2 Investigational Agent

Entolimod is provided as a sterile, clear, colorless or slightly yellow liquid for IM injection. The drug is supplied in 2 mL Type 1 DIN 2R glass vials secured with V35 bromo-butyl injection stoppers and sealed with aluminum white flip off caps. Each single-use vial is prefilled with 50 µg of entolimod in 0.5 mL (concentration of 10 µg/100 µL). Entolimod diluent comprises sodium chloride (8.0 mg/mL), potassium chloride (0.2 mg/mL), disodium hydrogen phosphate dehydrate (1.44 mg/mL), potassium dihydrogen phosphate (0.24 mg/mL), polysorbate 80 (Tween 80) (0.001 mL/mL), and water for injection (to 1 mL), with a pH of 7.4 ± 0.5. The diluent is supplied in 50 mL vials with extractable volume 40 mL.

A matching placebo is also provided as a sterile, clear, colorless to slightly yellow liquid for IM injection in prefilled vials that are identical in appearance to the vials containing active drug. The placebo comprises of the same liquid used as a diluent for the active drug formulation.

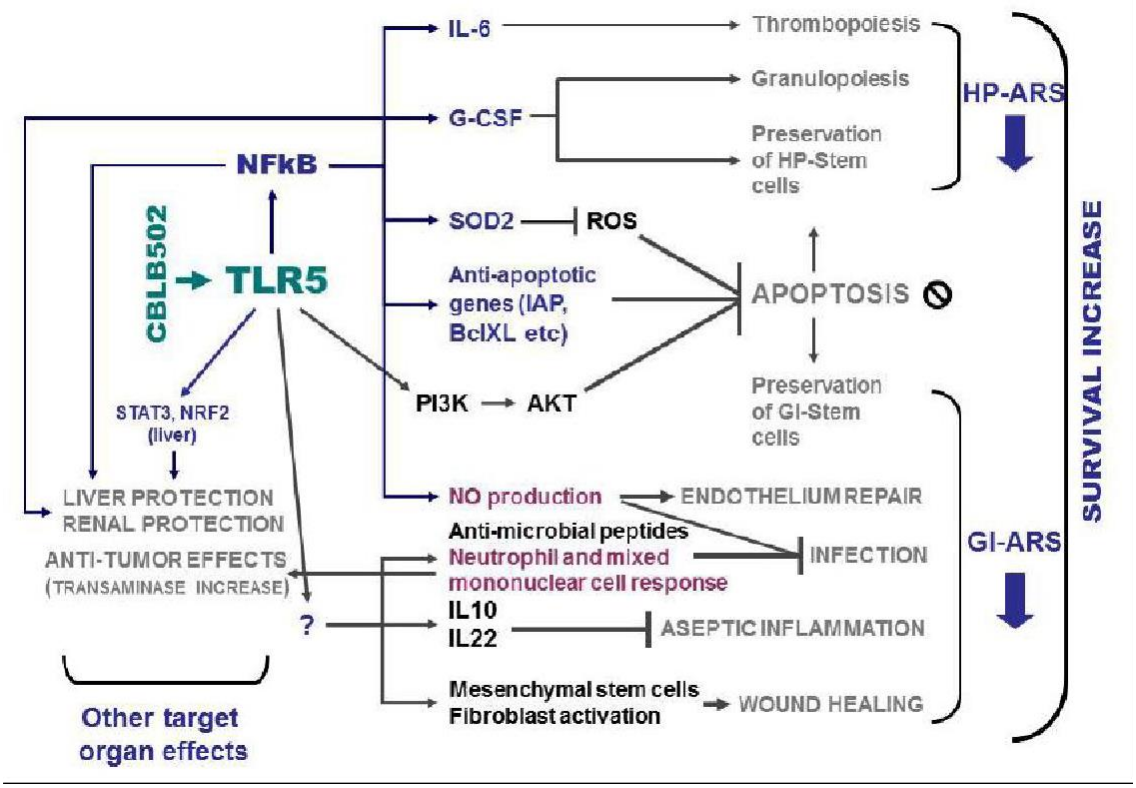
1.3 Preclinical Data

1.3.1 Entolimod's Mechanism of Action

The target of entolimod action, the TLR5 receptor, is found on the surface of both structural and immunological cell types, including multiple types of epithelia, intestinal crypt cells, intestinal endothelial cells on the basolateral surface of the intestinal epithelium, hepatocytes, mesenchymal stem cells, peripheral blood mononuclear cells, stromal elements of some hematopoietic organs, intestinal cluster of differentiation (CD) 11c+ dendritic cells and regulatory T-cells (T_{reg}).

Binding of entolimod to TLR5 specifically activates NF-κB-dependent pathways [6,7]. This results in increased production of multiple factors, including cytokines (in particular, G-CSF and IL-6) [3], antioxidants and antiapoptotic factors, and promotes multiorgan tissue protection and regeneration through multiple mechanisms as shown in Figure 1 [5,6,8–13]. Entolimod shows a similar half maximal effective concentration (EC50) for TLR5-mediated activity as its parent, flagellin (FliC), when assessed by a reporter system comprised of an NF-κB-dependent bacterial ß-galactosidase gene (LacZ) reporter [6].

Figure 1. Pharmacological Response to Entolimod Binding of TLR5



Abbreviations: AKT=protein kinase B, ARS=acute radiation syndrome, CBLB502=entolimod, G-CSF=granulocyte colony-stimulating factor, GI=gastrointestinal, HP=hemopoietic, IL=interleukin, NRF2=nuclear factor (erythroid derived 2)-like 2, P13K=phosphoinositide3-kinase, ROS=reactive oxygen species, SOD2=superoxide dismutase 2, STAT3=signal transducer and activator of transcription 3, TLR5=Toll-like receptor 5

1.3.2 Entolimod's Pharmacokinetics

The pharmacokinetics of entolimod have been evaluated in mice and in non-human primates (NHP). Because entolimod is a small protein (35 kD), is composed of natural amino acids and does not contain any nonbiodegradable moieties, degradation of the drug is expected to occur via ubiquitous proteases present in serum and via routes common to endogenous plasma proteins. For this reason, metabolism studies have not been performed. Nonclinical drug-drug interaction studies have not been performed.

To evaluate the PK of entolimod in mice, animals were injected IM with 1, 4, 12, 40, or 160 µg/kg entolimod. Serum samples were collected at 0 (baseline, pre-injection), 0.5, 1, 2, 4, 8, and 24 hours relative to entolimod injection. There were 36 mice in each dose group, providing 6 animals for serum collection at each time point. Due to assay sensitivity limitations, PK profiles for the 1 µg/kg and 4 µg/kg dose levels could not be determined. At doses of 12, 40 and 160 µg/kg, the time at which the maximum plasma concentration (C_{max}) was observed (T_{max}) was ~0.5 hours post-injection. Both C_{max} and AUC increased in a dose-dependent fashion. After the C_{max} was reached, serum concentrations of entolimod declined rapidly to undetectable levels by ~4 hours post-injection. Elimination showed first-order kinetics.

In healthy, research-naive, nonirradiated NHP given a single IM injection of 0.3, 1.0, 3.0, 6.6, 10, 40, or 120 µg/kg entolimod, the drug was rapidly absorbed (median T_{max} of ~1 hour) and rapidly eliminated (mean $T_{1/2}$ values ranging from 1.13 to 3.15 hours). Mean values for AUC_{0-t}, AUC_{0-24}, and $AUC_{0-\infty}$ following doses of 0.3 to 120 µg/kg ranged from 0.139 to 273 ng•hour/mL, 0.171 to 272 ng•hour/mL, and 0.199 to 273 ng•hour/mL, respectively, indicating that the majority of entolimod exposure occurs within 24 hours post-dose. Entolimod exposure showed no evidence of saturation over the dos range studied. An analysis of the dose-exposure relationship using dose-normalized C_{max} and AUC over the entire 400-fold dose range studied indicates that both peak exposure (C_{max}) and total exposures (AUC) were consistent with dose proportionality. Mean apparent clearance (Cl/F) values ranged from 536 to 1700 mL/hour/kg, which is ~20% to 65% of the liver blood flow (2616 mL/hour/kg) for a 5 kg monkey. Mean apparent volume of distribution values (V_d/F) ranged from 2090 to 3100 mL/kg, which is ~3 to 4.5-fold greater than total body water (693 mL/kg) for a monkey weighing 5 kg. If systemic bioavailability from the IM injection site was complete, these values would suggest moderate clearance and substantial distribution of entolimod into tissues. However, if distribution of this molecular-weight polypeptide molecule (MW ~35 kD) is limited to approximately the serum space (nominal serum volume for monkey = 44.8 mL/kg [14]), the bioavailability of entolimod would be approximately 1.3%-3.5%, and the molecule would have a clearance of approximately 75 mL/hour/kg after correcting for the bioavailable fraction.

There were no apparent sex differences in entolimod pharmacokinetic parameters in either mice or NHP.

1.3.3 Entolimod's Toxicology

A study was conducted in CD-1 (ICR) BR mice to evaluate potential toxic effects of entolimod after a single subcutaneous (SC) injection at doses of 0, 0.02, 0.06, 0.2, 0.6, 2.0 or 6.0 mg/kg. Dosing formulation assays revealed that the actual doses delivered were 0.067, 0.303, 1.22, and 4.71 mg/kg for the 4 highest entolimod dose groups. Each dose group included 10 males and 10 females.

Overall, there were no abnormal clinical signs observed, and no abnormalities in food consumption or body weight gain. Twenty-four hours after dosing, total white blood cell (WBC) counts were decreased in both males and females at entolimod doses ≥0.303 mg/kg. Animals given entolimod doses ≥0.067 mg/kg displayed decreases in serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin, and an increase in globulin 24 hours after dosing. Serum aspartate aminotransferase (AST) was increased 24 hours after dosing in females given 4.71 mg/kg of entolimod. Slight decreases in serum triglycerides were noted at all dosages. At 2 weeks after dosing, serum sodium was decreased in mice given entolimod doses ≥0.067 mg/kg; however, the means remained greater than or equal to the mean ±2 standard deviations reported for CD-1 mice. Serum calcium was also decreased 2 weeks after dosing at entolimod doses ≥1.22 mg/kg.

There were no abnormal findings at necropsy, no effects on organ weights, and no histopathological changes indicative of entolimod toxicity. Overall, the only change attributed to entolimod toxicity in this study was the increase in AST at the highest dosage; no other clinical

pathological changes were considered indications of toxicity. Accordingly, the entolimod no-observed-adverse-effect level (NOAEL) in this study was reported as 1.22 mg/kg.

Another study was conducted in rhesus monkeys to evaluate potential toxic effects of entolimod after a single IM injection at doses of 0, 1, 3, 10, 30 and 100 μg/kg. Formulation assays revealed the actual administered doses to be 0, 0, 0, 2.2, 13.2, and 62.5 μg/kg. Each dose group included 4 males and 4 females.

Administration of entolimod did not result in mortality or moribundity in any of the groups. Other than emesis on the day of dosing in 2 males in the highest dose group, no entolimod-related adverse clinical reactions were observed and there were no entolimod effects on body weight or temperature. Food consumption remained unchanged across all groups. Electrocardiograms (ECGs) and ophthalmology evaluations revealed only incidental, non-related findings.

Overall, there were no significant test article-related alterations in any of the parameters of the performed clinical chemistry, hematology, coagulation and urinalysis evaluations. Increases in absolute neutrophil count (ANC) and decreases in absolute lymphocyte count (ALC) were observed 24 hours post-dose at entolimod doses ≥10 μg/kg (2.2 μg/kg actual). Some of the leucocyte changes were still evident 14 days post-dose. Decreases in serum phosphorus were also observed 24 hours post-dose at entolimod doses ≥10 μg/kg (2.2 μg/kg actual). Activated partial thromboplastin time (aPTT) was increased 24 hours post-dose in males at 10 μg/kg (2.2 μg/kg actual) and 30 μg/kg (13.2 μg/kg actual), but not at 100 μg/kg (62.5 μg/kg actual). There were no abnormalities in urinalysis.

There were no abnormal findings at necropsy, no effects on organ weights, and no histopathological changes indicative of entolimod toxicity. The single occurrence of emesis on the day of dosing in some but not all high dose males was not considered to be an adverse event (AE). The observed changes in leukocyte counts were expected from the known pharmacological action of the drug (cytokine induction) and were not considered adverse. The increases in aPPT were not considered to be entolimod-related due to the lack of a dose response. Therefore, the NOAEL in this study was reported as the highest dose administered 100 μg/kg (62.5 μg/kg actual).

1.3.4 Immunosenescence and Entolimod

Aging of mammals is associated with accumulation of DNA damage in somatic cells, an increase in chronic systemic inflammation and reduced effectiveness of the immune system in clearing damaged cells. To a large extent, aging is the pathological manifestation of poisoning of the organism by these damaged cells and their products [15]. Therefore, a study was performed to determine whether the immunomodulatory activity of entolimod could have anti-aging effects.

Three experiments were performed in NIH Swiss mice. First, groups of male and female mice were treated with 5 daily injections of entolimod (or PBS as a control) at 18 (“young”), 55 (“middle-aged”) or 112 (“old”) weeks of age. Animal survival was monitored to determine effects of treatment on longevity (life span) and Physiological Frailty Index, a quantitative composite of multiple physiological parameters, was measured at different times post-treatment

to determine effects of treatment on health status (health span/biological age). The results showed that entolimod did not significantly increase mouse life span but did have beneficial effects on health span (reduced PFI) in male NIH Swiss mice specifically when administered at "middle-age". A second experiment then tested the immunomodulatory effects of entolimod delivered together with a vaccine (Prevnar13 pneumococcal vaccine) to young, middle-aged or old male NIH Swiss mice. Entolimod was found to enhance the efficacy of vaccination (boosted antigen-specific antibody production), but only in the middle-aged group of mice. In addition, flow cytometric analysis of the T cell repertoire in the spleens of middle-aged mice showed that, unlike in other age groups, co-administration of entolimod along with the vaccine resulted in a dramatic reduction in activated T cells, thus suggesting that entolimod enhances their recruitment to peripheral sites of infection. The concordance of these results supports the hypothesis that the immunostimulatory activity of entolimod can have anti-aging effects. The third experiment in the study confirmed that entolimod improved vaccination efficacy in middle-aged male mice using an independent model (Tdap vaccine) and demonstrated that such effects were not dependent on the route of entolimod administration and were not improved by addition of alum adjuvant.

Overall, this study demonstrated a stimulatory effect of entolimod on vaccination efficiency in a mouse model of age-related immunosenescence. This effect did not depend on the site of entolimod administration (indicating that it acts via a systemic mechanism) or on entolimod being part of the vaccine formulation. In addition, in "middle-aged" male mice, entolimod had a long-lasting positive effect on general health status that correlated with is immunomodulatory activity. Further studies are needed to clarify the mechanisms underlying the age- and gender-dependence of these effects. It should be noted that these effects were observed with doses of entolimod that are lower than its optimal efficacious dose in acute radiation syndrome.

1.4 Clinical Data to Date

1.4.1 Overview of Clinical Program

To date, clinical studies with entolimod have been performed in healthy human subjects and patients with cancer. Data from two clinical studies performed in 150 healthy subjects comprise most of the entolimod safety database. Entolimod has also been evaluated in 33 patients with advanced solid tumors and in 40 patients with colorectal cancer receiving entolimod as a neoadjuvant therapy before primary cancer surgery. Most recently, entolimod has been administered to 40 healthy subjects as an anti-addiction vaccine adjuvant. The safety data derived from studies in cancer patients provide safety information for older patients and those with comorbidities.

1.4.2 Clinical Studies in Healthy Subjects and Patients with Cancer

1.4.2.1 Phase 1 Studies in Healthy Subjects

Study HU-7014 was a first-in-human, sequential dose-escalation, single-dose study that evaluated entolimod doses of 2 (N=6), 6 (N=6), 12 (N=6), 24 (N=6), 30 (N=12), 35 (N=6), 40 (N=5), and 50 µg (N=3). Study HU-9001 was a subsequent randomized study of 4 groups of subjects that were administered one of the following dosing regimens: a single IM injection of 25 µg of entolimod (N=25), two IM injections of 30 µg of entolimod administered 72 hours apart (N=24), a single IM injection of 35 µg of entolimod (N=25), or a single IM injection of 35 µg of

entolimod preceded by 400 mg of orally administered ibuprofen (N=26). Dose selection for Study HU-9001 was intended to evaluate doses found to be readily tolerable in Study 7014, considering limited experience with higher doses in a range of subject sizes. Given the similarities of the subject populations and overlap of the dose groups, safety data from Studies HU-7014 and HU-9001 were integrated, focusing on the effects of single-dose administration.

In the combined healthy subject population of Studies HU-7014/HU-9001, subjects ranged in age from 18 to 55 years. Subjects were primarily Caucasian men of Hispanic ethnicity; however, women and African Americans were also represented. Body weights ranged from 46.0 to 109.5 kg but with few subjects at the extremes of the weight range. Demographic characteristics were reasonably well balanced across the dose groups. However, the 50 µg dose group was composed completely of women. Mean body weights in the lower and middle dose groups were between ~74 kg and ~83 kg. In contrast, mean body weights were lower in the 40 µg and 50 µg dose groups (66 kg and 58 kg, respectively), resulting in high body-weight-adjusted doses (ranging to 1.09 µg/kg) in these subjects.

Pharmacokinetic data revealed a lag in the appearance of entolimod in the serum after IM injection, which resulted in a mean time of maximum concentration (T_{max}) of 3 to 6 hours post-dose. Increases in maximum concentration (C_{max}) and AUC values with dose were somewhat greater than dose-proportional. Clearance from the circulatory system was rapid with the mean terminal elimination half-live ($t_{½}$) being ~3 hours. The pharmacokinetic profile of entolimod was not affected by pretreatment with the nonsteroidal anti-inflammatory drug (NSAID) ibuprofen.

Administration of entolimod resulted in rapid and substantial dose-related increases in plasma levels of G-CSF and IL-6. IL-8 and IL-10 were also elevated. Plasma cytokine levels typically peaked by 4 hours and decreased to pre-dose levels by 7 to 12 hours after injection. Increases in absolute neutrophil count (ANC) were observed in all dose groups after entolimod administration, peaking by ~7 to 24 hours and returning to pre-dose levels by 5 days (120 hours) after injection. Administration of ibuprofen prior to entolimod dosing appeared to augment the entolimod-mediated increases in plasma concentrations of cytokines (e.g., G-CSF, IL-6, IL-8, IL-10) relative to administration of entolimod alone.

Entolimod administration was associated with unpleasant, but transient and generally mild, adverse effects, which were primarily comprised of elements of a flu-like syndrome (malaise, fatigue, chills, fever, myalgias, and nausea) that are consistent with TLR agonism. As specifically evaluated in the development program, the constitutional components of the flu-like syndrome could be lessened either preemptively or reactively with ibuprofen while still preserving the desired pharmacodynamic activity of entolimod.

Transient increases in white blood cell count (WBC) and ANC and decreases in platelet count and absolute lymphocyte count (ALC) were seen in response to entolimod administration. These changes were considered to be components of the expected mechanism-related activity of entolimod, had no clinical sequelae, and were not considered adverse effects. Administration of entolimod was also associated with transient increases in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), without changes in serum levels of alkaline phosphatase (ALP) or bilirubin. Such hepatic transaminase changes are considered consistent with the mechanism of action of entolimod and may result from recruitment of large numbers of natural killer cells to sites of TLR5 activation such as the liver [16]. The data from

Study HU-9001 were modeled using the DiliSym™ proprietary software that is currently the focus of a collaboration between the U.S. Food and Drug Administration (FDA) and The Hamner Institutes for Health Sciences [17]. This analysis indicated that subjects given entolimod had a pattern of minor hepatocyte loss and recovery similar to that observed in healthy subjects receiving commercially available heparin drug products (e.g., heparin, dalteparin, enoxaparin, adomiparin). Other laboratory abnormalities associated with entolimod administration were asymptomatic hyperglycemia and transient hypophosphatemia. All of these laboratory abnormalities were perceived to be transient and self-limiting, have no serious clinical implications, and require no monitoring or intervention. Such effects were seen following administration of IL-6 in patients with cancer [18] and, thus, are likely secondary consequences of entolimod-mediated induction of this cytokine.

Transient decreases in blood pressure and increases in pulse rate observed in Studies HU-7014/HU-9001 were also likely the result of entolimod-mediated IL-6 induction [19]. Most of the changes in blood pressure were within the normal range and were imperceptible to subjects, although a minority of study participants did describe dizziness and orthostatic syncope occurred in 1 subject. For those subjects that developed blood pressures below the normal range, the time to return to normal averaged ~6 hours, indicating that hypotension following entolimod administration is typically not long lasting. No elevations of serum creatinine or blood urea nitrogen (BUN) consistent with renal hypoperfusion were evident in any subject. Observations of hypotension were managed at the investigator's discretion in both Studies HU-7014 and HU-9001. Consequently, such changes were addressed inconsistently, with neither the minimum absolute blood pressure nor the maximum decrease from baseline necessarily prompting an investigator decision to administer intravenous (IV) fluids. Only 13 of 150 subjects (8.7%) received IV fluids and only 1 subject (0.6%) was given IV dopamine; the rationale for administering a pressor agent to this subject was unclear since they only had transient asymptomatic Grade 2 hypotension and were discharged normotensive the following day.

Electrocardiograms (ECGs) were evaluated in both clinical trials in healthy subjects at screening, pre-dose, at 2 hours and 4 hours post-dose on Day 1, and at follow-up on Day 4 or 5 following entolimod administration. No changes in PR or RR intervals were observed and no other abnormalities were apparent. Changes in corrected cardiac QT interval (QTc) ≥60 msec or evidence of prolonged QTc (>501 msec) occurred in only 4 subjects but showed no dose-, exposure-, or time-dependency that suggested an adverse entolimod effect. Based on the available data in healthy subjects, there is no indication that entolimod caused disturbances of cardiac conduction or rhythm.

Overall, the results of these clinical studies in healthy human subjects indicated variable but dose-dependent increases in pharmacokinetic and pharmacodynamic parameters, but no clear dose-related pattern of adverse effects on safety parameters. Nevertheless, it remains possible that dose-dependent changes in safety parameters (i.e., symptomatic events, laboratory abnormalities, and hemodynamic measures) could have been obscured by differences in body weight among study participants.

1.4.2.2 Phase 1 Study in Patients with Cancer

Study I-196111 was a Phase I multi-center, dose-ranging study designed to determine the safety and tolerability of entolimod in patients with advanced cancers [20,21]. Entolimod doses of 5 (N=4), 10 (N=3), 15 (N=3), 20 (N=4), 30 (N=12), and 40 (N=7) µg were evaluated with IM

injections given once daily for 5 days (Days 1-5) on a dose-per-subject basis through the 20 µg dose level and with IM injections once per day on Days 1, 4, 8, and 11 at the 30 µg and 40 µg dose levels.

Subjects ranged in age from 27 to 82 years and were primarily male and Caucasian. Body weights ranged from 40.7 kg to 117.3 kg. Subjects had a variety of cancer types; colorectal cancer and lung cancer were most commonly represented.

The safety results of Study I-196111 confirm that the commonly occurring AEs following from entolimod administration are reflective of a "flu-like" syndrome, including chills, pyrexia, nausea, and vomiting. Expected transient treatment-emergent increases in leukocytes and neutrophils and decreases in lymphocytes and platelets, as well as treatment-emergent increases in serum ALT and AST levels, increases in serum glucose, and decreases in serum phosphate were observed. Expected transient, generally asymptomatic hypotension and tachycardia were also observed after entolimod injection; blood pressure recovered to pretreatment values with rest and fluids. When increases in QTcB occurred, they were almost universally low grade and did not show dose dependency; collectively, the ECG data do not suggest that entolimod causes clinically relevant disturbances of cardiac rhythm or conduction. Overall, the safety profile of entolimod in subjects with advanced cancers was found to be consistent with that observed in healthy subjects.

1.4.2.3 Phase 2 Study in Patients with Cancer

Study BL612-CBLB502 was a Phase 2, multi-center, randomized, placebo-controlled, single-blind study designed to assess the safety, pharmacodynamic effects, and preliminary efficacy of neoadjuvant therapy with entolimod in subjects with colorectal cancer who had never received antitumor treatment and were scheduled for surgical resection of the primary tumor. Entolimod doses of 0.35 µg/kg or 0.45 µg/kg (not to exceed 30 µg or 40 µg per injection, respectively) or placebo was administered as either a single IM injection or as two IM injections separated by 3 (±1) days, with the last injection administered 4 (± 2) days before surgical resection of the primary tumor. Absolute entolimod doses ranged from 18 to 40 µg. Subjects were given premedication of 400 mg ibuprofen orally 30 (± 5) minutes before each injection of study drug to mitigate the flu-like syndrome found to be associated with administration of entolimod in earlier clinical studies.

A total of 40 subjects were enrolled and randomized in a 3:1 ratio of entolimod (30 subjects) to placebo (10 subjects). Thirty-eight subjects received the planned number of injections of entolimod or placebo; 2 of the 30 entolimod-treated subjects who were assigned to receive 2 injections of 0.45 µg/kg entolimod did not receive the second of the 2 planned doses due to the occurrence of SAEs (one subject 69 y/female 30 µg entolimod - bigeminal ventricular extrasystoles, and the other subject 78 y/male 33 µg entolimod - myocardial infraction) attributed to a history of ischemic heart disease. Subjects ranged in age from 33 to 84 years and were 45% male and 55% female and primarily Caucasian. Body weights ranged from 43.0 kg to 107.2 kg. Most subjects had Stage II or Stage III colorectal cancer.

Both entolimod dose levels were well tolerated in this population. The commonly occurring AEs were reflective of the entolimod-associated "flu-like" syndrome, including chills, fever, headache, and nausea. Laboratory-related AE reports showed the expected pharmacologic effects of entolimod, including decreases in lymphocytes, increases in liver transaminases, an increase

in serum glucose, and a decrease in serum phosphate. Predicted findings of transient, generally asymptomatic hypotension and tachycardia were also observed after entolimod injection; blood pressure recovered to pretreatment values with rest and fluids. Predicted increases in plasma cytokines (G-CSF, IL-6, IL-8, IL-10) and increases in circulating neutrophils and decreases in circulating lymphocytes (including B cells, T-cell subsets, and NK cells) were observed in the entolimod-treated subjects. Overall, the safety and pharmacodynamic profiles of entolimod in subjects with colorectal cancer in this study were consistent with those observed in healthy subjects and in previous studies in subjects with advanced cancer.

1.4.2.4 Entolimod in Combination with Vaccine

The Kadvax Technology study was performed to evaluate the safety and efficacy of 1 μg of entolimod receiving a commercial tetanus-diphtheria (Td) vaccine in healthy human subjects. The study enrolled 40 male volunteers (16 Caucasian, 24 non-Caucasian) between the ages of 18 and 40 years old (mean age = 28 years). Fifteen volunteers were enrolled in the control group and given commercial Td vaccine alone while 25 volunteers received the Td vaccine in combination with 1 μg of entolimod. Tetanus- and diptheria-specific antibody titers were to determine if the addition of entolimod increased the vaccine response.

Mean baseline levels of tetanus toxoid (TT) -specific antibodies were relatively high in this study (2.95 IU/mL), and as a result, no subjects in the control (Td alone) or test (Td+entolimod) group had TT antibody responses above 6 IU/mL. Therefore, only subjects with pre-treatment-antibody titers below 3 IU/mL were included for data analysis (9 Td alone and 11 Td+entolimod). Eighty-two percent of the subjects that received entolimod with the Td vaccine showed a 2-fold or higher increase in their TT antibody titers, while only 33% of the control group showed this level of increase (chi sq = 4.3; p = <0.03). Two of the entolimod test subjects showed particularly strong increases in antibody titers (10- and 15-fold above baseline). A negative correlation was observed between baseline antibody titer and the change in antibody titer over 6 weeks. The correlation between lower baseline titer and greater post-vaccination change in titer was stronger in the Td+entolimod group than in the control group (r = -0.73 vs. -0.36). The negative correlation was statistically significant in the Td+entolimod test group with a p-value of <0.003. Analysis of anti-diphtheria antibody titers showed a greater increase in anti-diphtheria antibody titers for all 25 Td+entolimod test subjects versus the 15 Td alone control subjects (averaging 1.1 IU/mL vs. 0.9 IU/mL, respectively; p = <0.02). The negative correlation between baseline antibody titer and the change in anti-diphtheria antibody titer was also significantly stronger for the Td+entolimod group than for the control group (r = -0.91 vs -0.52, respectively).

During this 6-week clinical trial, there were no treatment-related adverse events reported. Thus, IM injection of 1 μg of entolimod together with Td vaccine was found to be safe and to significantly increase the Td vaccine response.

1.4.2.5 Entolimod Safety in Elderly Subjects (≥65 years old)

Entolimod-specific treatment emergent adverse event (TEAE) data for 25 geriatric (≥65 years old) subjects was extracted from the studies performed in patients with advanced solid tumors (Study I-196111; 14 geriatric subjects) and in patients with colorectal cancer prior to primary tumor resection (Study BL612-CBLB502; 11 geriatric subjects).

The geriatric subjects ranged in age from 65 to 84 years, with thirteen subjects between 65 and 70 years, nine subjects between 71 and 80 years and three subjects between 81 and 84 years. There were 8 females and 17 males with screening body weights ranging from 47.2 kg to 107 kg. Sixteen (64%) of these subjects had colorectal cancer, while lung cancer (n=4), melanoma (n=2), anal cancer (n=2) and urothelial cancer (n=1) were also represented. For analysis, subjects were stratified into 3 groups based on the administered entolimod dose per kg of body weight: (i) <0.25 mg/kg, (ii) 0.25 mg/kg to 0.40 mg/kg, and (iii) >0.40 mg/kg with mean body weights of 77.7 kg, 79.1 kg, and 70.8 kg, respectively.

All subjects experienced a TEAE, but there was little evidence of a dose response when considering the total frequencies of any given type of event. Altogether, 175 TEAEs were reported among the 25 (100%) geriatric subjects. There did not appear to be a dose trend within any of the system organ classes (SOC) including general disorders, administrative site conditions, and respiratory, thoracic and mediastinal disorders. A majority (147) of the TEAEs were mild (Grade 1) or moderate (Grade 2) in intensity. Grade 3 TEAEs were largely laboratory abnormalities, except for single instances of cardiovascular events, including hypotension, hypertension and extrasystoles. The frequency of TEAEs of Grade ≥3 was nonexistent in the group receiving <0.25 mg/kg entolimod (<19.4 mg based on 77.7kg group mean body weight) but more prevalent in the group receiving >0.25mg/kg of entolimod (56%). Only 3/25 (12%) subjects ≥65 years experienced a Grade 4 AE (1 subject with hypophosphatemia, 1 subject with increased lipase, and 1 subject with both lymphocytopenia and neutrophilia). These subjects received a substantially higher entolimod dose (between 0.35 mg/kg and 0.45 mg/kg) than what is proposed for the current study.

No deaths occurred within this age group. Overall, within the limits of confidence imposed by the available number of geriatric subjects, there appear to be no differences in the safety profiles of people ≥65 years of age versus younger adult study subjects.

1.4.2.6 Entolimod and Subjects with TLR5 Mutation

A mutation has been identified in the TLR5 gene in which a cytosine-to-thymidine transition at base pair 1174 changes an arginine at amino acid 392 to a stop codon ($TLR5_{392stop}$) [22,23]. The $TLR5_{392\ stop}$ mutation is reported to occur in approximately 10% of humans in a heterozygous form and in 0.5% of the population in a homozygous form. The completed entolimod clinical trials described above included six subjects with $TLR5_{392\ stop}$ mutations: 1 subject who was homozygous for the mutation and 5 subjects who were heterozygous for the mutation. The 1 homozygous subject and 1 of the heterozygous subjects had almost no pharmacodynamic responses to entolimod. Of the remaining 4 heterozygous subjects, two had moderate responses and two had wild-type responses. Due to the small number of subjects with TLR5 mutations, it is not possible to draw definitive conclusions, but the data suggest that it will not be useful to screen patients for the presence of mutations in TLR5 prior to administration of entolimod. However, retrospective analysis of TLR5 functional status will prove useful for analysis in being able to definitively exclude non-responsive mutated TLR5 individuals.

1.5 Dose Rationale

Based on pre-clinical and clinical studies described above (sections 1.3 and 1.4), we hypothesize that a single IM injection of entolimod may significantly increase levels of anti-influenza antibodies, promote increased immunity, and delay or alleviate aspects of frailty. Risk is reduced by using single doses 5- to 50-fold less than those which are likely to cause adverse events, such as hypotension. The route of administration provides local delivery to the site of vaccination where the initial immune response to influenza antigens will occur.

1.6 Risks and Benefits

Entolimod used at doses similar to those to be used in this study has not previously been associated with adverse reactions. Based on the known safety profile of entolimod used at doses 5 to 50 times those proposed in this study, the following potential risks may be associated with the investigational drug: (1) flu-like symptoms, (2) decreases in blood pressure, (3) increases in heart rate, (4) hepatic transaminase elevations, (5) hyperglycemia, and (6) hypophosphatemia. All potential risks, if realized, are expected to be transient. Since entolimod will be given only once, the likelihood of long-term consequences is expected to be very low. Potential benefits include: (1) increasing influenza vaccine efficacy in an older population prone to poor vaccine responses, and (2) knowledge that might reasonably be expected from the study results (e.g., predicting early vaccine responses, better understanding of immunosenescence, and associations of vaccine responses with frailty). Therefore, the risks of using this drug are likely minimal compared to the anticipated benefits and the knowledge that may be gained from these clinical investigations.

1.6.1 Definition of DLT

Reference should be made to the Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials for grading the severity of AEs and laboratory abnormalities where applicable. Based on the known safety profile of entolimod, flu-like symptoms are expected. Decreases in blood pressure, increases in heart rate, hepatic transaminase elevations, hyperglycemia, and hypophosphatemia are expected. DLT will be defined based on the sound clinical judgement of the investigator. DLTs will be considered adverse events and their time and grade severity will be recorded.

These risks and benefits are outlined in the Informed Consent Form and listed in the Investigators Brochure.

2 Study Objectives

Primary Objective

- To evaluate the effect of increasing dose of entolimod on enhancement of the influenza vaccine immunogenicity in the geriatric population (≥ 65 years old).

- To characterize the safety profile of entolimod within the geriatric population (≥65 years old) vaccinated against influenza.

Secondary Objectives

- To evaluate the pharmacodynamic effects of entolimod on immunological status, markers of senescence, viscoelasticity of tissue at the injection site, frailty indices and quality of life (QoL).

Exploratory Objectives

- To determine levels of anti-COVID-19 serum-circulating antibodies in a population of confirmed COVID-19 cases

- To determine the rate of the following occurrence in patients that become COVID-19 positive: (i) disease progression requiring hospitalization; (ii) acute respiratory distress syndrome (ARDS); (iii) and 30-day mortality following study drug administration.

3 Study Design

This clinical trial is a randomized, double-blind, placebo-controlled, dose-escalated, single-administration study evaluating the immunogenicity, pharmacodynamics, and safety of entolimod within the geriatric population (≥65 years old) vaccinated against influenza. After providing a written informed consent, subjects will undergo screening medical history, physical examination, vital signs, laboratory, and electrocardiogram (ECG) assessments within 28 days prior to study drug administration and influenza vaccination (see figure 2 below).

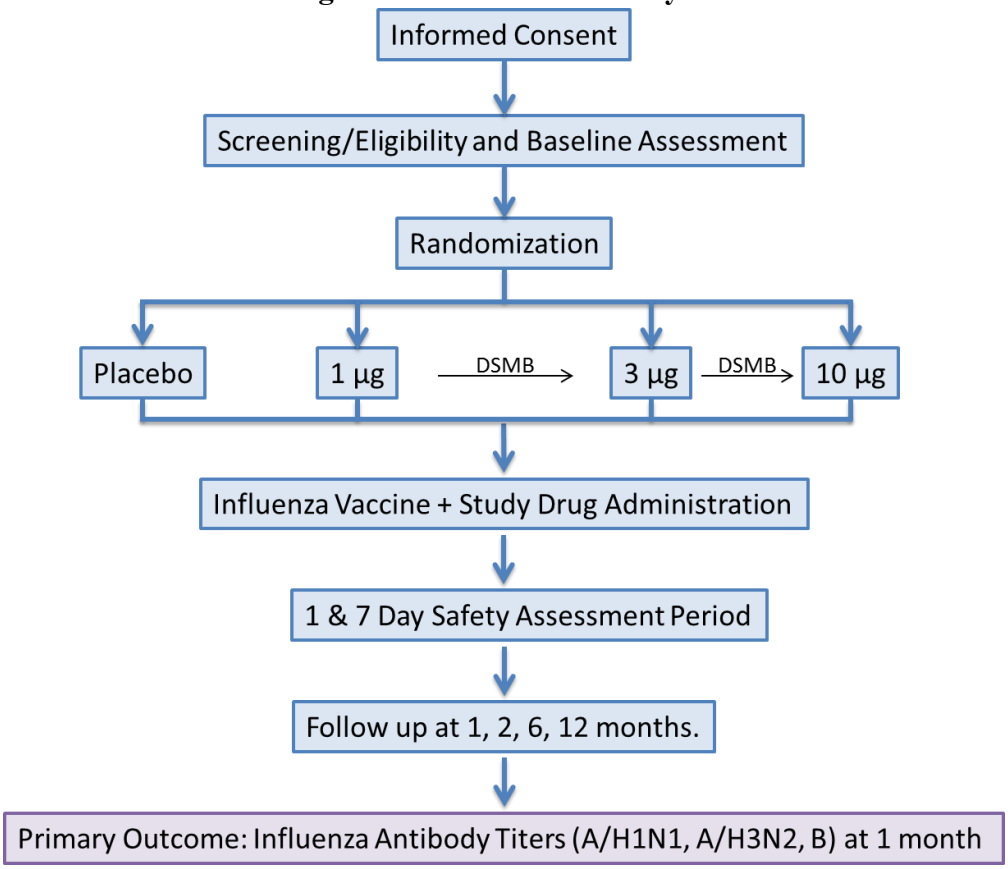
Eligible subjects will be seen at the clinical trial research unit (CTRU) to receive the influenza vaccination (Fluzone, high-dose split virion influenza virus vaccine, Sanofi Pasteur) and a single IM injection of the study drug (entolimod or placebo). For safety assessment, initially 8 patients were to be enrolled in arm 1 (1ug) and accrual temporarily halted until the DSMB had assessed early adverse events. Due to the late seasonal start only four patients were successfully accrued to the study by the end of the flu season. A safety report was generated and the DSMB endorsed proceeding with enrollment of arm 2 (3ug) and arm 1 will restart. After 8 subjects have been enrolled into arm 2, accrual for arm 2 will be temporarily halted until the DSMB has assessed early adverse events. A safety report will be generated and enrollment of arm 3 (10ug) will commence and arm 2 will restart. After 8 subjects have been enrolled into arm 3, accrual for arm 3 will be temporarily halted until the DSMB has assessed early adverse events. A safety report will be generated and enrollment of all three arms will continue until enrollment is complete.

Unblinded and subject-level adverse event data will be provided to the Data Safety Monitoring Board (DSMB). If there is any evidence that higher doses are associated with adverse events, those study arms may be dropped, and the remaining planned patients accrued and randomized to remaining dose levels (and placebo) in equal proportions.

Subjects will be evaluated at the CRTU on the day of study drug administration (Day 1), for ≥ 6 hours (2hrs, 4hrs and 6 hrs), thereafter day 2, weeks 1 and 4, and then on months 2, 6 and 12. Between month 2 and 6 and 6 and 12 AEs will be reported and occurrence of respiratory infections will be assessed via phone interviews. Assessments of adverse events (AEs), vital signs/oxygen saturation including orthostatic measurements, clinical chemistry and hematology parameters (i.e., complete blood count with differential), ECGs, plasma cytokines, leukocytes, anti- A/H1N1, anti-A/H3N2, and anti-B influenza serum circulating antibodies including cellular immune response outcomes will be performed to describe drug safety, pharmacodynamics, and immunogenicity.

Subjects will be screened using a questionnaire for potential viral infection of COVID-19 at Day 1 and monthly through the end of the study. Subjects will be asked to provide a nasal swab and serum sample at Day 1 and Month 12. If at any time the subject is evaluated for COVID-19, the study team will document the symptoms, test results and treatment until resolution.

Figure 2. Flowchart of Study Events



NOTE: Subjects will also be contacted every two weeks by phone for Adverse Event monitoring.
Day 7 Safety Assessment will be completed as a remote visit

3.1 General Description

3.2 Number of Subjects

A total of 100 individuals will be randomized into one of four treatment groups (placebo and three progressive dosage entolimod groups).

3.3 Duration of Participation

After consent, for each individual, the duration of participation will be for the length of time required to perform screening plus the 12-month follow-up period after the single-dose administration of entolimod. It is anticipated that the screening will occur within seven days of written content.

3.4 Primary Study Endpoints

Immunogenicity

- Changes of the anti- A/H1N1, anti-A/H3N2, and anti-B influenza virus strains serum circulating antibodies (as assessed using hemagglutination inhibition (HAI) assay) levels.

Safety

- All adverse events (AEs), including dose limiting toxicities (DLTs); laboratory abnormalities; oxygen saturation and vital sign changes, and adverse electrocardiogram (ECG) findings

3.5 Secondary Study Endpoints

Pharmacodynamics and Cellular Immune Responses to Influenza Vaccination

- Time to onset and the number of upper-respiratory infections, including (but not limited to) influenza viral infections (as indicated by subject self-reporting)

- Changes in the concentration of circulating plasma cytokines including (but not limited to): IL-6 and G-CSF as pharmacodynamic indicators of entolimod's activity (measured using MSD assay platform)

- Changes to 10 cytokine/chemokine mediators of adaptive immune function (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α) in PBMC culture supernatants at 0 and 24 hours after influenza (A/H1N1, A/H3N2 and B) viral stimulation as detected by Meso Scale Discovery, V-PLEX Proinflammatory panel human immunoassay kits.

- Changes in the quantification of IFNγ-positive cells as a marker of cell-mediated immunity (CMI) after vaccination using influenza virus-specific IFN-γ ELISPOT assay kits from R&D Systems.

- Changes in frailty indices and quality of life (QoL) of individuals receiving entolimod versus placebo

- Changes in measures of physical function (6-minute walk test, grip strength) and body composition

3.6 Exploratory Endpoints

Sub-hertz Analysis of ViscoElasticity (SAVE) in muscle tissue in response to local immunological reactions due to influenza vaccination

SAVE is a non-invasive imaging technique used to measure viscoelastic characteristics of tissue. This technique enables imaging biphasic characteristics of tissue which are related to both extra-cellular matrix changes as wells as disruption in the interstitial fluid mobility due to structural changes. A clinical study of this method in breast tumors has shown to provide effective separation of the benign from malignant breast lesions in patients [25].

In this study, SAVE will be used for quantification of muscle reaction in response to vaccination, which usually occurs within few hours of injection. Vaccine potency and efficiency are estimated by antigen-specific antibody titers and T-cell responses generated weeks after vaccination. Skeletal muscle contains few immune cells, but tissue resident and/or infiltrating immune cells encounter vaccine antigens for the first time at the site of administration. Thus, the magnitude of local innate immune responses starting at the vaccine delivery site (local inflammation) initially controls subsequent adaptive immune responses and may be captured by SAVE.

- Comparison of SAVE between left versus right deltoid muscles of the same individual.

Markers of Cell Senescence

- Changes in markers of senescence in blood and urine.

Efficacy

- Reduction of COVID-19 morbidity (detection of viral infection by PCR)

3.7 Primary Safety Endpoints

Safety assessments are undertaken with the measurement of safety laboratory tests and procedures, vital signs, and recording of adverse events.

3.8 Identification of Source Data

Source data will be obtained from the electronic medical record and documented in study specific case report forms (paper and/or electronic data capture). No information in source documents about the identity of the subjects will be disclosed.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Study candidates must meet all the following criteria within 1 week prior to entolimod administration to be eligible for participation in this study:

1. Men and women of age 65 years and older at the time of enrollment
2. Eligible to receive Fluzone High-Dose
3. Female subjects must be past menopause and not pregnant
4. No history of anaphylactic reaction to gelatin, neomycin, or other vaccine component
5. Must not have had the flu vaccine within the past 90 days
6. Medically stable with no exacerbations or changes in medication regimen for chronic diseases in the past 3 months and no hospitalizations in the past 6 months
7. Must be able to read/write English in order to provide informed consent and comply with study procedures
8. Expected to be available for the duration of the study

4.2 Exclusion Criteria

Study candidates who meet any of the following criteria within 28 days prior to entolimod administration will not be eligible for participation in this study:

1. Receipt of any other vaccines within the past 30 days prior to enrollment
2. Acute illness within the last 7 days without systemic signs and symptoms including fever > 99.5
3. History of hypersensitivity to the flu vaccine or its components (including gelatin, formaldehyde, octoxinol, thimerosal, and chicken protein).
4. History of Guillain Barré syndrome (GBS)
5. History of bleeding disorders
6. Medical contraindication to treatment with vaccine as indicated by a history of autoimmune disease, immune deficiency, or hypersensitivity to other vaccines.
7. Unstable major cardiovascular, renal, endocrine, immunological or hepatic disorder
8. Systolic blood pressure (SBP) < 110 mmHg or orthostatic hypotension [>20 mmHg fall in SBP or >10 mmHg fall in diastolic blood pressure (DBP) with standing] at the time of screening.
9. Evidence of an ongoing systemic bacterial, fungal, or viral infection (including upper respiratory tract infections) (within 14 days prior to entolimod administration). ***Note: Subjects with localized fungal infections of skin or nails are eligible.***
10. Baseline vital signs with ≥ Grade 2 abnormalities
11. Significant cardiovascular disease (e.g., myocardial infarction, arterial thromboembolism, cerebrovascular thromboembolism, venous thromboembolism) within 6 months prior to study drug administration; symptomatic dysrhythmias or unstable dysrhythmias requiring

medical therapy; angina requiring therapy; symptomatic peripheral vascular disease; New York Heart Association Class 3 or 4 congestive heart failure; or uncontrolled Grade ≥3 hypertension (diastolic blood pressure ≥100 mmHg or systolic blood pressure ≥160 mmHg) despite antihypertensive therapy.

a. Significant screening ECG abnormalities, including unstable cardiac arrhythmia requiring medication, atrial fibrillation, 2nd-degree atrioventricular (AV) block type II, 3rd degree AV block, or Grade ≥2 bradycardia (within 14 days prior to entolimod administration).

12. Inadequate hepatic function (within 14 days prior to entolimod administration):
    a. Serum alanine aminotransferase (ALT) ≥3 × upper limit of normal (ULN) (Grade ≥1).
    b. Serum aspartate aminotransferase (AST) ≥3 × ULN (Grade ≥1)
    c. Serum alkaline phosphatase (ALP) ≥5 × ULN (Grade ≥2)
13. Serum bilirubin ≥1.5 × ULN (Grade ≥ 1)
14. Positive antiviral serology:
    a. Positive hepatitis C virus (HCV) antibody or positive HCV ribonucleic acid (RNA) by quantitative PCR.
    b. Positive hepatitis B surface antigen (HBsAg) and negative hepatitis B core (HBc) antibody or undetectable hepatitis B (HBV) deoxyribonucleic acid (DNA) by quantitative polymerase chain reaction (PCR) testing.
15. Positive human immunodeficiency virus (HIV) antibody.
16. Use of medication that might interact with the flu vaccine including (but not limited to) specifically: aminopyrine, phenytoin sodium, theophylline, and warfarin sodium.
17. Any ongoing treatment with immunosuppressive or immune-stimulant therapy
18. Ongoing use of systemic corticosteroids.
19. Blood or blood products given within the three months prior to vaccination and two months after vaccination
20. Current and/or expected receipt of chemotherapy, radiation therapy or any other cytotoxic or immunosuppressive therapy [i.e. more than 10 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 3 months]
21. Receipt of another investigational pharmaceutical product within 60 days of treatment
22. Diagnosis of Parkinson's Disease, previous stroke, or significant cognitive impairment (defined as MMSE <20)
23. Other concerns that in the opinion of the PI would preclude a subject from participating in study procedures or from completing the study.

4.3 Subject Recruitment, Enrollment and Screening

Recruitment sources include: 1) Mayo Clinic Patients 2) Mayo – RST Classified Advertising 3) Flyers within the community, 4) Newspaper and Radio Advertising, 5) Existing lists/databases of individuals interested in being contacted about participation in research studies, and 6) Social Media including Facebook. Subject recruitment and advertisement will occur on Mayo campus and at various sites off campus (e.g., retirement centers). Permission will be obtained from each location before recruitment begins.

All patients who meet eligibility will be invited to participate in the study. Potential subjects will be approached by trained clinical coordinators who will assist in recruitment and carry-through of the protocol. Written and informed consent will be obtained by appointed, trained study personnel. Please see Inclusion and Exclusion criteria above. Documentation of recruitment and enrollment efforts will be maintained in a secure institutionally-supported database. Only those subjects who provide informed consent will be enrolled in the study; we anticipate enrolling 100 subjects.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

The following conditions describe the circumstances under which a subject may be withdrawn from the study prior to that subject completing all of the study related procedures:

- Any SAE that precludes further participation of the subject;
- With respect to subjects that have not received study drug, any SAE related to the study drug that in the opinion of the principle investigator or monitors as part of the Data and Safety Monitoring Plan (DSMP) which places subjects at high risk of experiencing the same SAE.
- If in the opinion of the principle investigator, there is doubt that a subject, for any reason, can continue to fully participate in the study; and
- Subject decision to withdraw from the study (withdrawal of consent).

See also Section 10.1, “Study Monitoring Plan”.

In the event of a subject withdrawal, the following will be performed:

- Review of data collected through the last in-person or remote visit for completeness;
- Replacement of study subject if the subject did not receive study drug (i.e., early withdrawal before study drug administration); and
- Follow-up for subjects withdrawn from study, if the treatment visit was completed. The exit visit will include those study assessments and procedures that would have been performed at the next scheduled visit.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a subject withdraws consent to participate in the study, for subject safety or other reasons, attempts will be made to obtain permission to collect follow up information whenever possible, including through medical chart review. As outlined in section 4.4.1, “When and How to Withdraw Subjects,” the exit visit will include those study assessments and procedures that would have been performed at the next scheduled visit. Although early withdrawal could be related to the safety profile of the study drug, it could also be related to other reasons, and thus follow-up assessments could be important in capturing both additional safety information as well as study outcomes that affect interpretation of risk versus benefit. Since the study drug will be given only once, long-term safety consequences related to participation are expected to be very limited.

5 Study Drug

5.1 Description

Study drugs (entolimod, diluent and placebo) are manufactured under current Good Manufacturing Practices (cGMP).

The study sponsor will provide entolimod as a liquid for IM injection in 2 mL prefilled, single-use vials containing 50 µg of entolimod in 0.5 mL (concentration of 100 µg/mL) of formulation. The formulation comprises phosphate-buffered saline (PBS) containing 0.1% polysorbate 80 (Tween 80).

For the dilution of 100 µg/mL entolimod the study sponsor will supply entolimod diluent in 50 mL single use vials filled with 40 mL of phosphate-buffered saline (PBS) containing 0.1% polysorbate 80 (Tween 80).

The study sponsor will also provide a matching placebo. The placebo has the same excipient composition as the active drug formulation (i.e., PBS containing 0.1% Tween 80) and fill-finished in the same type of 2 mL single use vials (0.5 mL per vial).

Vials of entolimod and placebo should be kept frozen at -70 +/- 10°C and -20 =/- 5°C until thawed for use. Entolimod contained in vials and stored at -70 +/- 10°C is stable for ≥7 years. The drug can withstand 3 freeze-thaw cycles. Entolimod should be thawed immediately before the dilution. Once thawed, study drug can be stored at 5^0 +/- 3^0C for up to 8 hours. Entolimod dilutions should be completed within the established periods of stability for drug in the thawed vials. Diluted entolimod should be administered immediately after dilutions preparation.

5.2 Treatment Regimen

A single IM injection of study drug (entolimod or placebo) will be administered to each subject on the morning of vaccination, Day 1 (to be administered within 1 inch and within 1 minute of flu vaccine). The appropriate amount of study drug will be aseptically withdrawn from the required number of study drug vials into a 1-mL tuberculin syringe calibrated in 10-µL units. The drug will be administered from the syringe through a 22-25-gauge needle (see table 1 below) at a 90°-angle to the skin surface into the deltoid muscle between the acromion process and the midaxillary line.

Table 1. Needle size.

Female or Male up to 69 kg	**22-25 gauge, 1 inch needle**
Female 70 kg to 91 kg, Male 70 kg to 118 kg	**22-25 gauge, 1 inch to 1.5 in needle**
Female > 91 kg, Male > 118 kg	**22-25 gauge, 1.5 inch needle**

5.3 Method for Assigning Subjects to Treatment Groups

Study subjects will be randomized in a dose-escalated 4-arm study of entolimod in 3 possible doses or placebo (see table 2 below). Beginning in period 1, the first eight patients in the 1ug arm will be evaluated for safety and tolerability by the DSMB prior to the enrollment of the remainder of the 1ug arm. In addition, after review we will proceed with recruitment in period 2 of the 3ug arm. Again, safety and tolerability will be assessed in the first 8 patients of the 3ug arm during period 2. Similarly, after evaluation by the DSMB we will proceed with the remainder of the 3ug arm. In period 3 we will evaluate safety and tolerability in the first 8 subjects of the 10ug arm. An evaluation by the DSMB will be completed and we will proceed with the remainder of the 10ug arm until all subjects have been accrued. We will recruit the balance of the subjects remaining to be enrolled (to obtain 25 subjects per arm) pending safety review by the DSMB.

Randomization will be performed using Medidata Balance software, integrated into the electronic case report forms Medidata RAVE. A permuted block design will be used, with stratification by sex. Subjects and study center personnel involved in the care of subjects will be blinded to study drug assignment (entolimod vs. placebo).

Table 2. Dose-Escalated 4-arm Study

Arm	Placebo	1ug	3ug	10ug
Period 1	2	6		
Period 2	2		6	
Period 3	2			6

Dose Levels
Cohorts of subjects will be sequentially enrolled at progressively higher starting dose levels. The following dose levels are planned:

- Dose Level 1: 1 µg
- Dose Level 2: 3 µg
- Dose Level 3: 10 µg

These doses are within the dose range of 1 µg to 10 µg and equal or greater than five times less than that which has been previously evaluated in healthy subjects and patients with advanced solid tumors.

5.4 Preparation and Administration of Study Drug

All dilutions of entolimod are performed in a biosafety cabinet under aseptic conditions by a licensed pharmacist on the day of injection. The drug dilution instructions for each dose level are described below and a summary of instructions is provided in Table 3.

Dose Level 1: 1 µg of entolimod per injection
Using 10 cc syringe remove 8.7 mL of diluent from 50 mL diluent vial and transfer this aliquot into 10 mL empty sterile vial. Using 0.3 or 1 cc syringe remove 0.3 mL of entolimod from the drug product vial. Holding the vial with 8.7 mL of diluent upside down inject 0.3 mL of

entolimod solution directly into the diluent in the vial (dilution 1:30, final entolimod concentration 3.3 µg/mL). Mix the vial by gentle rotation.

Dose Level 2: 3 µg of entolimod per injection
Using 3 cc syringe remove 2.7 mL of diluent from 50 mL diluent vial and transfer this aliquot into 10 mL empty sterile vial. Using 0.3 or 1 mL syringe remove 0.3 mL of entolimod from the drug product vial. Holding the vial with 2.7 mL of diluent upside down inject 0.3 mL of entolimod solution directly into the diluent in the vial (dilution 1:10, final entolimod concentration 10 µg/mL). Mix the vial by gentle rotation.

Dose Level 3: 10 µg of entolimod per injection
Using 1 cc syringe remove 1 mL of diluent from 50 mL diluent vial and transfer this aliquot into the vial of drug product containing 0.5 mL of 100 µg/mL entolimod (dilution 1:3, final entolimod concentration 33.3 µg/mL). Mix the vial by gentle rotation.

Table 3. Dilution of 100ug/mL Entolimod for IM Injections.

Dose level, µg	Diluent volume, mL	100 µg/mL entolimod volume, mL	Diluted entolimod volume, mL	Dilution factor	Diluted entolimod concentration, µg/mL	Injection volume, mL	Number of injection syringes that can be filled
1	8.7	0.3	9	30	3.3	0.3	up to 26
3	2.7	0.3	3	10	10	0.3	up to 6
10	1	-	1.5	3	33.3	0.3	up to 4

Placebo group
Remove 0.3 mL of placebo from placebo vial using 1 cc syringe.

Subjects will receive a single dose of study drug in the form of an IM injection. This injection is to be administered in the upper arm within 1 inch of the injection site of the influenza vaccine.

5.5 Subject Compliance Monitoring

Subject adherence to the study treatment will be monitored by requiring the subjects to have the study medication administered on-site in the Clinical Research and Trials Unit (CRTU). Records of study medication administration will be documented. Drug accountability will be noted.

5.6 Prior and Concomitant Therapy

Prior/concomitant medications will be assessed at every visit including remote visits.

5.7 Packaging

Entolimod, placebo and diluent will be shipped under cold conditions to the site in cardboard boxes containing 10 single-use vials per box placed into foam inserts. Entolimod vials should remain in the boxes in which they are supplied or stored in a manner that prevents glass-to-glass contact.

Both packaging boxes and individual vials will be labeled with the liquid contents, date of manufacture, lot number, manufacturer information, storage requirements, Mayo Clinic's IRB number, the name of the PI and the federal investigational new drug use warning. See Figure 3.

Figure 3. Labels for Experimental Study Drug Vials and Secondary Shipment Packages.

Labels for entolimod vials

Solution for Injection 0.1 mg/mL Entolimod, 0.35 mL
Date of Manufacture: 22 AUG 2011
Lot Number: [redacted]
Manufactured by: Wacker Biotech B.V. (former SynCo Bio Partners B.V. Amsterdam, Netherlands)
Store at: -70ºC
Mayo Clinic IRB # 19-004847;
Dr. R. Pignolo
Refer to Clinical Protocol for Dosing
Caution – New Drug Limited By Federal Law To Investigational Use

Labels for secondary entolimod package

Solution for Injection 0.1 mg/mL Entolimod, 0.35 mL
Date of Manufacture: 22 AUG 2011
Lot Number: [redacted]
Manufactured by: Wacker Biotech B.V. (former SynCo Bio Partners B.V. Amsterdam, Netherlands)
Store at: -70ºC
Mayo Clinic IRB # 19-004847;
Dr. R. Pignolo
Amount in Box: 10 vials
Refer to Clinical Protocol for Dosing
Caution – New Drug Limited By Federal Law To Investigational Use

Labels for entolimod placebo vials

Solution for Injection Entolimod Placebo, 0.35 mL
Date of Manufacture: 18 AUG 2011
Lot Number: [redacted]
Manufactured by: Wacker Biotech B.V. (former SynCo Bio Partners B.V. Amsterdam, Netherlands)
Store at: -70ºC
Mayo Clinic IRB # 19-004847;
Dr. R. Pignolo
Refer to Clinical Protocol for Dosing
Caution – New Drug Limited By Federal Law To Investigational Use

Labels for secondary entolimod placebo package

Solution for Injection Entolimod Placebo, 0.35 mL
Date of Manufacture: 18 AUG 2011
Lot Number: [redacted]
Manufactured by: Wacker Biotech B.V. (former SynCo Bio Partners B.V. Amsterdam, Netherlands)
Store at: -70ºC
Mayo Clinic IRB # 19-004847;
Dr. R. Pignolo
Amount in Box: 10 vials
Refer to Clinical Protocol for Dosing
Caution – New Drug Limited By Federal Law To Investigational Use

Labels for entolimod diluent vials

Entolimod sterile diluent, 40 mL per single use vial
Date of Manufacture: 25 MAR 2016
Lot Number: [redacted]
Manufactured by: AMRI (Glasgow, United Kingdom)
Store at: 2-8ºC
Mayo Clinic IRB # 19-004847; Dr. R. Pignolo
Refer to Clinical Protocol for Use
Caution – New Drug Limited By Federal Law To Investigational Use

Labels for entolimod diluent package

Entolimod sterile diluent, 40 mL per single use vial
Date of Manufacture: 25 MAR 2016
Lot Number: [redacted]
Manufactured by: AMRI (Glasgow, United Kingdom)
Store at: 2-8ºC
Mayo Clinic IRB # 19-004847; Dr. R. Pignolo
Amount in Box: 10 vials
Refer to Clinical Protocol for Use
Caution – New Drug Limited By Federal Law To Investigational Use

5.8 Masking/Blinding of Study

In order to minimize the study bias, this study will use a process for randomized assignment to entolimod or placebo. The investigational drug blind will be maintained using the Research Pharmacy processes for preparation of the investigational product. All subjects and study personnel except for those directly involved with study drug preparation will be blinded to study

drug assignment for the entire study. All unblinded dosing information must be maintained in a secured area, accessible only by unblinded personnel.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Per GPI request entolimod (study drug), entolimod placebo and entolimod diluent will be shipped overnight to Mayo Clinic from Almac (Souderton PA), clinical storage and distribution facility. Entolimod and entolimod placebo will be shipped on dry ice. Entolimod diluent will be shipped on cold packs. Temperature monitors will be enclosed in all packages.

5.9.2 Storage

All study medication will be provided by GPI. Mayo Clinic Research Pharmacy will be responsible for the storage of medication. The storage condition for the study drug will be described on the medication label. Medication must be stored in a safe, secure location with limited access.

5.9.3 Dispensing of Study Drug

The study drug is to be used exclusively in the clinical study according to the instructions of this protocol and directions for use. The Investigator's designee is responsible for providing subjects with the study drug and instructions for dosing and proper storage of the study drug.

The Investigator's designee will record the amount of study drug dispensed and date of dispensing.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

The specific study procedures and time of activities to be conducted for each subject enrolled in the study are presented in tabular form in Table S-1.

Physical examinations, other clinical evaluations, and additional laboratory studies or more frequent assessments may be performed consistent with appropriate medical care for the subject, but these data will not necessarily be collected.

In order to optimize scheduling convenience for the subject and for the study center staff, screening procedures may be performed over as many days as necessary within the specified screening period. For scheduled visits and follow-up periods the permitted visit windows are indicated in the table.

For procedures to be performed at a specified time post-dose, the acceptable margin for actual time is the specified time ±10 minutes. If multiple procedures are to be done at the same time point, the preferred order is vital signs, blood sampling, and then ECG, with blood sampling (particularly for pharmacodynamics) occurring as close as possible to the specified time.
Missed procedures or evaluations should be performed as close to the originally scheduled date/time as possible. Based on the investigator's judgment, an exception can be made when rescheduling becomes medically unnecessary because it is too close in time to the next scheduled procedure or evaluation. In that case, the missed evaluation may be omitted.

6.1 Visit 1, Screening Visit (-28 to -7 days)

A tabulated summary of all visits and assessments described in the following sections is provided in Table S-1, Schedule of Events. To the extent possible, subjects will be expected to adhere to the established visit schedule.

After discussing the study with the investigator/appropriate study staff and after agreeing to study participation by signing the consent, subjects will be assigned a subject number. For any subject, it is the responsibility of the investigator or study team member to obtain written informed consent (subject's signature) prior to performing any protocol-mandated assessment.

However, assessments performed as part of the routine care of the subject may be used to assess eligibility. The subject number will identify the subject throughout the study. In case of re-screening, the subject number assigned during the first screening procedure will be retained.

Patients meeting the entry criteria and consenting to participate in the protocol will undergo the following:

- Eligibility confirmation and informed consent discussion and documentation
- Review of medications
- Brief Physical examination
- Medical history
- Respiratory infection assessment
- Demographic Information
- Quality of Life (QoL) questionnaire
- Mini-Mental State Examination (MMSE) cognitive status assessment
- ECG – assessment of QTc interval
- Vital signs (orthostatic blood pressure, temperature, respiratory rate, height, weight, oxygen saturation and heart rate)
- Blood work – screening labs [serum chemistry, hematology, coagulation, CMV, serum virology, cytokines, study drug related antibodies, long interspersed nuclear elements-(LINE-1), peripheral blood mononuclear cells (PBMCs) and biomarkers of senescence]
- Urine collection for urinalysis
- Frailty Assessments (includes questionnaires, physical function testing and body composition)

6.2 Visit 2, Treatment Visit – Day 0

This visit will be recorded as "Day 0" and will require a visit to the research center where the following tests will be performed:

- Vital signs (orthostatic blood pressure, oxygen saturation, temperature, respiratory rate, weight, and heart rate) prior to vaccine and investigational product administration and 2, 4 and 6 hours post administration
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- COVID-19 Screening questionnaire
- Flu vaccine administration
- Investigational product administration
- Nasal swab
- Blood work for pharmacodynamics, vaccine related antibodies, LINE-1, serum antibody testing, and peripheral blood mononuclear cells (PBMCs) prior to vaccine and investigational product administration and 2, 4 and 6 hours post administration
- SAVE procedures (if applicable)

After the observation period (6 hours) and prior to discharge from the Clinical Research and Trials Unit, a reassessment of vital signs including orthostatic blood pressures will be conducted on all study participants.

6.3 Visit 3, Safety Follow-up –Day 1

This visit will be recorded as "Day 1" and will require a visit to the research center where the following tests will be performed:

- Brief physical examination
- Vital signs (orthostatic blood pressure, oxygen saturation, temperature, respiratory rate, weight, and heart rate)
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire
- ECG
- Blood work – labs [serum chemistry, Hematology, coagulation, cytokines, LINE-1, and vaccine related antibodies]
- Urine collection for urinalysis

6.4 Visit 4, Safety Follow-up –Day 7 (remote visit)

This visit will be recorded as "Day 7" and will be a remote visit where the following tests will be performed:

- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire

6.5 Visit 5, Follow-up - Month 1

This visit will be recorded as "Month 1" and will require a visit to the research center where the following tests will be performed:

- Vital signs (orthostatic blood pressure, oxygen saturation temperature, respiratory rate, weight, and heart rate)
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire
- ECG
- Blood work – labs [serum chemistry, Hematology, coagulation, CMV, cytokines, vaccine related antibodies, LINE-1, peripheral blood mononuclear cells (PBMCs) and biomarkers of senescence]
- Urine collection for urinalysis

6.6 Visit 6, Follow-up – Month 2

This visit will be recorded as "Month 2" and will require a visit to the research center where the following tests will be performed:

- Vital signs (orthostatic blood pressure, oxygen saturation temperature, respiratory rate, weight, and heart rate)
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire
- ECG
- Blood work – labs [serum chemistry, Hematology, coagulation, cytokines, vaccine related antibodies, LINE-1, and peripheral blood mononuclear cells (PBMCs)]
- Urine collection for urinalysis
- Frailty Assessments (includes questionnaires, physical function testing and body composition)

6.7 Visit 7, Follow-up – Month 6

This visit will be recorded as "Month 6" and will require a visit to the research center where the following tests will be performed:

- Vital signs (orthostatic blood pressure, oxygen saturation temperature, respiratory rate, weight, and heart rate)
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire
- ECG
- Blood work – labs [serum chemistry, Hematology, coagulation, CMV, cytokines, LINE-1, and vaccine related antibodies]
- Urine collection for urinalysis
- Frailty Assessments (includes questionnaires, physical function testing and body composition)

6.8 Visit 8, Follow-up – Month 12

This visit will be recorded as "Month 12" and will require a visit to the research center where the following tests will be performed:

- Vital signs (orthostatic blood pressure, oxygen saturation, temperature, respiratory rate, weight, and heart rate)
- Review of medications
- Review of adverse events
- Respiratory infection assessment
- Quality of Life (QoL) questionnaire
- COVID-19 Screening questionnaire
- ECG
- Nasal Swab
- Blood work – labs [serum chemistry, Hematology, coagulation, cytokines, and vaccine related antibodies, LINE-1, peripheral blood mononuclear cells (PBMCs) and serum antibody testing]
- Urine collection for urinalysis
- Frailty Assessments (includes questionnaires, physical function testing and body composition)

6.9 Monthly phone visits – Month 3- Month 11

The following tests will be performed remotely (phone visits):

- Review of adverse events
- Review of medications
- Respiratory infection assessment
- COVID-19 Screening questionnaire

6.10 Examinations and Procedures

- *Physical examinations* will be limited to cardiac, pulmonary, abdominal, integumentary, and gross neurological evaluation, and to other systems based on specific subject complaints.
- *Medical history* is to be documented at Screening for each subject
- *Vital signs*, including pulse, orthostatic blood pressure, heart rate, respiratory rate, oxygen saturation and temperature will be measured.
- *Height and weight* will be obtained at the screening visit; weight will be obtained at each additional visit. Body mass index (BMI) will be recorded.
- A *12-lead electrocardiogram (ECG)* will be performed. The Investigator will review and assess all abnormal results for clinical significance. Any post-baseline ECG abnormalities assessed as clinically significant will be recorded as AEs.
- *Prior/Concomitant medications* will be assessed at every site and remote visit.

6.11 Laboratory Assessments

The following laboratory assessments will be collected at the time points specified in the schedule of events. The Investigator will be provided all laboratory results and will review and assess out-of-range findings for clinical significance. Any post-baseline abnormal laboratory value assessed as clinically significant will be recorded as an AE.

Blood Tests:

- *Serum Chemistry* studies will include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total protein, albumin, ALT, AST, ALP, CK, LDH, GGT, total bilirubin, uric acid and CRP.
- *Hematology parameters* will include hematocrit, hemoglobin, erythrocyte count, absolute counts of leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count.
- *Coagulation studies* will include PT and aPTT.
- *Virology evaluation* includes serum CMV, HIV antibody, HBsAg antibody, HBc antibody, HCV antibody, Subjects will a positive antibody evaluation for HBc or HCV should undergo evaluation for HBV DNA and for HCV RNA to determine if the antibody test may be falsely positive.
- *PBMC Culture Supernatant Assessment* will include 10 cytokine/chemokine mediators of adaptive immune function (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α) test performed using Meso Scale Discovery, V-PLEX Proinflammatory panel human immunoassay kits.
- *Serum for study related antibodies* includes influenza antibody titers against A/H1N1, A/H3N2, and B viruses, LINE-1, and entolimod specific neutralizing antibodies.
- *Plasma for cytokines* will include G-CSF and IL-6 tests will be performed using the MSD assay platform as well as senescence biomarkers. These samples will be de-identified and shipped to the sponsor, GPI, in a coded format. Samples will be used for research purposes only as described in this protocol. The data will be reported to the Mayo Clinic team for the analysis. When the study is completed, the sponsor will destroy any remaining samples.
- *COVID-19 testing* will include COVID-19, PCR [SARS Coronavirus 2, Molecular Detection, PCR (NP)] and SARS-CoV-2 IgG Ab [SARS Coronavirus 2 IgG Ab, Serum]

Urine Tests:

- Urinalysis will include specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrate, leukocyte esterase as assessed by dipstick and microscopic urinalysis evaluating white blood cells, epithelial cell, bacteria, casts, and crystals.
- Urine will also be assessed for senescence biomarkers.

6.12 MMSE and Quality of Life (QoL) Assessments

The *Mini-Mental State Examination* (MMSE) is a 30-point questionnaire that is used to measure cognitive impairment. It includes tests of orientation, attention, memory, language and visual-spatial skills.

The *Short Form (36) Health Survey* will be administered to subjects. This survey consists of 36 questions and eight scaled scores (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health).

6.13 Frailty Assessments

Frailty phenotyping will consist of 5 measures, including grip strength, walking speed, body composition, and questionnaires that will assess endurance and energy, unintended weight loss and physical activity level.

Skin advanced glycation end products (AGEs) will be assayed using an *AGE Reader*®. This non-invasive method has a light source that illuminates a skin surface of approximately 4 cm^2 on the volar side of the forearm. The device uses an excitation light source with peak intensity of approximately 370 nm to excite fluorescent moieties in the tissue (skin autofluorescence), which then will emit light of a different wavelength. In the used wavelength band, the major contribution to fluorescence comes from fluorescent AGEs linked mostly to collagen. Emission light and reflected excitation light from the skin is measured with a spectrometer in the 300–600 nm range.

6.14 SAVE Assessments

SAVE is a non-invasive imaging technique used to measure viscoelastic characteristics of tissue. This technique enables imaging biphasic characteristics of tissue which are related to both extra-cellular matrix changes as wells as disruption in the interstitial fluid mobility due to structural changes.

6.15 Adverse Events

Adverse event monitoring will be conducted throughout the study for all subjects. The AE and serious adverse event (SAE) reporting period begins at the time of informed consent and continues through study completion. Adverse events will be assessed at every site and remote visit.

The Investigator will follow-up on all AEs observed or reported by the subject up to the end of the reporting period or until follow-up is no longer necessary. The Investigator will follow-up on SAEs until they are considered resolved or the outcome is known.

Definitions, documentation, and reporting of AEs are described in Section 8. Serious adverse events must be reported within 24 hours as described in Section 8.2.

Table S-1. Schedule of Events

PERIOD	Screening Visit	Treatment Visit	Safety Follow-Up		Monthly Follow-Up					
ON-SITE VISIT	**1**	**2**	**3**	**4 (remote)**	**5**	**6**	**-**	**7**	**-**	**8**
WEEK	**-4**	**0**	**Day 1**	**1**						
MONTH					**1**	**2**	**3 to 5[p]**	**6**	**7 to 11[p]**	**12**
Visit Window, week(s)	**-28 days**			**± 1 day**	**± 1 week**					
General Eligibility, Safety, and Frailty Assessments										
Eligibility assessment	X									
Written informed consent	X									
MMSE cognitive status assessment	X									
Frailty assessments [a]	X					X		X		X
Brief physical examination [b]	X		X							
Medical history	X									
Demographic Information	X									
Vital signs/oxygen saturation/Ht/Wt[c]	X[q]	X[d][q]	X[q]		X[q]	X[q]		X[q]		X[q]
12-lead ECG	X		X		X	X		X		X
AE assessment, including respiratory infection assessment	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Quality of life (QoL) questionnaire	X		X	X	X	X	X	X	X	X
Study Drug Administration										
Study drug		X								
Laboratory Assessments										
Serum chemistry[e]	X		X		X	X		X		X
Hematology [f]	X		X		X	X		X		X
Coagulation [g]	X		X		X	X		X		X
Serum virology [h]	X									
Urinalysis [i]	X		X		X	X		X		X
Serum for entolimod-reactive antibodies[j]	X		X		X	X		X		X
Plasma for pharmacodynamics[k]		X								
Serum for vaccine related antibodies [l]		X			X	X		X		X
Peripheral blood mononuclear cells (PBMC)[m]		X			X	X				X
COVID-19, PCR [SARS Coronavirus 2, Molecular Detection, PCR (NP)]		X								X
Exploratory Procedures										
SAVE[n]		X								
Biomarkers of senescence[o]		X			X			X		X
SARS-CoV-2 IgG Ab [SARS Coronavirus 2 IgG Ab, Serum]		X								X

	Footnotes
	[a] To be collected at screening, baseline, months 2, 6 and 12 (includes questionnaires, physical function testing, and body composition)
	[b] Physical examination will be limited to cardiac, pulmonary, abdominal, integumentary, and gross neurological evaluation, and to other systems based on specific subject complaints.
	[c] Height will only be collected at screening; weight will be collected at all in-person visits
	[d] To be collected pre-treatment and 2, 4 and 6 hours post treatment. Wt will only be performed prior to study drug administration.
	[e] Serum chemistry studies will include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total protein, albumin, ALT, AST, ALP, CK, LDH, GGT, total bilirubin, uric acid, and CRP.
	[f] Hematology parameters will include hematocrit, hemoglobin, erythrocyte count; absolute counts of leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils; platelet count.
	[g] Coagulation studies will include PT and aPTT.
	[h] Virology evaluation includes serum CMV, HIV antibody, HBsAg antibody, HBc antibody, HCV antibody. Subjects with a positive antibody evaluation for HBc or HCV should undergo evaluation for HBV DNA and for HCV RNA to determine if the antibody test may be falsely positive. Only CMV testing will be carried out on months 1 and 2.
	[i] Urinalysis will include specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrite, leukocyte esterase as assessed by dipstick and microscopic urinalysis evaluating white blood cells, red blood cells, epithelial cells, bacteria, casts, and crystals.
	[j] Test will be performed by GPI. Samples may also be used for additional testing related to the study objectives.
	[k] G-CSF and IL-6 tests will be performed using the MSD assay platform. Samples are to be collected pre-treatment and 2, 4 and 6 hours post treatment.
	[l] Influenza antibody titers A/H1N1, A/H3N2, and B assessed by HAI Assay
	[m] Meso Scale Discovery, V-PLEX Proinflammatory panel for 10 cytokine/chemokine mediators of adaptive immune function (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α) and Interferon gamma ELISPOT assay
	[n] Sub-hertz Analysis of ViscoElasticity (SAVE) will be performed 6 hours after study drug administration (or placebo) on left and right deltoid muscles in cohorts receiving entolimod (3μg and 10μg) and representing a total of 50 subjects.
	[o] Blood and urine will be collected for analyses of senescence
	[p] Subjects will be contacted bi-weekly via the telephone for remote follow-up visits
	[q] Orthostatic blood pressures will be performed at all visits

Abbreviations: AE=adverse event, ALP=alkaline phosphatase, ALT=alanine aminotransferase, aPTT=activated partial thromboplastin time, AST=aspartate aminotransferase, BUN=blood urea nitrogen, CK=creatine kinase, CMV = cytomegalovirus, CRP=C-reactive protein, CTCAE=Common Terminology Criteria for Adverse Events, DNA=deoxynucleic acid, ECG=electrocardiogram, GGT=gamma-glutamyl transferase, HBc antibody=anti-hepatitis B core antibody, HBsAg=hepatitis B surface antigen, HBV=hepatitis B virus, HCV=hepatitis C virus, HIV=human immunodeficiency virus, IV=intravenous, LDH=lactate dehydrogenase, PT=partial thromboplastin time, RNA=ribonucleic acid, SARS-CoV-2 IgG Ab = severe acute respiratory syndrome coronavirus 2 immunoglobulin G antibody; SAVE= Sub-hertz Analysis of ViscoElasticity, USP=United State Pharmacopeia, PBMC=peripheral blood mononuclear cells

Supportive Care

General Recommendations
Consistent with subject safety and comfort, administration of any prescription or over-the-counter drug products other than study medication will be minimized during the study period, except those currently used for chronic medical conditions at the time of enrollment or those prescribed by the subject's physician if not specifically contraindicated by the study design. Subjects will be discouraged from use of herbal remedies, self-prescribed drugs, tobacco products, alcohol, marijuana, or street drugs during their participation in the clinical study.

If considered necessary for the subject's well-being, drugs for concomitant medical conditions or for symptom management may be given at the discretion of the investigator. The investigator's decision to authorize the use of any drug other than study drug will take into account subject safety, the medical need, the potential for drug interactions, the possibility for masking symptoms of a more significant underlying event, and whether use of the drug will compromise the outcome or integrity of the study

Subjects will be instructed about the importance of the need to inform the clinic staff of the use of any drugs or remedies (whether prescribed, over-the-counter, or illicit) before and during the course of the study.

Recommendations with regard to specific types of concomitant therapies, supportive care, diet and other interventions are provided in the protocol. To minimize variations in supportive care, the recommended supportive care agents (e.g., loperamide, granisetron) should be used unless there is a medical rationale in a specific subject for use of an alternative product.

Anti-inflammatory or Antipyretic Drugs or Corticosteroids

Anti-inflammatory or antipyretic drugs may not be administered prophylactically. Thereafter, subjects may receive ibuprofen, 400 mg orally, as needed every 4 to 6 hours in response to constitutional symptoms, fever or malaise occurring following study drug administration. Other nonsteroidal anti-inflammatory drugs (NSAIDs) may be substituted as medically necessary. Post-entolimod use of acetaminophen, 650 mg orally as needed every 4 to 6 hours, may be used in response to constitutional or pyretic symptoms if medically necessary, but use of acetaminophen is not encouraged given its potential for adverse hepatic effects.

The use of systemic, topical, inhaled, or enteric corticosteroids during screening or on Day 1 should be avoided given that such drugs may confound interpretation of safety and pharmacodynamic responses in subjects on this study. However, such drugs are permitted after study drug administration if a subject develops an intercurrent condition that require corticosteroid therapy.

Support for Hypotension
In the event that a subject experiences hypotension (SBP <90 mm Hg or DBP <60 mm Hg; a > 20 mm Hg drop in SBP or > 10 mm Hg drop in SBP with symptoms at any time after administration of study drug or with orthostatics) it may be managed according to standards of

care until the SBP is ≥90 mmHg and the DBP is ≥60 mmHg on ≥2 determinations obtained ≥1 hour apart [or until BP approaches within 10% of baseline (pre-study drug values) with resolution of symptoms]. It should be emphasized that a subject's clinical condition, rather than blood pressure alone, should be the primary basis for any intervention or medical decision-making.

- As much as possible, subjects should be kept at rest following study drug administration.
- A subject requiring use of the bathroom should be evaluated for orthostatic changes. If the blood pressure is stable upon standing, and if the subject has no symptoms, the subject may ambulate to the bathroom, but study personnel should ensure the subject's safety. If there is a clinically significant drop in systolic blood pressure, or the subject complains of lightheadedness upon standing, the subject should be encouraged to use an emesis basin, urinal, bedpan, or bedside commode, as appropriate for the circumstances.
- For any hypotension more significant than minor dizziness, treatment should follow standard clinic protocol.

7 Statistical Plan

7.1 Sample Size Determination

A sample size of 25 per arm provides 90% power to detect a doubling (100% increase) in geometric mean influenza antibody titer A/H1N1 based on a one-sided two-sample t-test at alpha level 0.15. This calculation assumes a coefficient of variation of 2.0 for A/H1N1. Under the assumption of smaller coefficient of variation for A/H3N2 and B antibody titers of 1.4, 25 per arm provides 90% power to detect an 80% increase in these antibody titers at alpha level 0.15. Thus, we plan to enroll 25 to each arm, for a total sample size of 100. We anticipate greater power under the ANCOVA approach, adjusted for baseline levels of the titer.

7.2 Statistical Methods

The full analysis dataset will be based on a modified intent-to-treat principle. Randomized subjects receiving blinded study drug will be included and analyzed based on their randomized assignment. Patients meeting exclusion criteria or withdrawing after initial screening and randomization are excluded from all analyses. Safety outcomes will analyze patients based on treatment received. Evaluable analysis sets will be defined and will include data from subjects who have the necessary baseline and on study measurements to provide interpretable results for specific parameters of interest.

Descriptive Statistics

Data will be described and summarized by study drug assignment (entolimod 1μg, 3 μg, 10 μg, or placebo) and time point. Descriptive summaries will include sample size, mean and standard deviation, median, 25^{th} and 75^{th} percentiles, minimum and maximum for continuous variables and percentage for categorical variables. Baseline/screening data will be summarized by randomized group using median (25^{th}-75^{th}) percentiles and percentage for continuous and categorical data, respectively. Study drug administration, concomitant medication use, supportive care use, AEs, laboratory abnormalities, vital signs/oxygen saturation, body weight, virology data, quality of life and frailty index status will be described and summarized.

Primary Hypothesis: We hypothesize that entolimod will cause an increase in influenza antibody titers at 4 weeks in comparison to placebo

The distribution of antibody titers (A/H1N1, A/H3N2, B) during follow up is anticipated to be skewed based on reporting in prior studies and a log-transformation will be applied to these outcomes. Antibody titers are measured at multiple time points in the first week after treatment, 4 weeks, 2 months, 6 months, and 12 months. A hierarchical mixed-effects regression model will be fit separately for the 3 endpoints, adjusting for the baseline/screening observation of the titer (an analysis of covariance (ANCOVA) approach). The model will include random subject-specific intercepts and model time as discrete. The primary outcome comparison is a contrast analysis of the outcome at 4 weeks comparing each entolimod dose to placebo. As the overall aim of this study is to assess safety and identify potential efficacy of entolimod, especially the dose most likely to succeed in Phase III study, we apply a one-sided test at alpha level 0.15 to each dose comparison (vs. placebo) and antibody titer combination, without multiplicity adjustment. The primary analysis will be conducted using the modified intent-to-treat analysis dataset.

Secondary Hypotheses: Secondary and exploratory endpoints require a variety of standard statistical methods.

Key secondary endpoints include the frailty indices, measures of physical function (6 minute walk test and grip strength), and quality of life (QOL). Frailty indices are measured at 2, 6, and 12 months and QOL measured at each study visit. For these outcomes, linear mixed effects models will assess the relationship between randomized arm and outcomes over time. Discrete times will be considered corresponding to the study visit and secondary analyses may consider continuous linear time (constant slope over time) for changes in the outcome. For these outcomes, the 12 month observation is the primary comparison.

Upper respiratory infections, including influenza, will be recorded over the follow up period. We anticipate the total number of these will be infrequent. Thus, we compare the outcome of any infection (vs. no infection) across randomized arms using a Pearson chi-square test or Fisher's exact test. Secondary approaches may consider analysis of the ordinal count of the number of infections, analyzed using a trend test.

Cellular immune response and pharmacodynamics parameters, including concentration of circulating plasma cytokines and cytokine response to virial stimulation are outcomes compared using analysis of variance (ANOVA) methods across the four comparison groups. Pharmacodynamic measures will be listed and will be summarized using appropriate graphical and tabular methods. Statistical analysis evaluating pharmacodynamic parameters will be performed using contrasts in the context of analysis of covariance (ANCOVA).

Handling of Missing Data

In the analysis of primary outcomes, data are analyzed using a hierarchical regression approach assuming missing data are missing at random. In a secondary approach, patients with dropout or missing follow up due to influenza viral infection during follow up will be assumed missing not at random and missing data will be imputed to the baseline/screening value reflecting no increase

from baseline in antibody titers among these patients. Dropout for other causes will be considered similarly, except death unrelated to influenza, which will continue to be assumed as missing at random.

Interim Analysis
No interim analysis will be performed for efficacy assessment.

Multiplicity
No adjustments will be made for multiplicity correction to dose comparisons or the primary endpoints as the study aim is to identify promising doses for subsequent phase III study. Secondary outcomes will also not be adjusted for multiple comparisons.

7.3 Subject Population(s) for Analysis

The primary analysis uses a modified intent-to-treat principle. Patients will be analyzed based on randomized arm using methods described previously. Exploratory analyses will assess whether a differential sex-based response to treatment exists for the primary outcomes and secondary outcomes of FI and QOL, using interaction terms in regression models.

8 Safety and Adverse Events

For safety analyses, AEs will be classified using the Medical Dictionary for Regulatory Activities (MedDRA). The severity of AEs will be graded by the investigator according to the Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. Concomitant medication use will be coded using the World Health Organization Drug Dictionary (WHODRUG) into Anatomical Therapeutic-Chemical classification (ATC) codes; these data descriptions will particularly focus on supportive medications and care provided in response to any study-study-induced adverse effects and to therapies for GVHD. For ECG assessment of QT intervals, correction by both the Bazett and Fridericia methods will be applied, and the data will be summarized by CTCAE grading categories in terms of absolute QTc and maximal QTc change from baseline.

A safety analysis will be conducted after 20 patients (5 per arm) have been randomized and completed the one-week safety follow-up period. After the last subject has completed their one-week safety follow-up period, there will be a temporary halt in additional participation pending analysis of safety outcomes, anticipated to be no longer than seven days. A safety report will be generated after the 20th patient completes 1 week follow up, summarizing all adverse events overall and by study arm while maintaining blind (groups denoted as A-D in random order). If appropriate, as described below, unblinded and subject-level adverse event data will be provided. Adverse events, both short term (14 days) and long term (through 365 days), will continue to be collected with ongoing monitoring as specified in the Data Safety Monitoring Plan (DSMP).

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1)

death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as (365) days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if it changes from value within the normal range before treatment to one outside and worse than the normal range after treatment. Such changes will prompt a repeat test, telephone call to check the subject's status, a repeat visit, and/or referral to the subject's primary care physician.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery will **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.
- A visit to the emergency department or other hospital department <24 hours, that does not result in admission (unless considered an important medical or life-threatening event).
- Elective surgery planned prior to signing consent.
- Admissions as per protocol for a planned medical/surgical procedure.
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine mammogram).
- Medical or surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation will be obtained in these cases.

Recording of Adverse Events
At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.2 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.2.1 Sponsor-Investigator reporting: notifying the Mayo IRB

Information collected on the adverse event worksheet (*and entered in the research database)*:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously or after discontinuing treatment)
- Date of Resolution:

The Investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

Relationship
The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (PI) based on all available information at the time of the completion of the CRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

Severity
The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient’s daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.2.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Medwatch Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Medwatch Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Medwatch Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

The sponsor-investigator must also notify the FDA (and sponsors must notify all participating investigators) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under § 312.32(c)(1)(i)-(iv).

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.3 Unmasking/Unblinding Procedures

The investigational drug blind shall not be broken by the investigator unless information concerning the investigational drug is necessary for the medical treatment of the subject. In the event of a medical emergency, if possible, the sponsor-investigator or PI should be contacted before the investigational drug blind is broken to discuss the need for unblinding.

If the PI decides emergency unblinding is medically necessary, the PI needs to contact the Research Pharmacy. The sponsor-investigator must be notified as soon as possible if the investigational drug blind is broken. The date, time, and reason the blind is broken must be recorded in the source documents, CRFs or Event forms/logs.

8.4 Stopping Rules

A pre-specified safety assessment will be conducted after 20 subjects (5 per arm) have completed a one-week safety follow up period. Initial reports will maintain blinding of study arms. If any study arm reports a serious adverse event or ≥ 2 total adverse events that are possible, probable, or definite relation to the investigational study drug, study arms will be unblinded and data provided to an independent monitor as specified in the DSMB. Active high dose arms may be stopped early if safety concerns arise related to the investigational drug.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Internal Data and Safety Monitoring Board

A four person DSMB (clinical researchers) not affiliated with the study will be responsible for evaluating the progress of the study and will be provide unblinded data on a regular basis to monitor patient safety. This committee will communicate by meeting every other week during the enrollment phase as the study team will be utilizing a dose-escalated recruitment strategy. The DSMB members will be responsible for determining if and when the next dose can be administered in a subset of subjects or if the study must be discontinued as a result of excessive adverse events. Study data will be provided to the DSMB by the data coordinating center (including all adverse event reports). Data will be reviewed by the DSMB in an unblinded fashion. Randomization codes for each enrolled patient will be provided by the research pharmacist. The Committee will makes its recommendations by monitoring progress, data, outcomes, toxicity, safety and other confidential data, and may recommend stopping the clinical trial if an excessive number of serious adverse events are observed.

DSMB-Committee Members:

1) CHAIRPERSON: [redacted], Nephrology and Hypertension
2) STATISTICIAN: [redacted], Biostatistics
3) MEMBERS: [redacted], General Internal Medicine and [redacted], Community Internal Medicine

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. Case reports in the form of completed checklists will be kept to ensure inclusion/exclusion criteria and review of adverse events/toxicity. All data requested on the CRF will be recorded in indelible pen. Pencils are not to be used. Missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be written. If the item is not applicable to the individual case, "N/A" will be written. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. Errors will not be erased and "white-out" will not be used to correct errors. For clarification of illegible or uncertain entries, the clarification will be printed above the item, initialed, and dated. If the reason for the correction is not clear or needs additional explanation, details will be added related to the justification for the correction.

Data Management

The data will be housed in both hard copy case report forms (CRFs) and eCRFs through a system called Medidata RAVE.

Data Processing

Source documents and CRFs and original consents will be stored in secured locations. All data will be entered into a password protected, limited access database. Individually-identifiable patient history and medical record information will be stored in a database under coded accession numbers. Clinical laboratory values will be stored in the electronic medical record system, requiring protected password access. These data are monitored regularly for access and a formal policy regarding protection of personal privacy is in place. The key to identification of subjects will be maintained in a secure office environment under the direction of the principal investigators.

Data Security and Confidentiality

Source documents and CRFs and original consents will be stored in secured locations. All data will be entered into a password protected, limited access database. Individually-identifiable patient history and medical record information will be stored in a database under coded accession numbers. Clinical laboratory values will be stored in the electronic medical record system, requiring protected password access. These data are monitored regularly for access and a formal policy regarding protection of personal privacy is in place. The key to identification of subjects

will be maintained in a secure office environment under the direction of the principal investigators.

Data Quality Assurance
Manual and computerized quality checks will occur during data collection and analyses and any discrepancies will require Case Report Form (CRF) review and validation of correct data.

Data Clarification Process
Each eCRF contains edit checks and custom functions to ensure the highest possible data quality. Only necessary eCRF's are available for data entry to reduce the possibility of erroneous entry. After completion of data entry and resolving all outstanding queries, the database will be closed, and the data will be exported for statistical analysis.

9.4 Records Retention

The investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. The sponsor-investigator will retain the specified records and reports:

1. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted]
   - "2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated" OR
   - "2 years after the investigation is discontinued and FDA is notified if no application is to be filed or if the application has not been approved for such indication" OR
   - "Whichever is longer."

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will utilize a Data and Safety Monitoring Board (DSMB) to provide a system for appropriate oversight and attention to the protection of human subjects and quality assurance. The DSMB will have the following monitoring activities: (1) Review of a pre-specified safety assessment after 6 subjects in each arm have completed safety follow-up period day 1; If the DSMB determines that it is safe to proceed the next dose will be administered and a safety assessment will be performed to determine if it is safe to continue to the highest dosing of 10ug; (2) prospective review of any issues related to the safety of subjects; and (3) regular review (i.e., twice yearly) of measures designed to protect the validity of the data and the integrity of the research study. With regard to monitoring activity (2), safety issues will include the timely review of all SAEs (within 24 hours) and the regular review (every 3 months) of all AEs. With regard to monitoring activity 3, the DSMB will review with the principle investigator and lead study coordinator the methods in which data is collected, stored, and protected, including review of randomly selected subject case report and other forms. The DSMB will also identify when to terminate a subject's participation by following prescribed individual stopping rules and when to terminate the study by following prescribed study stopping rules. Prescribed individual stopping rules will include the following: (1) Any SAE that precludes further participation of the subject; (2) With respect to subjects that have not received study drug, any SAE related to the study drug that in the opinion of the principle investigator or DSMB places subjects at high risk of

experiencing the same SAE; (3) if in the opinion of the principle investigator, there is doubt that a subject, for any reason, can continue to fully participate in the study. Prescribed study stopping rules will include the following: (1) dose-limiting toxicities as defined elsewhere in this document in $\geq$ 2 subjects for the lowest dose concentration of study drug. See also section 8.4, *Stopping Rules*. This will not preclude the analysis of pre-existing data.

In addition to ongoing review of study progress by the Sponsor-Investigator, and delegated research staff, the key study source data, the study database and regulatory documents will be monitored on a periodic basis by the Mayo Clinic Office of Research Regulatory Support for the Mayo Clinic sites.

Study monitoring is provided as a service for the sponsor-investigator to assist with compliance activities and to assess protection of research subjects and data validity. Monitoring may include but is not limited to a Study Initiation Visit and periodic interim monitoring visits as the study progresses. Written reports will be issued to the Sponsor-Investigator and Study Coordinator and should be provided to the IRB by Mayo Clinic study staff at the time of continuing IRB review.

The Principle Investigator will allocate adequate time for such monitoring activities, give access to all study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.) as needed, and provide adequate space to conduct monitoring inquiries.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study will be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the Mayo Clinic Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the Mayo Clinic IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, will be obtained before that subject undergoes any study procedure. The consent form will be signed by

the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study will be financed by Genome Protection Inc.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, *etc*.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

12.3 Subject Stipends or Payments

Remuneration will consist of up to $600.00 if the subjects complete the entire study. Subjects will receive remuneration for each visit as follows:

1. Visit 1: Screening	$100.00
2. Visit 2: Baseline/Randomization	$75.00
3. Visit 3: Follow-up Day 1	$75.00
3. Visit 4: Follow-up Day 7	$25.00
4. Visit 5: Monthly Long-Term Month 1	$75.00
5. Visit 6: Monthly Long-Term Month 2	$75.00
6. Visit 7: Monthly Long-Term Month 6	$75.00
7. Visit 8: End of Study Month 12	$100.00

Completion of a study period means that the subject followed all study-related procedures for each day as described in the consent form. In addition, subjects will receive parking passes or taxi reimbursement or cost per mile traveled to the research center for the time involved with completing the study visits or travels to the research center for study visits. Subjects will be directed where to park in order to receive the parking passes. For subjects who travel greater than 50 miles to the research center subjects will be eligible to receive reimbursement for travel expenses including mileage at the current IRS mileage rate and parking. In order to receive reimbursement, subjects will be required to provide a copy of the original receipts for those expenses.

13 Publication Plan

It is the intention of the principle investigator to publish the results regarding the primary, secondary, and exploratory endpoints of this study. Criteria set by the International Committee of Medical Journal Editors will be used to establish authorship. The primary responsibility for publication of the study results, including determination of authors and order of authorship, will be with the principle investigator of record. It is understood that the primary investigator of record will work in collaboration with a designee of the sponsor, and all co-authors to produce

manuscript(s) suitable for publication and with the approval of all authors. There will be agreement between the principle investigator of record and the sponsor designee before any study-generated information can be used or passed on to a third party. All publications will refer to the ClinicalTrials.gov registration of the study and will adhere to any regulations related to posting of results to ClinicalTrials.gov (e.g., within 12 months of final data collection for the primary outcome).

14 References

1. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine*. 2006;24(8):1159-1169. doi:10.1016/j.vaccine.2005.08.105
2. Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. Biology of immune responses to vaccines in elderly persons. *Clin Infect Dis*. 2008;46(7):1078-1084. doi:10.1086/529197
3. Henry C, Zheng N-Y, Huang M, et al. Influenza Virus Vaccination Elicits Poorly Adapted B Cell Responses in Elderly Individuals. *Cell Host Microbe*. 2019;25(3):357-366.e6. doi:10.1016/j.chom.2019.01.002
4. Rolfes MA, Yousey-Hindes KM, Meek JI, Fry AM, Chaves SS. Respiratory Viral Testing and Influenza Antiviral Prescriptions During Hospitalization for Acute Respiratory Illnesses. *Open forum Infect Dis*. 2016;3(1):ofv216. doi:10.1093/ofid/ofv216
5. Burdelya LG, Brackett CM, Kojouharov B, et al. Central role of liver in anticancer and radioprotective activities of Toll-like receptor 5 agonist. *Proc Natl Acad Sci U S A*. 2013;110(20):E1857-66. doi:10.1073/pnas.1222805110
6. Burdelya LG, Krivokrysenko VI, Tallant TC, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*. 2008;320(5873):226-230. doi:10.1126/science.1154986
7. Yoon S, Kurnasov O, Natarajan V, et al. Structural basis of TLR5-flagellin recognition and signaling. *Science*. 2012;335(6070):859-864. doi:10.1126/science.1215584
8. Krivokrysenko VI, Shakhov AN, Singh VK, et al. Identification of granulocyte colony-stimulating factor and interleukin-6 as candidate biomarkers of CBLB502 efficacy as a medical radiation countermeasure. *J Pharmacol Exp Ther*. 2012;343(2):497-508. doi:10.1124/jpet.112.196071
9. Vijay-Kumar M, Aitken JD, Sanders CJ, et al. Flagellin treatment protects against chemicals, bacteria, viruses, and radiation. *J Immunol*. 2008;180(12):8280-8285. doi:10.4049/jimmunol.180.12.8280
10. Burdelya LG, Gleiberman AS, Toshkov I, et al. Toll-like receptor 5 agonist protects mice from dermatitis and oral mucositis caused by local radiation: implications for head-and-neck cancer radiotherapy. *Int J Radiat Oncol Biol Phys*. 2012;83(1):228-234. doi:10.1016/j.ijrobp.2011.05.055
11. Jones RM, Sloane VM, Wu H, et al. Flagellin administration protects gut mucosal tissue from irradiation-induced apoptosis via MKP-7 activity. *Gut*. 2011;60(5):648-657. doi:10.1136/gut.2010.223891
12. Kojouharov BM, Brackett CM, Veith JM, et al. Toll-like receptor-5 agonist Entolimod broadens the therapeutic window of 5-fluorouracil by reducing its toxicity to normal tissues in mice. *Oncotarget*. 2014;5(3):802-814. doi:10.18632/oncotarget.1773
13. Krivokrysenko VI, Toshkov IA, Gleiberman AS, et al. The Toll-Like Receptor 5 Agonist Entolimod Mitigates Lethal Acute Radiation Syndrome in Non-Human Primates. *PLoS One*. 2015;10(9):e0135388. doi:10.1371/journal.pone.0135388
14. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res*. 1993;10(7):1093-1095.
15. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. *J Clin Invest*. 2013;123(3):958-965.

doi:10.1172/JCI64096
16. Public summary of opinon on ophan designation (EU/3/15/1607): Entolimod for the tratment of acute radiation syndrome. 2016 Jan 11. European Medicines Agency. https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3151607. Published 2016. Accessed July 9, 2019.
17. Howell BA, Siler SQ, Shoda LKM, Yang Y, Woodhead JL, Watkins PB. A mechanistic model of drug-induced liver injury AIDS the interpretation of elevated liver transaminase levels in a phase I clinical trial. *CPT pharmacometrics Syst Pharmacol*. 2014;3:e98. doi:10.1038/psp.2013.74
18. Weber J, Gunn H, Yang J, et al. A phase I trial of intravenous interleukin-6 in patients with advanced cancer. *J Immunother Emphasis Tumor Immunol*. 1994;15(4):292-302. http://europepmc.org/abstract/MED/7520334.
19. Roberts AW. G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors*. 2005;23(1):33-41. doi:10.1080/08977190500055836
20. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. *Science*. 1987;236(4806):1229-1237. doi:10.1126/science.3296190
21. Dainiak N. Radiation response: Changing concepts and emerging paradigms. *Exp Hematol*. 2003;31(6):435-436.
22. Hawn TR, Verbon A, Lettinga KD, et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J Exp Med*. 2003;198(10):1563-1572. doi:10.1084/jem.20031220
23. Hawn TR, Wu H, Grossman JM, Hahn BH, Tsao BP, Aderem A. A stop codon polymorphism of Toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. *Proc Natl Acad Sci U S A*. 2005;102(30):10593-10597. doi:10.1073/pnas.0501165102
24. Hawn TR, Wu H, Grossman JM, Hahn BH, Tsao BP, Aderem A. A stop codon polymorphism of Toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. *Proc Natl Acad Sci U S A*. 2005;102(30):10593-10597. doi:10.1073/pnas.0501165102
25. Bayat M, Nabavizadeh A, Kumar V, et al. Automated In Vivo Sub-Hertz Analysis of Viscoelasticity (SAVE) for Evaluation of Breast Lesions. *IEEE Trans Biomed Eng*. 2018;65(10):2237-2247. doi:10.1109/TBME.2017.2787679