Impact of Host and Vaccine Characteristics on Immune Responses following Influenza Vaccination

Dissertation proposal by

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# Project Summary

Current influenza vaccines often perform poorly against novel strains. The development of a universal influenza vaccine is hindered by a lack of understanding of factors driving variation in the immune response to influenza. We will use longitudinal cohort data from multiple sources to explore how characteristics of vaccines interact with characteristics of the vaccine recipient to clarify potential drivers and mechanisms of the immune response to vaccination. We aim to explore how antigenic differences between the vaccine strain and historical strains (a surrogate for potential future strains) interact with the preexisting immune repertoire of the recipient, and how vaccine dose and prior vaccination history can modulate this relationship. In addition, we will propose metrics for the fair evaluation of vaccine candidates to multiple historical strains, which can allow the selection of an optimal broadly reactive vaccine. Our analysis will allow for improved development and evaluation of universal vaccine candidates.

# Project Narrative

Influenza is a respiratory disease that occurs in seasonal epidemics worldwide. The Centers for Disease Control and Prevention recommend annual vaccination against seasonal influenza with a modified vaccine for adults in the United States, but the effectiveness of these vaccines varies greatly across seasons and individuals. Improved understanding of the drivers of the immune response following vaccination is crucial for improving influenza vaccines.

# 1. SPECIFIC AIMS

Recent concerns about H5N1 influenza spillover events highlight the need for a universal influenza vaccine with the ability to mitigate future pandemic events. However, a combination of rapid antigenic evolution and heterogeneity in individual host response makes developing such a universal vaccine difficult. Previous literature has characterized the importance of prior immunity, vaccine design, and antigenic distance between the vaccine and host strain. The goal of my thesis is to understand how pre-existing immunity affects individual response to the seasonal influenza vaccine, and how this effect is modified by other factors. A better understanding of the host immune response will inform the design and evaluation of vaccines which induce a broader, more robust response.

We will use Bayesian hierarchical models, causal inference, and machine learning methods to quantify these effects. Our models will account for interactions between predictors, nonlinear effects, and clustered measurements. We will analyze longitudinal influenza vaccination data from the UGAFluVac cohort study (PI: Ross), wherein individuals provided serum samples, potentially for multiple years, and these samples were tested against panels of historical viruses. We will also combine the UGAFluVac data with other data sources provided by Ben Cowling, Andrea Sant, and potentially other investigators involved with the DIVERsity study (NIH project number 1R01AI170116-01) or the CIVR-HRP NIH CIVICs site (NIH project number 75N93019C00052) to answer questions about vaccine dose and pre-existing immunity.

**Aim 1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth.** Using the UGAFluVac data, we will analyze the relationship between immune response and *antigenic distance*, a measurement of how different the assay strain and the vaccine strain are. We will develop metrics for quantifying the overall strength of the immune response to a panel of heterologous strains, and the breadth of the response – intuitively, how the immune response diminishes as antigenic distance increases. We will also use subsamples of the UGAFluVac data to analyze the robustness of these metrics across differing panels of historical viruses.

**Aim 2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.** Influenza vaccinations provide diminishing boosts for recipients with high antibody titers, called the “antibody ceiling” effect. However, the threshold and rate of diminishing boosts depend on several other host and vaccine factors, including prior vaccination history, vaccine dose, and antigenic distance. We will use hierarchical statistical models and mechanistic models to disentangle the individual effects and interactions.

**Aim 3. Explore how age and vaccine dose interact to effect the antibody response.** The UGAFluVac cohort allows participants over the age of 65 to choose whether they receive FluZone standard dose or FluZone high dose, and Andrea Sant’s cohort study administered FluZone HD to individuals aged 18 – 49. By combining the two datasets, we can use causal inference and hierarchical modeling techniques to understand the effect of dose, and how this relates to previous mechanistic modeling predictions.

# 2. SIGNIFICANCE

Influenza is a rapidly evolving pathogen that evolves to escape current vaccines (1,2). All seasonal influenza vaccines currently available to the public focus on four lineages of influenza, which are predicted to be the dominant circulating strains for the upcoming season. The seasonal vaccine is thus not effective at preventing circulation of new antigen phenotypes that arise spontaneously from zoonotic spillover events, leading to pandemics like the 2009 H1N1 outbreak (3). The highly pathogenic H5N1 spillover cases in early 2023 (4) acutely demonstrate the need for a “universal” influenza vaccine that is broadly protective, and provides protection against emergent strains.

Designing a universal influenza vaccine has proven to be challenging (5–7). Understanding the immune response to influenza is complicated by rapid pathogen evolution and the accompanying change in vaccine formulation. Since the immune response depends on an individual’s history of influenza infection and vaccination events, the susceptible population displays an incredible diversity in immune repertoires even after controlling for effects like imprinting and prior vaccination. Decomposing the immune response to novel influenza strains into a set of mechanisms, and quantifying the relative contribution of each of these mechanisms is an open problem. Dissecting the components of the response would yield key insights into the design of vaccines which are reliably broadly-protective for individuals regardless of their immune state at time of vaccination.

**Aim 1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth.**

There is no universally accepted way to quantify the breadth of the immune response to a vaccine. Since direct trials of vaccine efficacy are expensive, correlates of protection (CoP) are typically used to measure the immune response to a given influenza vaccine. Several CoPs are currently in use with no clear consensus on which is best (8), but the most common CoP used in practice is hemagglutination inhibition (HAI) titer. We will focus on HAI titer in this project, but the methods we propose could be applied to any quantitative CoP.

HAI is correlated with protection from influenza with a 50% protection titer of 1:40 (9,10). If an individual’s reciprocal titer is 40 or greater for a particular strain of influenza, the individual is said to be *seroprotected* against that strain. If a vaccine induces a 4-fold or greater increase in HAI titer, with the final post-vaccination measurement above 40, the individual is said to have *seroconverted* against that strain. Seroconversion and seroprotection are commonly used dichotomous clinical endpoints for assessing the immunogenicity of an influenza vaccine candidate.

The traditional method for evaluating the breadth of an individual’s immune response following vaccination is to perform CoP assays against a variety of historical strains of influenza. The breadth of the response is then taken as either the count or proportion of strains to which the individual seroconverted (11,12). While this method is easy to quantify in a laboratory setting, the estimates of breadth are biased by the selection of the panel of historical strains, and variation in panels between laboratories makes comparing these estimates across studies difficult (13).

Modern methods for assessing the antigenic distance between strains of influenza allow the development of a consistent framework for assessing the breadth of a vaccine candidate. The simplest method for computing antigenic distance is the *time-based* method, where the antigenic distance is taken as the difference in isolation year between strains (14,15). Other methods include sequence-based methods, which assess the similarity of the genetic or protein sequence of the two strains (16–20); and antigen-based methods, which use immunogenicity data to inform distance between strains (21–25). There is no clear consensus on which measures of distance are most useful for informing vaccine evaluation. While previous work has explored the quantitative comparison of these so-called “antibody landscapes” (referring to the CoP measurement as a function of antigenic distance) (14), such approaches have not been widely utilized.

We will explore metrics for evaluating vaccine candidates that explain the **strength** of the response to the homologous strain; the **breadth** of the response explaining how the immune response is related to antigenic distance; and the **overall** response, which will weight the strength and breadth into an overall metric for the immune response induced by the vaccine. Using our framework for vaccine evaluation, we will compare measures of antigenic distance. We also plan to test the robustness of our metric to the selection of the virus panel in order to compare our framework to the traditional method. We will explore simple regression models, flexible spline models, and functional data analytic techniques for their potential to characterize the overall strength and breadth of the immune response. For this aim, we will use longitudinal cohort data with a wide panel of heterologous responses for each individual collected by Ted Ross (26–28).

**Aim 2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.**

Several characteristics of both the vaccine and the recipient are known to be associated with the immune response to the vaccine. In addition to antigenic distance between the vaccine strain and the strain of interest, several details of the vaccine formulation are associated with immunodeficient including dose (29–31), route of administration (32–34), and vaccine type (35–37). Promising vaccine candidates have been developed using intranasal, intramuscular, and subdermal routes of administration, and recombinant protein or mRNA vaccines may prove to be superior to the traditional split-inactivated or live attenuated vaccine types.

In addition to vaccine design choices, “intrinsic” characteristics – baseline characteristics of the vaccine recipient – are also known to potentially alter the vaccine response. These characteristics fall into two large categories: multifaceted characteristics that vary with every member of the susceptible population, and factors which can be aggregated at the population level. In the former category are genetic differences (38–40), epigenetic modifications, and differential gene expression (27,41–45), all of which play a significant role in the immune response to influenza. In the latter category are individual characteristics like sex, obesity, and age, which are easier to measure and understand.

Birth sex and circulating hormones may influence the immune response to flu through both sex-associated genetic differences or through sex-differentiated hormonal signaling, although results are ambiguous with no mechanism yet discovered (46–50). Obesity, typically measured through BMI, is associated with a decreased response or with more rapid waning of antibodies (51). As individuals age, they undergo immunosenescence and a gradual decline in immune protection (12,52).

In addition to the immunosenescence effect of age, an individual’s birth year is predictive of the immune response to specific strains of influenza, because birth year is strongly associated with the first strain of influenza to which an individual is exposed (53–55). The theory of imprinting (also called original antigenic sin) predicts that an individual’s first influenza exposure leads to the development of a memory response to that strain. Future exposures can then activate the memory response to the original strain (assuming the strains are somewhat similar), which dominates the novel immune response to the new strain, as influenza antibodies reach a saturation level called the “antibody ceiling”, which can vary between individuals (56–58)

One consequence of the imprinting effect and of the antibody ceiling effect is a strong negative relationship between pre-vaccination immunity and the response to a vaccine (36,59). Prior and repeat vaccination also has a strong effect on vaccine response (12,60), potentially independent of the antibody ceiling effect. In fact, the reactivation of the memory response at every exposure to a somewhat similar strain makes the response to vaccination dependent on an individual’s entire history of influenza infection and vaccination (15,23,56,61). In addition, the immune response to influenza may be affected by prior exposure to other pathogens, including herpesviruses such as Epstein-Barr virus (62) or cytomegalovirus (63), or through antigen-independent effects which modify the baseline immune state and induce a differential response (64).

While measuring all of these effects simultaneously is impossible, we intend to model the effects of vaccine dose, pre-vaccination titer, prior vaccination, and antigenic distance using data from the cohort studies conducted by Ted Ross. While our analysis will be limited to a single vaccine (Sanofi Pasteur’s FluZone, with standard and high dose formulations), we have data on an extensive panel of historical viruses along with reported vaccination history for each patient. We will incorporate predictive machine learning approaches and hierarchical bayesian modeling to understand the individual contributions of these factors to the overall immune response. We also plan to modify previously-developed mechanistic models for multiple influenza epitope responses (65–67) to include a degree of similarity between epitopes (conceptually representing antigenic distance), and compare the results from the updated model to our data.

**Aim 3. Estimate the effect of vaccine dose and age on vaccine response.**

The role of vaccine dose is so important in vaccine response that determining a dose that balances efficacy and side effects is a crucial part of drug approval in the United States (5,68,69). High-dose influenza vaccines are approved for use in older adults and can substantially improve the immune response for older and otherwise immunocompromised individuals (31,70,71). While otherwise healthy recipients generate substantive immune responses even to fractional doses of influenza vaccine (72,73), mechanistic models predict that increased dose may be useful in overcoming the negative effects of prior immunity and original antigenic sin (65,66).

These mechanistic models allow for simulation of the immune response following vaccination for a wide range of inoculum doses, and predict that as the inoculum dose is increased, the effect of prevaccination titer is mitigated. That is, an individual with a higher prevaccination titer could potentially receive a higher dose than an individual with a low prevaccination titer, and observe the same fold-change in titer as a result of vaccination. To date, a randomized clinical trial comparing standard and high dose formulations of available vaccines has not been conducted in otherwise healthy adults 18 - 49, so these claims have not yet been evaluated in groups that are not elderly or immunocompromised.

We will combine observational data from multiple sources to estimate the effect of dose on influenza vaccine response while controlling for the effects of prevaccination titer, age, and other relevant effects as enumerated previously. In this analysis, we specifically plan to focus on the effect of dose on homologous vaccine response by combining the UGAFluVac data, where high dose vaccines were only administered to individuals aged 65 or older, with data collected by Angela Branche and Andrea Sant (36,74). We will obtain the average causal effect of dose, after controlling for age and other confounders, and compare the predictions to those of the mechanistic model, which does not currently account for age. Together, these analyses will provide a better substantive understanding of the effect of dose on the immunogenicity of a standard influenza vaccine.

# 3. APPROACH

## 3.1 Data description

We will combine data from multiple influenza vaccination cohort studies for our analyses. At the time of writing, we have data from two cohort studies conducted by Ted Ross, one study conducted by Andrea Sant and Angela Branche (see (36)), and a collaboration with Ben Cowling, who is the PI of several similar studies and has offered to share data with us. The two studies conducted by Ted Ross provide a unique opportunity to study heterologous antibody responses to influenza vaccination, as a wide historical panel of assays was conducted for each individual for several years of the study. Combining Ross’ data with data from Sant and Cowling will allow for us to compare the immunogenicity of multiple vaccines, and increase the power of our analyses of host factors.

We will refer to both of Ross’ cohort studies conjointly as the **UGAFluVac** study. This data set consists of longitudinal HAI measurements taken at three different study sites. From fall 2013 to spring of 2016, participants were recruited at one of two study sites in either Pittsburgh, PA or Stuart, FL. Sample collection is further detailed in (26), among other references. Briefly, the study is a prospective open cohort design. Participants were adults aged 18 and up who were allowed to repeat each year, and the data includes a unique ID per participant that allows for the identification of longitudinal measurements. Each participant received a pre-vaccination blood draw, and was then administered a split-inactivated Fluzone seasonal influenza vaccine (Sanofi Pasteur). Patients aged 65 or older could opt to receive Fluzone High Dose instead. At the PA study site in 2013, some patients were administered an intradermal vaccine rather than the standard intramuscular Fluzone. Followup whole blood draws were targeted for 21 days post-vaccination.

Processed sera were used for HAI assays following standard protocols against the homologous vaccine strains as well as a heterologous panel of historical strains. The starting dilution was 1:10 and assays which did not agglutinate at the starting dilution were coded as 1:5. For all further analyses, we will use the reciprocal titer transformed as

This transformation serves to set 0 as the limit of detection on the log scale.

Additionally, the following data were collected from patients by a survey prior to vaccination: year of birth, age, gender (the covariate is listed as gender but is coded as male/female only), and race/ethnicity.

In January 2017, the study moved to the University of Georgia, Athens, GA. The paper (28) contains a description of the study, but at the time of writing, no published paper contains a complete description of the study cohort. The study design was similar, with additional covariate information collected: complete date of birth, sex assigned at birth, race/ethnicity, BMI, height, weight, and questions about smoking and comorbidities. Beginning in the 2017-2018 flu season, participants aged 10 and older were additionally recruited for the study. The HAI assays were conducted in the same way. The post-vaccination time point target was changed to Day 28 beginning in fall 2018. Finally, additional subcohorts were administered other vaccines at certain points during the study. See [Figure 3.1](#fig-michael) for details.

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| Figure 3.1: Study diagram showing the subcohorts and associated time points collected during the ongoing UGA study. The diagram was created by Michael Carlock, the CIVR-HRP research director. |

We will refer to the data provided by Andrea Sant and Angela Branche as the **RocFluVac** study. We have not yet finished cleaning and processing the data, but it contains similar HAI measurements for homologous strains only, participants were administered a variety of vaccines (including Fluzone HD administered to participants aged 18 - 49), and also contains data on T cell responses to the vaccine. See (36) for one brief explanation of the data.

## 3.2 Aim 1

For this aim, we will use the cohort data collected by Ted Ross.

### 3.2.1 Preliminary results

We have calculated several metrics of antigenic distance, and will compare the year-based method (15), the dominant *p*-epitope sequence based method (17), and a distance based on antigenic cartography (21,75). To compute the dominant *p*-epitope distance, the pairwise Hamming distances are computed between the amino acid sequences of each of the five hemagglutinin head epitopes, and these are divided by the length of their respective sequences. The dominant *p*-epitope measurement is the maximum of each of these probabilities, representing the different between the two strains at their most different epitope.

Antigenic cartography analyses were conducted by Amanda Skarlupka, PhD, who continues to work with us on this project. In short, antigenic cartography uses a matrix where each row represents an individual in the study and each column represents an influenza strain. The cells of the matrix are populated with individual ’s titer to strain . Multidimensional scaling is used to reduce the matrix to a specified column dimension, while minimizing the change in the Euclidean distances between measurements. After performing iterative MDS on a variety of target dimensions, we found that two dimensions was satisfactory, and our maps were similar to those in (21). After calculating the MDS maps, we then obtained antigenic distances as the Euclidean distance between map coordinates of the vaccine strain and the other strains in the panel following the method of (75). For the purposes of this analysis, we ignored longitudinal measurements between individuals, and treated each observation of an individual as a unique measurement. Finally, all of the antigenic distance were normalized *for each vaccine strain*. Therefore, each vaccine strain had a distance of 0 with itself, and the most distant historical strain had a distance of 1 with that strain. (We normalized all three of the distance measurements in this way.)

After computing the normalized distance measurements, we fit simple linear regression models with either post-vaccination titer (measuring the absolute immune response post-vaccination) or fold-change in titer (measuring the relative boost post-vaccination) against antigenic distance. [Figure 3.2](#fig-amandafits) shows sample linear regression models following this protocol for two vaccine strains.

Our proposed metrics for evaluating the **strength**, **breadth**, and **overall response** are the intercept of the regression line, slope of the regression line, and AUC respectively. The intercept measures how strong the response is to the homologous strain, the slope describes how the response weakens linearly as antigenic distance increases, and the AUC provides a framework for combining the strength and breadth measurements into one measurement of overall response.

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| Figure 3.2: Fitted models with titer outcomes and antigenic distance as the only predictor. The metrics reported are for titer increase for simplicity. |

As a case study to motivate our framework, we plan to analyze differences in these three metrics between the Fluzone SD and Fluzone HD vaccines, to determine if the high dose vaccine elicits a stronger or broader response in our cohort, as shown in [Figure 3.3](#fig-distdose). Our preliminary results suggests that this effect differs qualitatively across vaccines, and we plan to analyze which vaccines show the most notable differences between strains.

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| Figure 3.3: Linear models for titer increase vs. antigenic distance, stratified by vaccine dose. The same metrics are reported for both doses. |

### 3.2.2 Proposed studies

Going forward, we plan to consider more flexible models than the simple linear regression models we used in this step of the analysis. We will consider restricted spline GAMs and LOESS to fit a potentially nonlinear effect of antigenic distance, which we would expect in the presence of original antigenic sin if our panel contains strains which are distant enough to confer no cross-protection. We will also implement Bayesian multilevel generalizations of both the linear and spline models, in order to incorporate between-subject variability into the overall model fit.

We will then compare our metrics to traditional metrics, notably the mean titer increase, HAI composite score (76), and proportion of seroconverted strains, all calculated for each individual. In order to determine whether our proposed metrics are more robust than traditional metrics, we will subsample measurements from our cohort in order to mimic the use of different virus panels across labs. For labs, we will subsample strains from our panel, plus the homologous strain. For each of these panels, we will calculate the metrics for each individual, and then analyze the variability of mean metrics across labs.

Finally, we will explore weighting schema for our metrics. The unweighted AUC assigns equal weight to strains of all antigenic distances, but perhaps we would prefer to weight the response to distant strains higher or lower than similar strains–for example, if we are primarily considered with boosting the response to a specific pandemic strain, we could assign less weight to distant strains. But if we are considering candidates for a broadly reactive vaccine, we could weight distant strains higher in order to favor vaccine candidates that induce responses to the most distant strains. Examples of weighting schema are shown in [Figure 3.4](#fig-weights).

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| Figure 3.4: Example weighting schema for AUC measurements. In the linear scheme, weights decrease linearly with antigenic distance. In the 2 antigenic unit scheme, all strains which are within two antigenic units on the cartography map (or equivalent with the other distances) are weighted equally, while strains that are further away are not included in calculating the AUC. |

### 3.2.3 Expected outcomes

1. The presence of an nonlinear relationship between titer increase and antigenic distance depends on the breadth of the panel. If the panel includes a wide variety of similar and dissimilar strains, such as with the A(H1N1) data containing both the pandemic-like and non-pandemic-like clades, a nonlinear U-shaped relationship would be consistent with the theory of original antigenic sense. However, if the vaccine fails to elicit any cross-protective immunity to the HA antigen of the most distant strains in the panel, we would still expect to see a (potentially nonlinear) monotonically decreasing relationship.
2. We expect our proposed metrics to be more robust to the subsampling experiments than traditional metrics.
3. In older adults, we expect the strength, breadth, and overall immune response to the Fluzone HD vaccine to be greater than the standard-dose formulation, based on currently available immunogenicity studies.

## 3.3 Aim 2

For this aim, we will use the UGAFluVac data, specifically the portion collected at the University of Georgia. The PA/FL data does not have prior vaccination history and thus we can only use the UGA data for this aim.

### 3.3.1 Preliminary results

We first explored the homologous case in order to identify simple first-order effects of each of the covariates of interest. [Figure 3.5](#fig-mada-eda) shows plots of fold change in titer (titer increase) plotted against available covariates. The only covariate which independently explained a substantial proportion of the variance in the outcome was prevaccination titer. Note that these results do not consider interaction effects (or subgroup specific effects that can be modeled as interactions): for example, we would not expect to see an effect of dose in the entire population since otherwise healthy adults under the age of 65 were not offered high dose vaccine. The metrics also only capture linear trend–if a predictor acts through a primarily nonlinear (especially true for nonmonotonic effects) the will drastically underestimate the strength of association between the two variables.

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| Figure 3.5: Titer increase plotted against each of the (normalized) antigenic distance measurements. The weighting schema are illustrated by shading the area under the curve in accordance with the weight given to that point. |

From our exploratory analyses, we also know that the effect of prevaccination titer on the homologous immune response varies across vaccine strain and dose ([Figure 3.6](#fig-pvt-lm)). We fit stratified linear models with both parallel slopes (A and C) and varying slopes (B and D) with these two factors. We further expect to see a third-order interaction between vaccine strain and dose. Note that these preliminary figures show all individuals, and the effect of dose will be further modified by age, since high dose vaccine was only offered to elderly participants.

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| Figure 3.6: Titer increase plotted against pre-vaccination titer. Models are stratified by vaccine strain type (A and B) or by vaccine dose (C and D). Figures A and C show a parallel slopes model where only the intercepts are allowed to vary (corresponding to the model with no interaction term, only main effects for both variables), while Figures B and D show the varying slopes (interaction and main effects) models. The varying slopes models improve R-squared marginally, but there are several limitations to these analyses that may hide larger differences. |

We further conducted an analysis at the strain-specific level, fitting separate models for each combination of vaccine strain and assay strain represented in the data ([Figure 3.7](#fig-ns-panel)).

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| Figure 3.7: Relationship between titer increase and prevaccination titer for each of the assay strains which were used as part of a historical panel. Only assays for individuals who received H1N1-Michigan-2015 containing vaccine are shown for simplicity. We can see that the intercept and slope have the largest magnitude for the homologous strain and for the previous vaccine strain (H1N1-California-2009), and decreases with other assay strains. |

We can further stratify these strain specific analyses by other factors like dose ([Figure 3.8](#fig-dose-panel)). These analyses reveal a non-constant modification of the effect of prevaccination titer by dose, which differs across vaccines and across assay strains within vaccines.

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| Figure 3.8: The same analyses as shown in [Figure 3.7](#fig-ns-panel), but stratified by vaccine dose. Additionally, only individuals aged 65 and older are included in these models, since younger individuals were not eligible to receive high dose vaccines. Some relationships are distorted by small sample effects, but in general the dose appeared to make little difference for this particular vaccine. However, we see varying, potentially real effects in the MI/15 and CA/09 effects. The effect of dose also varies across vaccine strains (data not shown). |

Differences in strain-specific models for the same vaccine demonstrate the interaction between antigenic distance, prevaccination titer, and other stratifying factors (like dose and prior vaccination history). We conducted a preliminary analysis of the strength of this interaction by compiling the slopes of each model (as shown in [Figure 3.7](#fig-ns-panel), but incorporating data from all vaccine strains that were used in the study), and plotting these slopes against the antigenic distance between the vaccine strain and the assay strain for that model, shown in [Figure 3.9](#fig-slope-plots). Note that in this framework, vaccines were only compared against assays of the same type. HAI assays for influenza B strains which predated the divergence of the Victoria and Yamagata lineages were compared against both B-Yamagata and B-Victoria vaccines.

For the type A influenza assays, we see a strong positive correlation between antigenic distance and slope of the strain-specific model. As the vaccine strain and the assay strain become more distant, pre-vaccination titer has less of an effect on the amount of boosting produced by the vaccine.

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| Figure 3.9: The same analyses as shown in [Figure 3.7](#fig-ns-panel), but stratified by vaccine dose. Additionally, only individuals aged 65 and older are included in these models, since younger individuals were not eligible to receive high dose vaccines. Some relationships are distorted by small sample effects, but in general the dose appeared to make little difference for this particular vaccine. However, we see varying, potentially real effects in the MI/15 and CA/09 effects. The effect of dose also varies across vaccine strains (data not shown). |

We also used machine learning models to predict response to the homologous vaccine strain based on all of the covariate data that was available to us. We used permutation variable importance to score the importance of the included predictors ([Figure 3.10](#fig-vip)). We found that prevaccination titer was, by far, the most important covariate in predicting vaccine boosting. However, we also observed differences between influenza seasons, individuals of different ages, and the vaccine strain type. Future models will include antigenic distance measurements instead of indicators for strain types.

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| Figure 3.10: Permutation variable importance for each feature included in the final tuned random forest model. The variable coding indicates the following features: prevactiter is the pre-vaccination titer; season is the current influenza season; age is the individual’s age; the strain\_type variables are indicators for influenza strain types for each assay; times\_vaccinated is the total number of times the individual repeated the study; dose\_HD is an indicator for the individual receiving high-dose vaccine; and male and white are demographic indicator variables for sex and race/ethnicity respectively. |

### 3.3.2 Proposed studies

First, we will expand and refine our preliminary machine learning approach to include all predictors of interest. We will apply similar models to quantify the predictive power of each variable using permutation importance. For the final models, we will perform more in-depth feature engineering to ensure that predictors have optimal predictive power while retaining biological meaning. These models can learn nonlinear and interaction effects without prior specification and thus will allow us to understand how well all we can expect inferential models to capture trends in the outcome. We will also fit models using both the post-vaccination titer and the titer increase as outcomes, to determine if the drivers of the raw immune response are less influenced by pre-vaccination titer. We will also fit separate models for elderly participants in order to gain a better understanding of the effect of vaccine dose in this subgroup.

After we understand how much of the post-vaccination titer can be explained by the data we have, we will build Bayesian hierarchical models to take advantage of the clustered structure of the data. These hierarchical models will be allow us to make inferences about the relationships between variables, rather than only quantify the predictive power. Furthermore, we can implement random effects in these models to partially absorb unmeasured confounding, which is not possible within a predictive machine learning framework. However, we can compare the overall predictive power of our inferential models to the best predictive models.

Finally, we will modify previously developed mechanistic models (65,66) which model steric hindrance between multiple epitopes of the same antigen. The model for steric hindrance is mathematically similar to how we would model differences in binding avidity between antigenically distance strains, so by incorporating a notion of antigenic similarity, we can use these models to predict how the relationship between dose, prior immunity, and vaccine response varies with antigenic distance. We will compare the model predictions with the predictions made by our machine learning and inferential models. A notable limitation of the mechanistic models in this case is that we will not be able to make predictions about the effect of serial repeated vaccinations as we hope to do for the hierarchical models.

### 3.3.3 Expected outcomes

1. The following results from the machine learning models are consistent with our exploratory data analyses. We expect pre-vaccination immunity and antigenic distance to be the strongest predictors of the post-vaccination titer. Age will be the most important demographic predictor, with small to moderate contributions from other demographic data. Any effect of prior vaccination status will be detected through models like random forest or gradient-boosted trees which detect interaction between predictors and nonlinear effects.
2. We expect hierarchical Bayesian models which respect the clustered structure of the data to predict the outcome fairly well. In these models, we expect pre-vaccination titer and antigenic distance to interact with each other, with potential interactions with prior vaccination status. We also expect models that allow nonlinear relationships for the predictors to fit better (after adjusting for the increasing number of parameters in the model).
3. Dose will likely show little impact in models which include all participants from the study. However, in subset models for elderly participants only, we expect the effect of vaccine dose to be stronger. Some previous literature also suggests that the effect of sex should be stronger in older individuals.
4. We expect mechanistic model predictions to be similar to predictions from machine learning models and hierarchical models. Similarities in model results would support competition for B cell binding as a plausible mechanism to explain the repeated activation of the influenza memory response in the presence of similar but drifted antigen.

## 3.4 Aim 3

For this aim, we will combine the UGAFluVac data, RocFluVac data, and any applicable data provided by Ben Cowling.

### 3.4.1 Preliminary results

While working with the UGAFluVac data, we have constructed a small DAG exploring only the necessary adjustment variables that affect the causal pathway between dose and post-vaccination titer ([Figure 3.11](#fig-dag)). In this reduced framework, the total and direct causal effects of dose would be the same, and the only factor we would need to adjust for to obtain the true causal estimate of dose is age. Although age may be a common cause of several other factors that affect post-vaccination titer, vaccine dose is a quality of the intervention (vaccination) and thus the only factors that affect vaccine dose are factors that determine participation in the study.

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| Figure 3.11: A directed acyclic graph (DAG) showing a graphical causal model for the effect of dose (the exposure, in green) on post-vaccination titer (the outcome, in blue with an I symbol). Age is a confounder and is colored pink in the DAG. |

We have also conducted preliminary comparisons of the UGAFluVac and RocFluVac data, primarily to compare the patterns in antibody responses observed in the UGAFluVac data with the T cell responses observed in the RocFluVac data ([Figure 3.12](#fig-rocted)). We also have HAI data for the RocFluVac data, and thus we can compare the effect of dose on the immune response in the 18 - 49 year olds in the RocFluVac study with the effect in the 65+ year olds in the UGAFluVac study.

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| Figure 3.12: Antibody immune response measured via HAI in the UGAFluVac cohort compared to T cell immune response measured via Elispot in the RocFluVac cohort. |

### 3.4.2 Proposed studies

First, we will ensure that our theoretical causal model is accurate, and we will update our model to include other common causes which are observed in both data sets. Our causal model will be formulated as a DAG, which we will use to generate a minimal sufficient adjustment set for the causal effect of vaccine dose on post-vaccination titer and on titer increase. We will analyze the conditional independencies of the DAG by comparing the observed standardized covariance matrix to the covariance matrix predicted by the DAG in order to determine how consistent our data are with the identified DAG.

We will apply traditional regression methods using either cluster-robust standard error estimation or hierarchical modeling to obtain a causal estimate of the effect of dose, using a minimally sufficient adjustment set identified by our DAG. We will use quantitative bias analysis methodology to estimate the effect of unmeasured confounding on our estimate. Finally, we will obtain the same estimate using a targeted maximum likelihood estimation (TMLE) framework, which is doubly robust to model misspecification, and we can compare the TMLE estimate to the regression estimate.

We will explore the interaction of age and dose by estimating the causal effect of dose in both the UGAFluVac and RocFluVac studies separately and comparing these estimates to the combined study estimate. We note that differences in these estimates could be due to sampling variation, systematic variation between the two source populations, or due to the interaction effect with age. We can furthermore estimate interaction effects in all three situations.

### 3.4.3 Expected outcomes

1. We expect the average treatment effect of dose on post-vaccination titer to be positive. We also expect age to modify this effect, with a stronger positive effect on dose in elderly participants.
2. Within each study, age will act as an effect modifier on the effect of dose. If there are noticeable differences between the two studies, there will be a discontinuity in effect modification that occurs in-between the age ranges for the two studies.
3. While unmeasured confounding is certainly present in our estimate (as with any observational study), the amount of unmeasured confounding required to reverse the direction of the ATE will be unreasonably large after we control for age.
4. The TMLE estimate and regression estimate will both be positive and of the same order of magnitude, but may not necessarily make the same quantitative predictions. Since the quantitative effect of dose is likely to vary significantly across individuals and study sites, such a result would still be consistent with our primary expected outcome for this aim. Zero may be a plausible value for both the TMLE and regression estimates, due to attenuation of the causal effect by non-differential measurement error in the outcome.

## 3.5 Timeline

[Figure 3.13](#fig-timeline) shows the expected timeline for our project. The expected completion date for our project is Friday, March 14, 2025, and a dissertation draft will be sent to the committee no later than the following Monday. My dissertation defense will be planned for early April to accommodate Graduate School deadlines.

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| Figure 3.13: Proposed project timeline. We expect the majority of research to be conducted during the 2023-2024 academic year, extending into 2025 as necessary. All research will be complete by March 1, 2025 in order to finalize results for the written dissertation. |

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