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The S.h.i.v.e.r.i.n.g. Trials: A Novel Vaccine Strategy And Analysis Of Serologic Durability In Patients With Plasma Cell Dyscrasias

Eamon Duffy

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Eamon Duffy
Yale Schools of Medicine and Management
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ABSTRACT

Background: Patients with plasma cell dyscrasias are at an increased risk for infections due to their dysfunctional immune system. Each year these vulnerable patients are advised to receive the flu shot, but this vaccine has been shown to induce a serologic response that is not sufficiently protective in these patients. More effective methods for vaccinating patients with plasma cell dyscrasias are necessary.

Methods: The Study of High-Dose Influenza Vaccine Efficacy by Repeated dosing IN Gammopathy patients (SHIVERING 1) Pilot Trial was implemented at the Yale Cancer Center during the 2014-2015 flu season. Patients with plasma cell dyscrasias (n=51) received the high-dose inactivated trivalent influenza vaccine followed by a booster dose of that same vaccine 30 days later. The SHIVERING 2 randomized, double-blind, placebo-controlled, interventional trial was implemented at the Yale Cancer Center during the 2015-2016 flu season. The experimental arm (n=81) received the two dose regimen and the control arm (n=41) received the standard of care. In both trials, patients were followed throughout the flu season for evidence of flu infections, and sera was collected for hemagglutinin titer analysis and correlation with clinical characteristics and patient demographics.

Results: SHIVERING 1 demonstrated that the double high-dose regimen was safe and resulted in significantly higher rates of seroprotection than have been previously reported. There were no grade ≥ 2 adverse events. The seroprotection rate increased from 4% at baseline, to 47% after the first vaccine, and to 65% after the second vaccine. SHIVERING 2 demonstrated significantly higher rates of seroprotection at day 60 and a lower rate of laboratory confirmed flu infections in the experimental arm versus the control arm. Analysis of the durability of serologic protection demonstrated a significant difference in HAI titer growth and direction between the two arms, with the standard of care arm experiencing a decline in HAI titer levels during the day 30 to day 60 interval following vaccine administration. Additionally, patients with early disease in the experimental arm were significantly more likely to remain seroprotected at study end than patients with advanced disease in the control arm. Finally, patients that are female and those that had undergone an autotransplant in the past were significantly more likely to remain seroprotected at study end.

Conclusion: These trials suggest that a boosted high-dose influenza vaccine regimen is safe and results in lower rates of infection and higher rates of seroprotection in patients with plasma cell disorders. Patients with early stage disease are able to mount a more durable serologic response than patients with advanced disease. Larger studies will be needed to confirm these preliminary findings.

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INTRODUCTION

This investigation attempts to answer a very simple question: can we improve how we vaccinate against the flu in patients with multiple myeloma and other plasma cell dyscrasias? To answer that question, we embarked on the Study of High-dose Influenza Vaccine Efficacy by Repeated dosing IN Gammopathy patients – the S.H.I.V.E.R.I.N.G. Trials. This investigation began in September of 2014 and continues today.

Multiple Myeloma

Multiple myeloma is a neoplastic plasma cell disorder characterized by the clonal proliferation of plasma cells in the bone marrow, monoclonal protein in the blood, and the systemic organ dysfunction that is caused by this neoplastic process.[1] The disease accounts for 1% of all cancers and 13% of hematologic cancers. In Western countries, its age-adjusted incidence is 5.6 cases per 100,000 persons. This neoplastic process occurs across a wide range of ages: the median age at diagnosis is 70 years, but 37% of patients are younger than 65 years and another 37% are 75 years or older at diagnosis.[2] Despite significant improvements in treatment options – autologous stem cell transplantation and the development of the next generation of anti-cancer chemotherapies – in patients presenting at age 60 years or younger, the 10-year survival rate is 30%.[3]

The disease is thought to begin by a premalignant process whereby plasma cells proliferate but the host remains asymptomatic. This stage is known as monoclonal gammopathy of undetermined significance, or MGUS. The host then progresses, due to both genetic mutations and environmental influences, to smoldering myeloma and then to symptomatic myeloma. Patients are diagnosed based on the presence of at least 10% clonal plasma cells in their bone marrow and monoclonal protein in their serum or urine.

Once diagnosed, patients are classified as either asymptomatic or symptomatic, depending on the presence of related organ or tissue dysfunction. This most often takes the form of hypercalcemia, renal insufficiency, anemia, and bone disease. The C.R.A.B. acronym for these sequelae of multiple myeloma has been passed on to patients and the medical students who study them for decades. A patient's progress is then further delineated in parallel with their signs and symptoms using a staging system based on serum β_2-microglobulin, serum albumin, and the presence of high- or low-risk chromosomal abnormalities.

Treatment of symptomatic myeloma, or "active disease," begins immediately. Asymptomatic myeloma is clinically observed, as no benefit comes from treatment with conventional chemotherapy.[4] The treatment algorithm for patients with active disease is dependent on their age and stage, and consists of six main types of treatment: immunomodulatory drugs, proteasome inhibitors, monoclonal antibodies, traditional chemotherapies, corticosteroids, and stem cell transplantation.[5] The medical team and the patient together balance the benefits of each piece of this arsenal against their associated side effects. This is a disease that cannot be cured, but only managed.

Risk for Infections Multiple Myeloma

Part of that yearly management comes in the form staying otherwise healthy. Infections are a significant cause of morbidity and a leading cause of death in patients with multiple myeloma. Immunologically, this makes sense: those with a cancer of the immune system have a dysfunctional defense in place that is further weakened by immunosuppressive chemotherapies and steroids. These patients represent a near-perfect host for a variety of infectious organisms. Dexamethasone and other steroids induce a T-

cell immunodeficiency in the host, leaving the patient vulnerable to viral (influenza, CMV, VZV, HSV), fungal, and mycobacterial infections. Constant transfusions can lead to iron overload, creating an environment in which all bacteria thrive, but aspergillus and zygomycetes feel particularly at home. Defects in the complement cascade and functional hypogammaglobulinemia put the host at risk for infections by the encapsulated bacteria. The severe neutropenia induced by rounds of chemotherapy leaves the host open to infection by both gram negative and gram positive bacteria.[6] Similar to the progression of opportunistic infections seen in patients with HIV/AIDS, bacterial infections with *Streptococcus pneumonia*, *Haemophilus influenza* and *Escherichia coli* predominate in the early stages of the disease and then give way to viral and fungal infections as the myeloma advances.[7] It is no surprise that if a patient with multiple myeloma presents with a fever, they are to be considered infected until proven otherwise.

A study of population-based data from Sweden comparing all patients diagnosed with multiple myeloma between 1988 and 2004 (n = 9,253) to 34,931 matched controls quantified the exact increased risk for infection.[8] Overall, patients with multiple myeloma had a seven-fold (hazard ratio = 7.1; 95% C.I. = 6.8-7.4) risk of developing any type of infection compared to matched controls. Multiple myeloma patients had the same seven-fold (hazard ratio = 7.1; 95% C.I. = 6.8-7.4) risk of developing a bacterial infection and a ten-fold (hazard ratio = 10.0; 95% C.I. = 8.9-11.4) risk of developing a viral infection. At one year follow up, infection was the cause of death in 22% of patients. A separate study of over 3,000 patients with multiple myeloma that investigated early deaths (within the first 6 months after diagnosis) found that 45% of early deaths were due to infections.[9]

These studies could change the acronym of multiple myeloma sequela from C.R.A.B. to the more appropriate, "C.R.A.B.I.," with an "I" for "infection."

In addition to the risk that these infections pose to the immunocompromised patient, the inflammatory response that follows the infection, particularly in the bone marrow microenvironment, may contribute to the progression of the host's multiple myeloma. Similar to how HPV can lead to cervical cancer, research has focused on finding a viral trigger of multiple myeloma, with HHV-8 as the prime suspect.[10] The tie between inflammation and cancer initiation and progression has been well-studied, while the tie between inflammation and initiation or progression of multiple myeloma remains an exciting area of current research.[11] A recent study by the Dhodapkar lab investigated the chronic inflammation caused by specific lipid subtypes as a trigger for multiple myeloma in a mouse model of Gaucher disease, a disease associated with higher rates of multiple myeloma.[12] Other studies have examined and illustrated differences in toll-like receptor (TLR) expression in these neoplastic plasma cells, providing further molecular evidence that inflammation and the body's innate immune response following an infection is key to driving the hospitable microenvironment in which multiple myeloma thrives.[13] Put simply, infections drive inflammation and inflammation drives multiple myeloma. It is thus no wonder that these studies call for a greater focus on improving treatment of and prophylactic measures against infections in this vulnerable population.

The Influenza Vaccine

Vaccine prophylaxis against the influenza virus in patients with multiple myeloma and other plasma cell dyscrasias provided the basis for this current investigation. The influenza virus, or "the flu," is a significant cause of morbidity and mortality in the

United States. The severity of disease varies year by year, and the Center for Disease Control (CDC) estimates that the flu is the cause of between 9,200,00 to 35,600,000 illnesses, 140,000 to 710,000 hospitalizations, and 12,000 to 56,000 deaths each year.[14] A 2007 study by Molinari et al. calculated the total annual economic burden of the influenza virus to be $87 billion.[15] The influenza virus is an RNA virus that has three main subtypes – A, B, and C. The various subtypes of A and B are the main cause of the annual flu epidemic and the targets that the influenza vaccines attempt to protect against.

The World Health Organization (WHO) and the Center for Disease Control (CDC) recommend yearly vaccination against the influenza virus for all people over the age of 6, with rare exception. The vaccine that the vast majority of people receive is an inactivated mixture of the likely circulating viruses for that year. More specifically, each year the vaccine includes a recent H1N1 virus, a recent H3N2 virus (both of which are A viruses), and an influenza B virus. Together these three form the "trivalent" vaccine. The vaccine induces the production of antibodies against the viral attachment protein hemagglutinin, thus inhibiting viral entry and neutralizing the virus.[16] The serum hemagglutinin-inhibition (HAI) assay is the means by which one can assess the HAI antibody response to the influenza vaccine. Higher levels of HAI antibody are correlated with clinical protection against influenza virus infection.[17] The target seroprotection rate, defined as the proportion of individuals who achieve an HAI titer of >1:40, is accepted to be >70% in the healthy population. Yet this target protection rate is not always achieved with the current regimen or, more often, the vaccine simply misses the target and protects hosts against the incorrect subtypes. Each year the CDC reports whether it was a "good" or a "bad" year for the accuracy of the flu vaccine, and the numbers rarely inspire

confidence in the current trivalent approach. A recent meta-analysis of pooled influenza vaccine effectiveness (VE) (the percent reduction of disease in the vaccinated group compared with the unvaccinated group) concluded that "vaccine improvements were needed." Pooled VE was 33% for H3N2, 54% for type B, and 67% for the H1N1 subtype.[18] Given the yearly disease burden and the sub-optimal accuracy of the vaccine each year, much work has been done to improve the traditional single, trivalent inactivated influenza vaccine regimen.

As our current influenza season approaches, experts in the United States are watching the southern hemisphere with great anxiety. Experts pay particularly close attention to Australia, the last stop for the approaching influenza strain on route to North America. Australia has experienced a record-high number of laboratory-confirmed influenza notifications and higher-than-average numbers of hospitalizations and deaths. By mid-October, toward the end of the Australian flu season, notifications had reached 215,280, far greater than the 59,022 cases reported during the 2009 H1N1 influenza pandemic, with the influenza A (H3N2) subtype the predominate subtype.[19] The preliminary estimate of vaccine effectiveness against this H3N2 subtype was a mere 10%. Unfortunately, it is likely the 2017 influenza season will highlight the need for improved accuracy and efficacy in our influenza vaccine development process.

Vaccine Durability

In addition to the question of vaccine accuracy, there is also the key question of vaccine durability: how long does the serologic response last and is the patient protected for the entire flu season? Antibody titers decline over time, but does that decline matter clinically, and if so, at what rate, and in whom? Does peak titer level impact duration of

protection? After matching the vaccine with the circulating strains, the immunization process must then be timed to optimize serologic protection during a flu season that occurs with variability between November and April. Many studies have looked at the durability of the serologic response to both flu infection and the flu vaccine. Durability of serologic defense is determined by both host and vaccine characteristics. Key host factors include age, comorbidities, and prior exposure to the antigen or the vaccine. Key vaccine characteristics include mode of delivery and type of vaccine (live attenuated, inactivated, subunit, or toxoid). Live attenuated vaccines are known to produce a more robust and durable immunity, as these vaccines activate memory B cells, memory helper T cells and memory killer T cells. The downside is that live attenuated vaccines are, as their name suggests, alive. Inactivated vaccines, the most commonly administered version of the flu vaccine, pose no danger of infection but do not stimulate as robust or durable an immune response, and therefore often require booster vaccines to induce a truly protective response.

The 1918 H1N1 influenza virus pandemic (the "Spanish Flu") killed over 50 million people worldwide and survivors of that pandemic still possess, over 90 years later, highly-functional, virus-neutralizing antibodies to that historically aggressive virus.[20] Not nearly as immunogenic as this strain, the modern influenza vaccine takes 2-4 weeks to illicit seroprotection that will peak at 4-6 weeks and last for 6-12 months in adults. However, this durability has been called into question, most notably in the elderly, a population most at risk for influenza infection and related complications. From 1990 to 2006, advisory committees in the United States and Canada advised that providers delay the administration of the influenza vaccine to the elderly until later in the fall season, as

"antibody levels may fall below protective levels within 4 months" in these patients.[21] A 2008 meta-analysis of this claim that, "Influenza vaccine induced antibody decline more rapidly in the elderly, falling below seroprotective levels within 4 months" found, "no compelling evidence for more rapid decline in the elderly as compared with young adults." [22] In response to this work and similar findings, the advisory committee abandoned their advice to delay vaccination. The titer decline rate for patients with multiple myeloma or other immunocompromising illnesses is not currently known, and should be investigated. Although the elderly may remain protected throughout the entire flu season, it is possible that patients with multiple myeloma are unable to mount a truly robust or sufficiently durable response to the vaccine, and are left vulnerable for the second half of the flu season or longer.

Strategies to Improve Vaccine Efficacy

Much work has been done in recent decades to improve on our current lackluster defense against the flu. The studies that aim to improve protection against the flu that are most relevant to this investigation include the development of a "high-dose" vaccine and the addition of a second "booster" vaccine to increase HAI antibody titer levels. The high-dose, trivalent inactivated influenza vaccine, known as Fluzone High-Dose and produced by Sanofi, contains four times the hemagglutinin as the standard dose vaccine. It was first licensed in 2009, and in 2014 a multi-center, randomized, double-blind, active-controlled trial compared the high-dose (HD) vaccine to the standard dose (SD) vaccine in 31,989 patients over the age of 65 years with laboratory-confirmed influenza illness as the primary endpoint.[23] The HD group had lower rates of infection versus the SD group (1.4% versus 1.9%) and significantly higher 28-day post-vaccination HAI titers

and seroprotection rates. This study established the high-dose vaccine as the standard of care for patients over the age of 65.

The idea of using a first vaccine to prime the immune system followed by a booster vaccine to spark antibody production is not new and has historically been applied to pediatric patients. The CDC recommends that all children who have not been vaccinated against the flu in the past receive this two dose, or "prime-boost" regimen.[24] This booster regimen has since gained momentum for use with other vulnerable populations. Both patients that are positive for HIV and hemodialysis patients have been shown to benefit from a boosted influenza vaccine regimen.[25,26] However, a recent meta-analysis showed no clinical benefit to this practice in patients on hemodialysis.[27] A third actively-researched approach to improving the influenza vaccine involves the addition of an adjuvant to the antigen in an attempt to increase the immunogenicity of the vaccine. As Sanofi pursued the high-dose approach, Novartis developed the M59 adjuvanted vaccine that has shown some promise in the elderly population.[28] The goal of using an adjuvanted protein is to aim the vaccine at a less fickle target and proteins that do not so rapidly undergo genetic drift. Along with many other strategies, these novel approaches continue to fuel the search for a better flu vaccine.

Vaccine Efficacy in Patients with Multiple Myeloma

Even in the face of these developments, the current standard of care fails to protect patients with multiple myeloma. Their dysfunctional immune systems leave them unable to effectively defend against the flu or mount a sufficient response to the vaccine. Several recent studies have examined the efficacy of vaccinations in patients with multiple myeloma. A study from Great Britain, published in 2000, looked at the

seroprotection rates 4-6 weeks following vaccination against influenza, streptococcus pneumonia and haemophilus influenza in 52 patients with multiple myeloma. At 4-6 weeks post-vaccination only 19% of patients reached protective HAI antibody titer levels. Response to the Pneumovax II vaccine against streptococcus pneumonia was also weak, as 39% of patients had low titers following vaccination. Response to the heamophilus influenza vaccine was statistically no different from the healthy population.[29] More recently, investigators have begun to trial the novel regimens described above in patients with multiple myeloma.

A recent retrospective study examined the immune response of a single dose versus boosted influenza vaccination in patients with multiple myeloma.[30] The secondary aim of the trial was to correlate this immune response with multiple myeloma parameters and myeloma treatment regimens. In 48 patients with smoldering or active myeloma, a single dose of the standard influenza vaccine resulted in a seroprotection of 14.6%. The rate of seroprotection more than doubled to 31.3% after a second dose of the standard vaccine 4 weeks later, representing a statistically significant improvement. The trial study concluded that, "There are no systemic studies on the efficacy of influenza vaccines in patients with multiple myeloma. Double vaccination against influenza in multiple myeloma patients seems to enhance protection and should be systematically studied. A larger and stratified cohort of patients would be needed for systematic assessment of associations between immunization results and clinical parameters."

The goal of the SHIVERING Trials was to do this systematic assessment of a novel influenza vaccination regimen in patients with multiple myeloma and other plasma cell dyscrasias. The flu is a major source of morbidity and mortality in this patient

population. The current regimen does little to protect these patients against that threat. Even with only the current resources available, we can do better for these patients. SHIVERING I, a safety trial, and SHIVERING II, a randomized, double-blind, placebo-controlled clinical trial, aimed to do better by investigating a novel vaccine regimen: the Fluzone High-Dose influenza vaccine at day zero followed by that same high-dose vaccine as a booster 4 weeks later in all patients, regardless of age.

STATEMENT OF PURPOSE AND HYPOTHESES

The purpose of this investigation is to test a novel influenza vaccine regimen in patients with multiple myeloma, and to better define the nature of their response to this vaccine. If this regimen improves clinical outcomes and serologic protection it should be further studied and should be considered for the standard of care in this select group of patients.

The aim of this investigation is to trial a novel influenza vaccine regimen in patients with multiple myeloma and other plasma cell dyscrasias to determine the rates of flu infection, the levels of serologic protection against the flu, the durability or lasting nature of that defense, and to find clinical characteristics of these patients that correlate positively or negatively with response to this vaccine regimen.

I hypothesize that the durability of the serologic protection – how long patients remain seroprotected throughout the flu season – will correlate positively with the rise in titer level during the day 30 to day 60 time interval and will correlate negatively with disease stage and number of previous anti-cancer therapies. I hypothesize that this vaccine regimen will be safe and well-tolerated by all patients in the study. I hypothesize that the high-dose plus a booster strategy will result in lower rates of clinically diagnosed influenza, higher rates of serologic protection, and that an improved serologic response to the vaccine will correlate with more mild stages of myeloma and less aggressive stages of anti-cancer treatments.

Statement on Personal Contribution

I joined the SHIVERING trial team during the fall of my second year of medical school at the invite of Andrew Branagan MD, a hematology-oncology fellow at Yale in the Clinical Scholars Program. Dr. Branagan, with Dr. Dhodapkar as senior PI, led the SHIVERING Trials throughout the 2014-2015 and 2015-2016 flu seasons. As a sub-investigator, my primary roles included screening and consenting patients, helping to process the daily flow of patient sera, and EPIC chart reviews and data acquisition on each patient's demographics, influenza history, disease status and treatment history. Following each trial, I was intimately involved with analysis of the results, manuscript writing and poster and oral presentations of these results at academic conferences. The background research, data collection, and statistical analysis of the question of durability of serologic protection was done independently, as was the writing of this thesis. My work on the SHIVERING trials was done in parallel with my academic coursework at the schools of Medicine and Management over the last four years. The work on durability of serologic defense was done during dedicated research time in October and November of 2017.

STUDY ENDPOINTS

SHIVERING 1:

Primary:

1. To study the rate of disease control throughout the study period as determined by lack of disease progression requiring new or different therapy.

2. To study the safety profile of this high-dose booster regimen.

Secondary:

1. To study the rate of influenza-related morbidity and mortality at the end of the flu season following the high-dose booster strategy.

2. To study the rates of serologic protection (defined as HAI titer > 40) following each interval of the high-dose booster regimen.

3. To study preliminary correlations between serologic protection and clinical characteristics and demographics of the study population.

SHIVERING 2:

Primary:

1. To study the influenza infection rate between patients who receive the high-dose booster regimen and those that receive the standard of care.

2. To study the rate of disease progression determined by lack of disease progression requiring new or different therapy in patients who receive the high-dose booster regimen and those that receive the standard of care.

Secondary:

1. To study the rates of serologic protection (defined as HAI titer > 40) following each interval and between both the experimental and control groups.

2. To study the durability of serologic protection to better define the endurance of the standard of care in this patient population and determine the impact of the high-dose booster regimen on serologic peak and durability.

METHODS

SHIVERING 1

Clinical Trial Design

The SHIVERING I trial was implemented from September 2014 through May 2015 at the Yale Cancer Center. Patients were eligible for inclusion in the study if they were (1) able to understand and sign the informed consent form, which was provided in by English and Spanish, (2) greater than 18 years of age at the start of the study, and (3) had a diagnosis of a monoclonal gammopathy: asymptomatic or active multiple myeloma (MM), asymptomatic or active Waldenstrom Macroglobulinemia (WM), or Monoclonal Gammopathy of Undetermined Significance (MGUS). Patients were deemed ineligible for inclusion in the study if they (1) had already received the influenza vaccine that year, (2) had an egg allergy or (3) were pregnant or planning to become pregnant during the study period. All patients in the study, regardless of age, received the study intervention of one dose of the trivalent Fluzone High-Dose influenza vaccine followed by a second dose of that same vaccine thirty days later. Blood samples were taken from each patient before the first vaccine at day 0, before the second vaccine at day 30, and 30 days after the second vaccine at day 60.

Study Oversight

The study was approved by the Yale School of Medicine Institutional Review Board and conducted in accordance with the International Conference on Harmonization Good Clinical Practices guidelines. All patients in the trial provided written informed consent before enrollment. The trial was registered at ClinicalTrials.gov (identifier: NCT02267733).

Study Vaccine

The Fluzone High-Dose influenza vaccine (produced by Sanofi) is a trivalent inactivated vaccine that is administered intramuscularly. The vaccine contains a total of 180 μg of influenza virus hemagglutinin, made up of 60 μg from each of the three influenza strains chosen that year for the vaccine: the A/California/7/2009 (H1N1) virus, the A/Texas/50/2012 (H3N2) virus, and the B/Massachusetts/2/2012 (B) virus.

HAI Titer Measurements

Serum was isolated from the whole blood samples within 24 hours of each blood draw using a standardized protocol (Appendix 1). HAI assays were performed using a standardized protocol (Appendix 2). Using the accepted definitions, seroprotection to the influenza virus is defined as achieving an antibody titer of ≥1:40 and seroconversion to acceptable protection against the influenza virus is defined as a fourfold increase in the antibody titer level.

Patient Screening and Surveillance During Study Period

Each patient was screened by their hematologist or a study investigator for eligibility for the trial. Each patient's disease status was assessed and recorded at the first visit, as was their most recent quantifiable disease marker (m-spike or serum free light chain count). Patient surveillance was a top priority for the study team throughout the study period. The flu can go un-diagnosed if the patient does not seek treatment and can mimic other illnesses even when patients do seek treatment. Our study endpoint was laboratory-confirmed influenza infections and the team therefore needed to keep a very close eye on each patient. The team was assisted in this effort by the fact that these patients are already closely followed by their multi-layered clinical care teams. At each

study visit, each patient was asked to fill out a "Flu Morbidity Screen" (Appendix 3) and was reminded to reach out to study team if any worrisome symptoms (fever, fatigue, headache, body ache, cough, sore throat) developed before their next visit. In addition to the clinical surveillance that took place throughout the study period, the team performed a retrospective review of each patient's medical record to check for (1) a laboratory diagnosed influenza infection and (2) to count the number of times each patient had contact (in person or by phone) with a clinical provider during the study period. On average, each patient in the study had contact with a clinical provider once every 11 days. This high regularity of contact gives us assurance that if one of the study patients was ill with the flu or with any other illness, the team knew about it. It should be noted that there was a large range in the number of contacts, dictated primarily by the patient's diagnosis and progression of their disease.

Assessment for Adverse Events and Safety

At each study visit each patient was assessed for any adverse events immediately following the administration of the vaccine or in the time since the last vaccine. The study team used the National Cancer Institute's Cancer Therapy Evaluation Program Adverse Event Recording System (CTEP-AERS), which grades possible adverse events according to attribution (can that event be attributed to the intervention) severity, what occurred, and what was the action, therapy and outcome. All adverse events were recorded at each visit with the study team.

Patient Demographics and Clinical Characteristics

Following the conclusion of the study period, the team performed a chart review to collect data on each patient in the following categories: (1) demographics (age,

gender), (2) disease type (immunoglobulin and light chain type), (3) history of flu vaccine or laboratory confirmed influenza diagnosis during the previous flu season (2013 – 2014), (4) disease response to therapy for those patients that received therapy (from progressive disease to complete response), (5) treatment regimen (anti-cancer chemotherapies, immunomodulatory therapies, and steroids), and (6) autotransplant status at the start of the study period.

Statistical Analysis

The McNamer test, used for paired nominal data sets, was used compare seroprotection and seroconversion rates from baseline to after the first vaccine and to after the second vaccine. Generalized Estimating Equations were used to correlate the binary outcomes (clinical correlates) with seroprotection and seroconversion. For statistical analysis of the durability of seroprotection, a simple linear regression model was used. All statistical analysis was performed using either Prism 7, STATA or Excel and the statistical significance was set at $P<0.05$m using a two-tailed T-test.

SHIVERING 2

Clinical Trial Design

The SHIVERING 2 Trial was implemented from September 2015 to May 2016 and was a multi-center, randomized, double-blind, placebo controlled trial at the Yale Cancer Center and several surrounding satellite care centers. The inclusion and exclusion criteria for SHIVERING 2 were the same as for SHIVERING 1, as detailed above. Patients were randomized in a 2:1 allocation to the experimental arm and the standard of care arm. The recruitment goal was to reach 100 patients in the experimental arm and 50

patients in the standard of care arm. Patients in the experimental arm received the Fluzone High-Dose influenza vaccine at day 0 and then again at day 30. Patients in the standard of care arm received the Fluzone High-Dose influenza vaccine if they were 65 years of age or older and then a placebo second vaccine at day 30. If they were younger than 65 years of age, they received a standard dose of influenza vaccine at day 0 and then a placebo second vaccine at day 30. For all patients, regardless of their study arm, the study team recorded an assessment of disease status (SPEP and serum free light chains) at both day 0 and at their end of study visit in May. For all patients, regardless of their study arm, the study team took a research blood draw at day 0 before the first vaccine, at day 30 before the second vaccine, and 30 days following the second vaccine. Patients in both arms were encouraged to participate in an optional day 7 and end of study research blood draw. As in SHIVERING 1, at each study visit all patients were assessed for any laboratory-confirmed (by direct fluorescent antibody or "DFA") flu infections, flu-like symptoms or illnesses, flu-related hospitalizations or deaths. Additionally, as in SHIVERING 1 and described above, at each study visit all patients were assessed for any adverse events.

Study Oversight

The study was approved by the Yale School of Medicine Institutional Review Board and conducted in accordance with the International Conference on Harmonization Good Clinical Practices guidelines. All patients in the trial provided written informed consent before enrollment. The trial was registered at ClinicalTrials.gov (identifier: NCT02566265).

Study Vaccine

The Fluzone High-Dose influenza vaccine (produced by Sanofi) is a trivalent inactivated vaccine that is administered intramuscularly. The vaccine contains a total of 180 μg of influenza virus hemagglutinin, made up of 60 μg from each of the three influenza strains chosen that year for the vaccine: the influenza H1N1 (A) virus, the influenza H3N2 (A) virus, and the influenza B virus.

The remainder of the methods for SHIVERING 2 (HAI titer measurements, patient screening and surveillance, adverse events assessment, patient demographics and clinical characteristics, and the statistical analysis) were the same as those performed in SHIVERING 1, as outlined above.

RESULTS

This results section will begin with a highlight of the key findings from the two SHIVERING trials and then focuses on an analysis of the durability of the immunologic defense produced by this vaccine regimen.

SHIVERING 1

The SHIVERING I trial enrolled a total of 51 patients during the 2014-2015 flu season. Each patient received two doses of the Fluzone High-Dose influenza vaccine and study blood draws were taken at the appropriate dates. Baseline patient demographics are summarized in Table 1. The median age of the patients was 65 years old and the trial was 61% male. Of the 51 patients, 49 had a diagnosis of multiple myeloma and 2 had a diagnosis of Waldenstrom macroglobulinemia, with a heavy predominance toward the IgG immunoglobulin type and the kappa light chain type. With regard to flu vaccine and

flu infection history, 76% of patients received the flu vaccine the previous year and 6% had a laboratory confirmed influenza infection. The majority of patients with active disease were on active therapy and on a steroid medication.

Table 1: SHIVERING 1 Patient Demographics and Clinical Characteristics

Characteristic	All Patients (N=51) % (n)
Median age	65 years
Male	61% (31)
Heavy chain type: IGG	59% (30)
Heavy chain type: IGA	16% (8)
Heavy chain type: IGM	4% (2)
Light chain only	21% (11)
Light chain type: kappa / lambda	80% / 20% (41 / 10)
Asymptomatic PCD	
Asymptomatic Myeloma	6% (3)
Asymptomatic WM	2% (1)
MGUS	12% (6)
PCD Requiring Therapy	
Myeloma	78% (40)
WM	2% (1)
Median Prior Therapies	2

Safety

No patients in the study experienced a grade 2 or greater adverse event that was attributed to the intervention. The most common side effects were soreness at the injection sight, fatigue, and malaise.

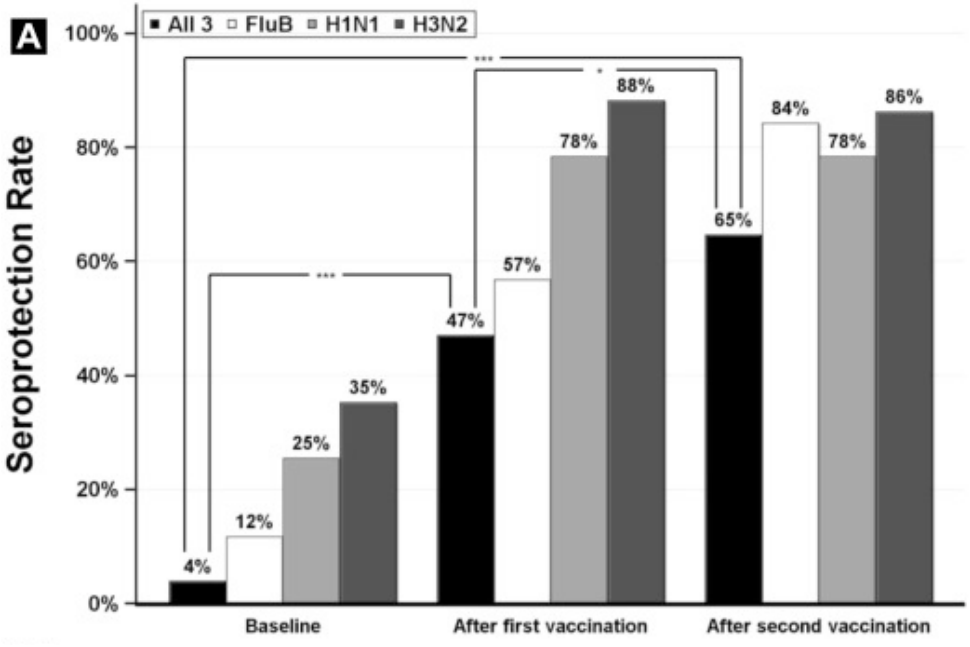
Influenza Infection Rate

Over the study period, 3 of the 51 total patients developed laboratory-confirmed influenza infections. This represents 5.9% (95% CI, 1.2%-16.2%) of study participants, a significant decrease from the Center for Disease Control estimate of 20% in this population (P=0.01).

HAI Titer Response Rate

There was a statistically significant increase in seroprotection against all three strains from baseline (4%) to after the first vaccine (47%) and from baseline to after the second vaccine (65%) ($P<0.001$, Figure 1). Additionally, there was a statistically significant increase in seroprotection against all three strains from after the first vaccine to after the second vaccine ($P<0.01$). There was a statistically significant increase in seroconversion from after the first vaccine (39%) to after the second vaccine (55%). The seroconversion rate excludes those patients who have seroprotection at the start of the study.

Figure 1: SHIVERING 1 HAI titer response rates at each study point.

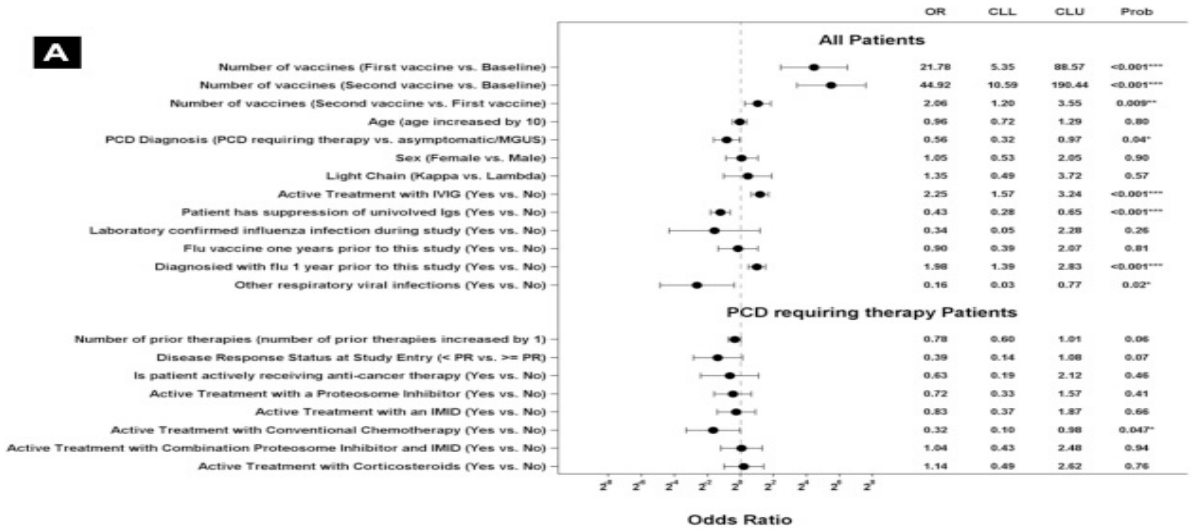


Clinical Correlates of Serologic Response

Figure 2 illustrates those clinical variables that are associated with complete seroprotection and seroconversion following the full, boosted regimen. There were three variables that were significantly associated with increased odds of seroprotection: (1) receiving both doses of the study vaccine, (2) active treatment with intravenous

immunoglobulin (IVIG), and (3) if the patient was diagnosed with the flu one year prior to this study. There were four variables that were significantly associated with decreased odds of seroprotection: (1) diagnosis with a PCD requiring therapy (vs. asymptomatic or MGUS), (2) active therapy with conventional chemotherapy, (3) suppression of uninvolved immunoglobulins, and (4) laboratory-confirmed diagnosis of a viral respiratory infection other than influenza during the study period. A fifth variable, disease response to therapy status of less than a partial response, showed a trend toward significance (P=0.07). In total, the study identified these five clinical variables that, even with this high-dose boosted regimen, are associated with poor response to the vaccine.

Figure 2: Clinical correlates of seroprotection against all 3 flu strains.



SHIVERING 2

The SHIVERING 2 randomized, double-blind, placebo-controlled, interventional trial enrolled 122 patients with plasma cell dyscrasias. The patient demographics and characteristics are highlighted in Table 2. 41 patients were randomized to receive a single standard of care influenza vaccination and 81 patients were randomized to receive two doses of the Fluzone High-Dose vaccine. The characteristics of the SHIVERING 2

participants closely resemble those who participated in SHIVERING 1, save for a slightly older and a greater percentage of males than in the pilot trial.

Table 2: Patient demographics and clinical characteristics.

All Patients (N=122) Characteristic	Interventional Arm (n=81) % (n)	Standard of Care Arm (n = 41)
Median Age	68 years	67 years
Male Sex	48% (39)	56% (23)
Heavy chain type: IGG	56% (45)	44% (18)
Heavy chain type: IGA	20% (16)	12% (5)
Heavy chain type: IGM	8% (6)	22% (9)
Heavy chain type: IGD	3% (2)	0% (0)
Light chain only	13% (11)	22% (9)
Ligh chain type: kappa / lambda	68% / 32% (53 / 25)	66% / 34% (27 / 13)
Asymptomatic PCD		
Asymptomatic myeloma	10% (8)	2% (1)
Asymptomatic WM	0% (0)	8% (3)
MGUS	14% (11)	10% (4)
PCD Requiring Therapy		
Myeloma	65% (53)	70% (28)
WM	7% (6)	10% (4)
Other (AL Amyloid, IGA LPL)	4% (3)	0% (0)
Median prior therapies	2	2

Safety

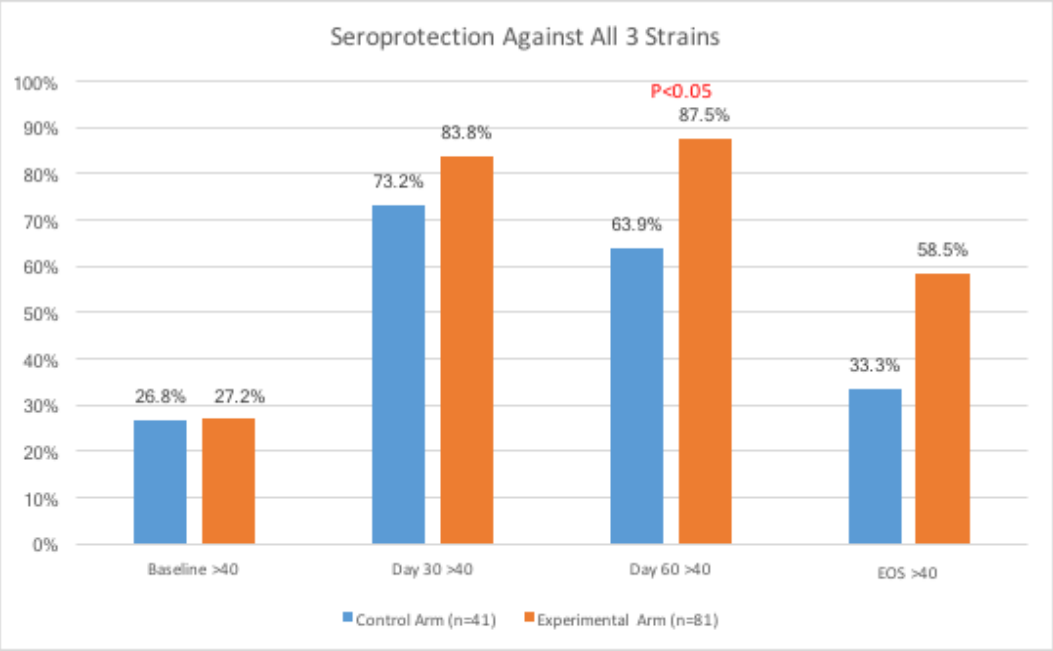
No patients in the study experienced a grade 2 or greater adverse event that was possibly attributed to the intervention. The most common side effects were soreness at the injection sight, fatigue, and malaise.

Influenza Infection Rate and HAI Seroprotection Rate

Significantly fewer patients that received the intervention of two Fluzone High-Dose vaccines developed laboratory-confirmed influenza versus those patients that received a single standard of care vaccine (4.0% vs. 8.3%, $P<0.05$). This is based off an intention-to-treat analysis, which includes all subjects according to their randomization status. Figure 3 displays the rates of seroprotection across the study period for each arm.

There was no significant difference in seroprotection rate at baseline (26.8% in control vs. 27.2% in experimental) or at day 30 (73.2% in control and 83.6% in experimental). At day 60, the experimental arm had a significantly higher rate of seroprotection than the control arm (63.9% in control vs. 87.5% in experimental, P<0.05). At the end of study time point, the difference between the two arms was not statistically significant (33.3% in control vs. 58.5% in experimental, P=0.07).

Figure 3: Seroprotection against 3 strains at each time point by study arm.



Clinical Correlates of Serologic Response

The data revealed those variables that are associated with increased or decreased odds of achieving total seroprotection against all three strains at 60 days. Those variables significantly associated with increased odds for seroprotection include: the female gender (OR 1.84, 1.12-3.02, P=0.02) and a documented flu infection during the previous year (OR 2.03, 1.04-3.96, P=0.04). Those variables significantly associated with decreased odds for seroprotection include: a diagnosis of clinical plasma cell dyscrasias versus

MGUS or asymptomatic disease (OR 0.36, 0.19-0.67, P=0.001), an increase in age of ten years (OR 0.75, 0.57-0.98, P=0.04), whether the patient was receiving IVIG treatment during the study period (OR 4.54, 1.21-16.99, P=0.02), and whether the patient was receiving cytotoxic chemotherapy (OR 0.39, 0.16-0.97, P=0.04). There was a trend toward significance for the following variables: an increase in the number of prior therapies by one was associated with a greater likelihood of seroprotection (OR 0.83, 0.68-1.02, P=0.07) and receiving the flu vaccine the year prior was associated with an increased likelihood of seroprotection (OR 1.60, 0.97-2.63, P=0.07).

Durability of Serologic Protection

SHIVERING 2 included an optional end of study (EOS) blood draw for the specific purpose of assessing serologic protection throughout the entire flu season in both arms. In total, 56 patients chose to take part in the EOS blood draw and were included in the following results. For the following results, "loss of seroprotection" indicates that the patient attained seroprotection (HAI≥40) and then lost that protection (HAI<40) by the end of the study period. Figure 4 illustrates the percentage of patients in each arm that were no longer seroprotected (HAI≥40) at EOS. At EOS, the standard of care arm lost seroprotection to all three strains at a higher rate compared to the experimental arm, but no comparative difference is statistically significant.

Figure 4: Loss of seroprotection by EOS by strain and study arm.

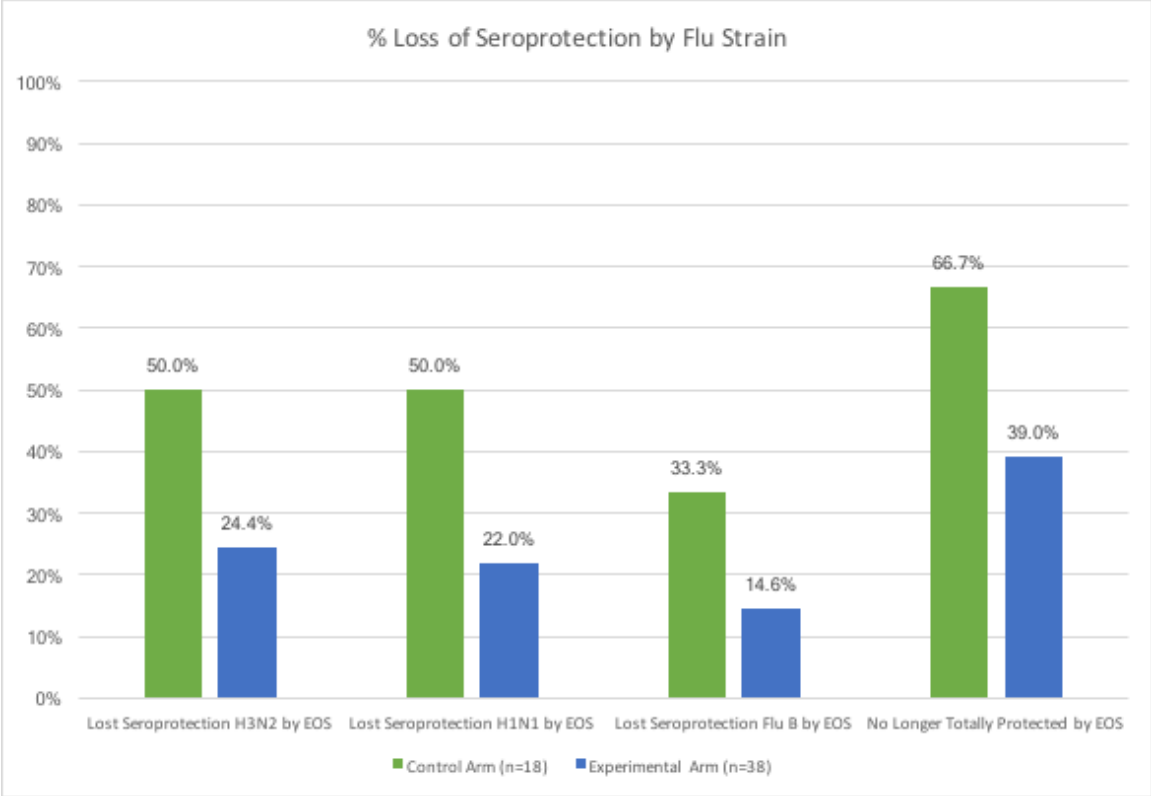


Figure 5 illustrates the percentage of patients in each arm, separated by disease stage (early versus advanced), that lost seroprotection to each strain at EOS. In both the experimental and control arms of the study, patients with advanced disease lost seroprotection by EOS at a higher rate. Patients in the advanced control group lost seroprotection at a significantly higher rate than patients in the early experimental group (73% vs. 22%, $P<0.05$).

Figure 5: Loss of seroprotection by strain, disease stage and study arm.

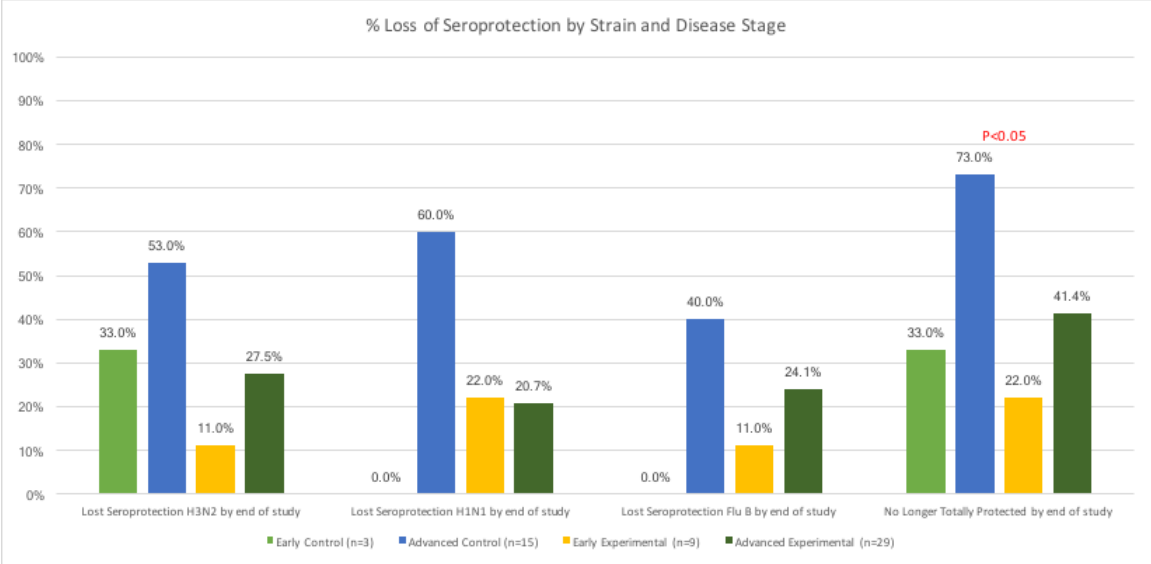


Figure 6 illustrates the average percentage change in HAI titer across all three strains at each time interval: day 30 to 60, day 60 to EOS, and day 30 to EOS. During the day 30 to day 60 interval, the average change in HAI titer level for the experimental arm (78.4%) was significantly greater than for the control arm (-8.6%) ($P<0.05$).

Figure 6: Average % Change in HAI titer during each time interval by arm.

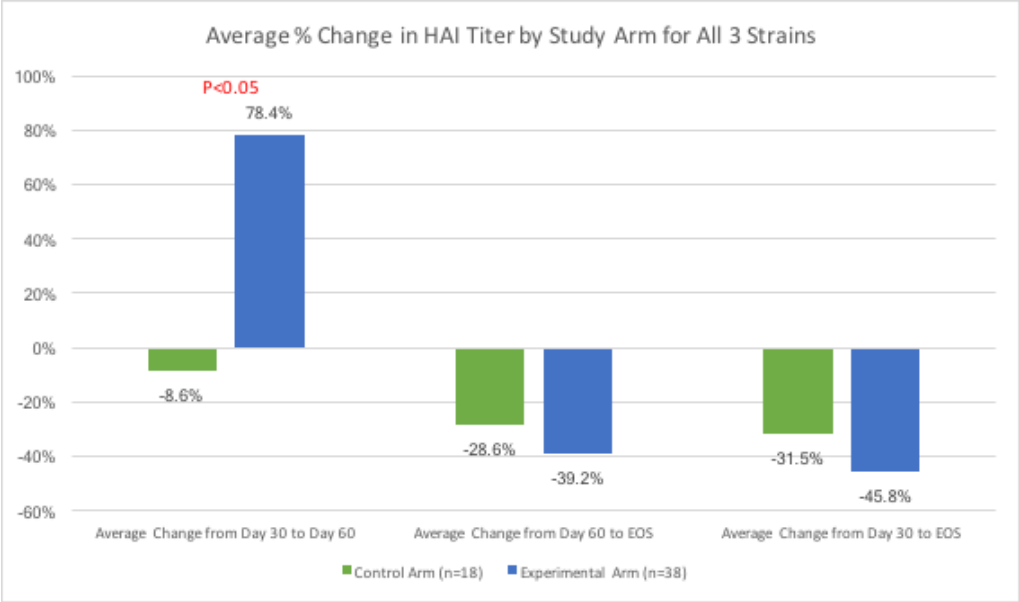
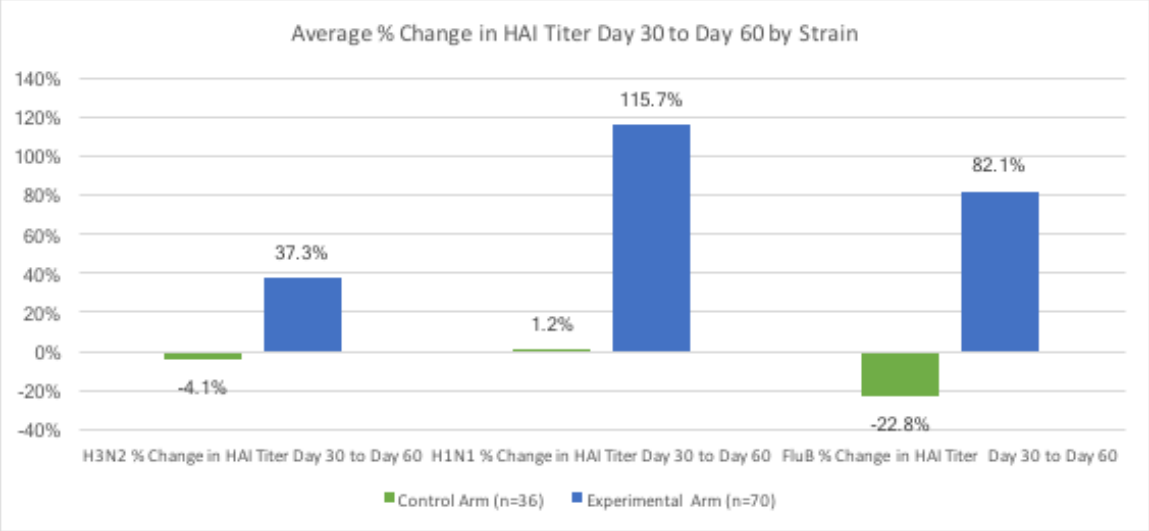


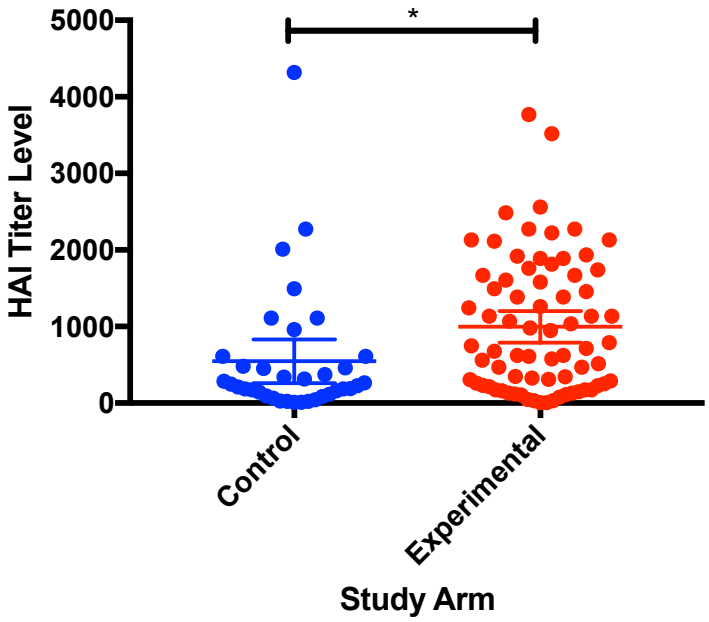
Figure 7 focuses on the day 30 to day 60 interval and illustrates the percentage change in HAI titer during that time interval by strain. During that time interval and across all three strains, the average HAI titer level of the experimental arm increased at a higher rate than the control arm, with the experimental arm positive in each case and the control arm negative or just above zero (1.2%) in each case. None of these comparisons were statistically significant.

Figure 7: Average % Change in HAI titer by strain and arm during the day 30 to day 60 time interval.



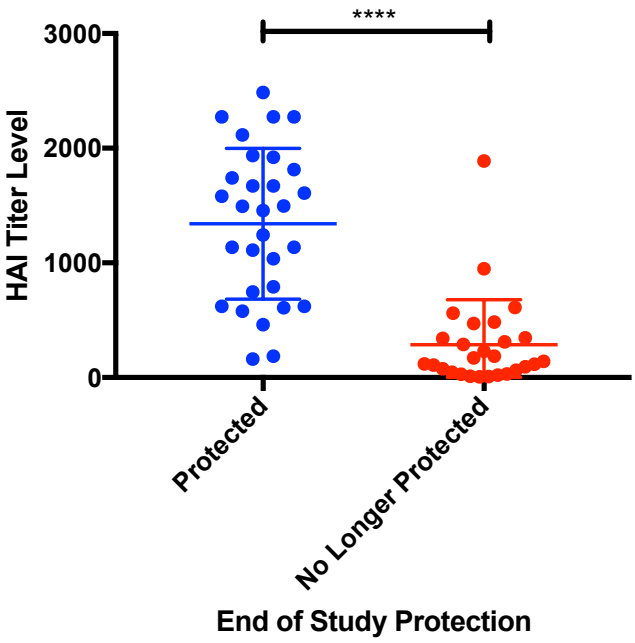
Next, the investigation focused on an estimated peak HAI titer value, rather than the change in HAI titer, as a possible indicator of vaccine durability. To estimate the peak titer values in a uniform fashion each patient's day 60 titer was used. Patients in the experimental arm had a significantly higher peak HAI titer level (996.2 ± 102.7 vs. 547.1 ± 140.8, P=0.0124, Figure 8).

Figure 8: Peak HAI Titer Level by Study Arm



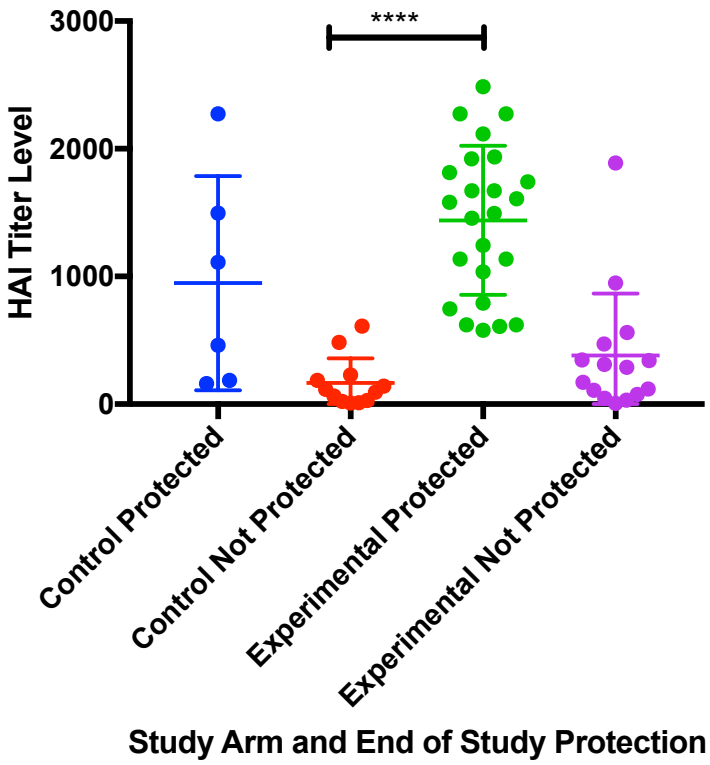
Patients that were protected against all three strains at the end of the study had significantly higher peak HAI titer levels than patients that lost protection by the end of the study (1341 ± 120.1 vs. 285.3 ± 75.72, P<0.0001, Figure 9).

Figure 9: HAI Titer Peak by End of Study Protection



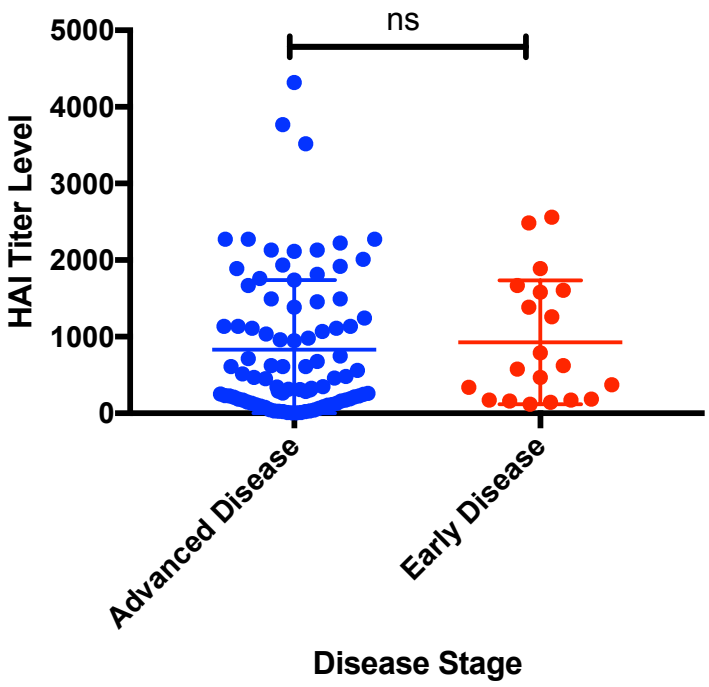
Patients in the experimental arm that were protected at the end of study time point had significantly higher absolute peak titer levels than patients in the control arm that were not protected (1440 ± 119.4 vs. 166.1 ± 55.78, P<0.0001, Figure 10.)

Figure 10: Peak HAI Titer by Study Arm and EOS Protection



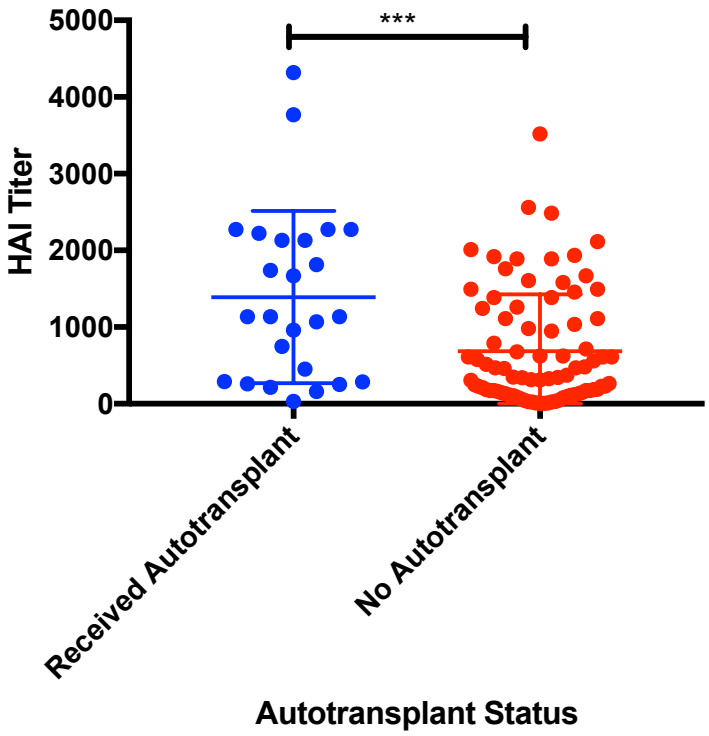
There was no significant difference in peak HAI titer level between patients with advanced disease versus those with early disease (928.2 ± 180.9 vs. 829.8 ± 96.3, P=0.66, Figure 11).

Figure 11: Peak HAI Titer by Disease Stage



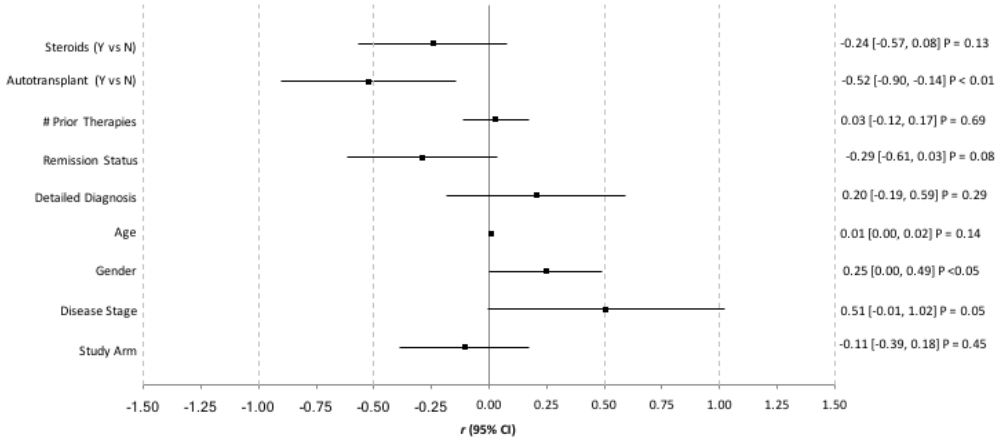
There was no significant difference in HAI titer levels due to steroid use (979.3 ± 124.5 not on steroids vs. 660.9 ± 100.6 on steroids, P=0.065) or gender (998.8 ± 121.2 for females vs. 715.2 ± 117.5 for males, P=0.096), but there was a significant difference in peak titer level between patients who received an autotransplant and those that did not (1390 ± 224.6 vs. 686.6 ± 80.73, P=0.004, Figure 12).

Figure 12: Peak HAI Titer by Autotransplant Status



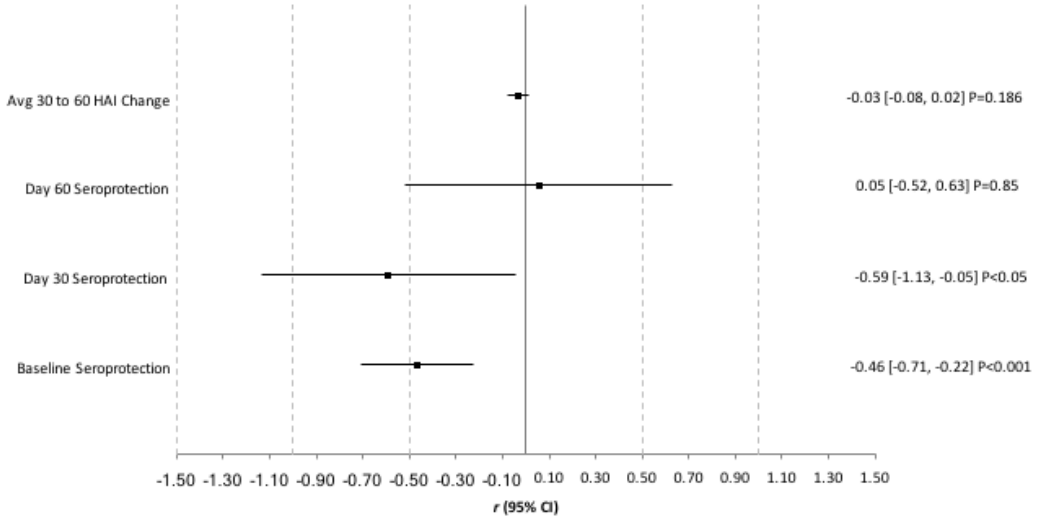
Next, the investigation focused on statistical correlations between patient demographics or clinical characteristics and a loss of seroprotection by the end of the study. Figure 13 illustrates the Forest Plot results from a regression analysis of the loss of seroprotection against study arm (experimental vs. control), disease stage (early vs. advanced), gender, age, detailed diagnosis (active disease vs. asymptomatic disease), baseline disease remission status (>partial response to therapy vs. <partial response to therapy), number of prior therapies, autotransplant recipient status (yes vs. no), and current steroid therapy (yes vs. no). The variables of gender (O.R. 0.25, 0.002–0.49, $P<0.05$) and autotransplant status (O.R. -0.52, -0.9 – -0.14, $P<0.01$) are statistically significant, while the variable disease stage (O.R. 0.51, -0.009–1.02, $P=0.054$) is trending toward significance.

Figure 13: Clinical correlates with loss of seroprotection at study end.



Finally, the investigation focused on statistical correlations between the loss of seroprotection at the end of the study period and the following variables: seroprotection against all 3 strains at baseline, seroprotection against all 3 strains at day 30, seroprotection against all 3 strains at day 60, and the percent change in HAI titer during the day 30 to day 60 time interval. Figure 14 illustrates the Forest Plot results for this model. The variables for seroprotection against all 3 strains at baseline (O.R. -0.46, -0.7--0.22, P<0.001) and for seroprotection against all 3 strains at day 30 (O.R. -0.59, -1.13--0.46, P<0.05) are both statistically significant and correlated with decreased odds of losing seroprotection.

Figure 14: HAI titer level correlates with loss of seroprotection at study end.



DISCUSSION

These clinical trials have investigated a novel vaccine regimen in patients with plasma cell dyscrasias. The study reconfigured an old vaccine in a new and novel way, and the results show that this regimen could have practice-changing implications in the field of myeloma and beyond. In addition to lowering the clinical infection rate, these trials have provided key insight into the way in which patients with plasma cell dyscrasias respond to vaccines, how the serologic response rises and then falls, and what clinical or demographic factors encourage or impede the development of immunologic defense over time.

The pilot clinical trial was focused on establishing the safety and efficacy of the high-dose booster regimen in patients with plasma cell dyscrasias. In this study's cohort of patients, the regimen was safe and well tolerated, with no grade 2 or greater adverse events attributed to the vaccine throughout the trial. The pilot trial suggests that the high-

dose booster regimen provides improved serologic protection and is associated with a significantly lower rate of influenza infection than what is expected with the standard of care in this population. After a single dose of the high-dose vaccine seroprotection against all three strains increased from 4% to 47%, and after the booster vaccine the seroprotection rate increased further to 65%. This high a level of seroprotection against all three strains has not been previously recorded and is on par with what most healthy adults reach with the standard of care vaccine. Furthermore, the laboratory-confirmed infection rate of 6% was significantly lower than expected in this population, suggesting a real clinical benefit of this regimen.

Finally, the correlation of serologic response with demographics and clinical characteristics revealed several interesting findings that merit further investigation. This study has identified several variables correlated with improved serologic response. Patients of the female gender and those that endured a documented flu infection during the previous year were at significantly increased odds of attaining seroprotection. An infection the previous year may have primed the immune system to respond to the vaccine or it is possible a more robust response the year prior made these patients serologically closer to seroprotection during the current year. This study has identified several variables that correlate with lower odds of reaching seroprotection: active PCD diagnosis, increased age, currently on IVIG treatment, and currently on cytotoxic chemotherapy. Taken together, these variables suggest that sicker patients are less likely to form a serologically robust response to the vaccine regimen.

Taking a step back, this study raises multiple questions: does everyone need this regimen or will some plasma cell dyscrasia patients, possibly those with milder disease,

achieve equally favorable results with the current standard of care? Are there some patients who will benefit more than others from this regimen? These data suggest that sicker patients, those older patients with an active PCD diagnosis that has them on IVIG and conventional chemotherapy, need this regimen more and should perhaps be targeted to receive this regimen in place of the standard of care. However, this was only a pilot trial which was implemented during a particularly benign flu season that saw lower rates of flu infection across the country. To truly extract the actionable clinical and scientific insights a larger cohort of patients and an experimental, randomized, double blind, placebo-controlled, phase 2 trial was necessary.

SHIVERING 2 was this trial. It provided the opportunity to test the hypotheses that the high-dose booster regimen is superior to the standard of care and results in improved clinical outcomes, more robust and longer-lasting serologic protection, and that healthier patients, as was the case in SHIVERING 1, respond better than sicker patients. Once again, the regimen appears to be safe, with no grade two or greater adverse events attributed to the vaccine. This is no small point. In an age when vaccines are front page news as often for their harm as for their protection the fact that giving patients the high-dose vaccine plus the booster, a total of eight times the inactivated antigen, did not lead to more common or more serious adverse events is a key finding.

Supporting the findings in the pilot trial, patients in the experimental arm had a significantly lower rate of laboratory-confirmed flu infection than patients in the control arm. This finding has practice-changing implications: patients who receive the high-dose booster regimen were significantly less likely to get the flu than patients who receive the

standard of care. A larger trial would be needed to confirm or refute these findings, but these results suggest that patients on this regimen get the flu less often.

A comparison of the HAI seroprotection rates of the control arm and the experimental arm demonstrates the serologic impact of the high-dose booster regimen. At day 30 there was no significant difference in seroprotection rate, suggesting that the single high-dose vaccine, in this case, did not lead to significantly higher titer levels in the experimental arm. The booster high-dose vaccine, however, did just that and by day 60 patients in the experimental arm had significantly higher rates of seroprotection than the control arm. This suggests that the booster high-dose vaccine is necessary for the regimen to be superior to the standard of care. At the end of the study period, the rate of seroprotection was not significantly higher in the experimental arm than in the control arm (P=0.07). This possible trend led the team to further investigate the drivers and correlates of HAI titer durability.

Moving on to the durability of the produced serologic defense, the key driver of this thesis. As described above, there is little research into how long the flu vaccine protects the recipient. Is it the full year? Is it 6 months? Or 3 months? Do those eager vaccine recipients, who line up for their shot when it first becomes available in September, lose protection by the height of the flu season in January and February? And what impacts that durability? Is it age or gender? Is it dependent on the previous flu season, either a past infection or previous vaccines? Shockingly little is known about this major source of morbidity and mortality around the world each year, and a better understanding of the flu vaccine is the best shot at standing up to this yearly scourge. If our healthcare system is going to stand a chance against future flu pandemics (the

Spanish Flu killed more people than World War I), better vaccines are needed and our current vaccination tools must be used with greater dexterity.

This picture becomes even more interesting when investigating a cohort of patients with multiple myeloma and other plasma cell dyscrasias. Even less is known about the robustness and durability of serologic defense in these particularly vulnerable patients. This analysis of the durability of HAI titer levels sheds some much needed light on this important question. Although the data is limited by sample side (only 56 patients opted for an end of study blood draw) there still exist several exciting findings in these data to report and continue to investigate.

Figure 4 (page 32) highlights the loss of seroprotection by strain at the end of study time point. For each strain and for all three strains combined, the control arm lost seroprotection at a higher rate than the experimental arm. This is not surprising, but a key point when advocating for the need for this high-dose booster regimen. Additionally, these data give rare insight into how the standard of care performs over time in this study population. Half of all patients in the control arm lost seroprotection against both Flu A (H1N1 and H3N2) strains by the end of study point. This standard of care vaccine, in this population, does not appear to withstand the test of (flu season) time. A larger trial is necessary to determine if these suggestive findings are significant and clinically applicable.

Figure 5 (page 33) further breaks down this question by highlighting the loss of seroprotection against each strain by disease stage: early disease versus advanced disease. The data suggests that patients with advanced disease are likely to lose seroprotection against each strain: 53% lost H3N2 protection, 60% lost H1N1 protection and 40% lost

Flu B protection. These data points are in stark comparison with patients with early disease in the experimental arm, which suggests that they form a more long-lasting immunity: 11% lost H3N2 protection, 22% lost H1N1 protection, and 11% lost Flu B protection. The power and statistical significance of these data are limited by the small sample size, but this work represents an initial investigation into which patients lose seroprotection and how long it takes them to do so. The most interesting and statistically significant finding is in the comparison of advanced-control to early-experimental patient groups. The advanced-control patients were significantly more likely to lose seroprotection when compared with the early-experimental patients ($P<0.05$). As hypothesized and evident in SHIVERING 1, the healthier the patients the more robust and sustainable their response is to the high-dose booster regimen. This finding suggests that these healthier patients benefit most from this new regimen. As for the advanced patients, other approaches may be necessary. It is possible they may need a vaccine in September and then the booster 60 or 90 days later, rather than the 30 day interval applied in this regimen.

Figure 6 (page 33) highlights when and to what extent serologic protection levels diverge between the two arms across the study period. The difference in HAI titer change between the control and experimental arm was significant during the day 30 to day 60 interval. The experimental arm had just received its booster and, interestingly, control arm HAI titer levels on average already began to decline by 8.6%. As HAI titer levels peaked higher on average in the experimental arm than in the control arm, it is not surprising or clinically significant that they then declined on average to a greater degree by the end of the study. Figure 7 (page 34) demonstrated the day 30 to day 60 interval

change for each strain, to reveal any differences in the serologic responses to each strain during that key time internal. Each strain showed a large jump in HAI titer average, with H1N1 increasing the most (115.7%). Likely due to the high variances and small data set, none of these comparisons were statistically significant. Once again, however, this analysis gives interesting insight into the serologic durability of the standard of care. In this study, during this key time interval, when HAI titer levels of the experimental arm are on average rising, those of the control arm are flat or in decline.

The analysis of how peak titer level predicts serologic durability revealed several interesting findings. Peak titer level is more indicative of durability than percent change in titer level and likely more practical, as it represents an absolute rather than relative value. The findings in Figures 8, 9 and 10 are not surprising: patients that received the high-dose booster regimen had significantly higher peaks and the higher the peak the higher the likelihood of a durable defense. The higher the peak the further it has to fall and the longer the patient remains protected. What is more interesting is determining who can produce those higher peaks. These data illustrate that gender and steroid status do not have a significant impact on peak titer, but a history of an autotransplant is associated with the ability to produce a high peak titer level. This is consistent with the finding in Figure 13 that illustrates the protective impact of an autotransplant on loss of seroprotection by the end of the study. These findings on the role of peak titer levels could have clinical significance in the future. If confirmed, it may make sense to give an initial vaccine, check the titer level 60 days later, and give a booster vaccine if the titer level is below a certain level. Additionally, certain factors, like an autotransplant, may be

protective in this process and a booster vaccine may deserve consideration in those patients that have not undergone such a procedure.

Similar to the analysis of SHIVERING 1 looking at the clinical correlates of seroprotection, Figures 13 (page 39) and 14 (page 40) examine the clinical correlates of serologic durability. The finding of the impact of gender is quite interesting and suggests that male patients are significantly more likely to lose seroprotection than female patients, controlling for other considered variables. This finding is consistent with the literature, as it has been shown that in older individuals there are differences between the genders in response to the influenza, tetanus, pertussis, shingles, and pneumococcal vaccines.[31] Crediting the impact of sex steroids, epigenetic regulation of the X chromosome, and the microbiome as possible mechanistic etiologies, a recent review on the topic reports, "The efficacy of vaccines recommended for older-aged adults is consistently greater for females than for males." Additionally, the autotransplant variable requires further investigation and possibly an entirely separate study looking just at vaccine efficacy in patients who undergo an autotransplant. These results suggest that patients that have had an autotransplant are significantly less likely to lose seroprotection by the end of the study. This model controls for disease stage, so the more durable response in patients that have undergone autotransplant cannot be attributed to the stage of their disease (early vs. advanced). Additionally, the relevant question is not just if the patient received a transplant, but when exactly that transplant occurred. It has been shown that the efficacy of a vaccine in this setting is influenced by the time elapsed since transplantation, the nature of the hematopoietic graft, the use of serial immunization, and the presence of graft-versus-host disease.[32] As mentioned previously, a separate study

that enrolled only patients who had undergone autotransplant would be needed to determine which clinical variables impact vaccine efficacy in plasma cell dyscrasia patients, as only 24% of patients in this study underwent an autotransplant in the past.

Finally, the analysis of how HAI titer levels early in the flu season correlate with the loss of seroprotection by the end of the flu season yielded interesting results that may be clinically relevant. Figure 14 (page 40) demonstrates that patients who were seroprotected against all three strains at baseline were significantly less likely to lose seroprotection by the end of the study. It is not possible for patients to have their HAI titer levels checked before each flu season, but it is possible to determine which patients received the flu vaccine the prior year and are thus more likely to be protected at baseline and thus less likely to lose that protection by season's end.

The SHIVERING trials represent an important investigation into a novel influenza vaccine regimen in patients with plasma cell dyscrasias. This regimen of a high-dose vaccine followed by a booster is safe and the results from a randomized, double blind, placebo-controlled, experimental trial suggest that it is more efficacious than the standard of care with regard to protecting against flu infections and sparking a robust and durable serologic defense for these vulnerable patients with plasma cell dyscrasias. In addition to improved efficacy over the standard of care, these trials suggest several demographic and clinical correlates that are associated with improved or impaired response to this novel vaccine strategy. Finally, the analysis of serologic timing represents the first detailed investigation into when patients with plasma cell dyscrasias develop and lose protection, what clinical or demographic factors impact this timing, and how the high-dose booster regimen should be used in this population.

The SHIVERING trials are an initial investigation into a growing field of research on vaccine efficacy in patients with cancer. These results will not immediately change clinical practice, but will hopefully move the needle toward using this high-dose booster regimen in patients with plasma cell dyscrasias. Morbidity from the influenza virus will likely continue to rise, with a record number of cases already occurring during the 2017 to 2018 season, and more such research is required to better protect vulnerable and healthy people from this dangerous virus. There are countless directions in which this research could be expanded upon in the future. First, a much larger study population is necessary to prove superiority of this regimen. The trial that led to the approval of the high-dose vaccine in people above the age of 65 enrolled 31,989 participants over two years across 126 health centers in the United States and Canada.[24] Second, about 80% of the patients in each SHIVERING study were receiving therapy at the time of the trial, and these therapies undoubtedly impact the patient's vaccine response. Each patient was on a combination of immunomodulatory drugs, steroids, cytotoxic chemotherapies, and proteasome inhibitors, in addition to their non-cancer related therapies. It is possible patients should not receive a vaccine on the same day they receive cancer therapies or steroids. This seems reasonable but in practice most patients try to batch their reasons for coming to the hospital. This question of vaccine timing in relation to anti-cancer treatment should be investigated further. Third, as discussed previously, the impact of stem cell transplant timing on vaccine response should be more clearly defined. Such a study would impact patients not just with plasma cell dyscrasias, but those with all hematologic malignancies for which transplant is a treatment option. The results from

this trial suggest that there is a significant difference but the optimal time interval between transplant and vaccines should be studied.

These are just a few of many additional questions that could be asked of this regimen and potential vaccine strategies in patients with plasma cell dyscrasias. The SHIVERING trials have pushed the envelope of how to vaccinate vulnerable, immunocompromised patients, but much work remains to be done before our influenza vaccine meet our patients' needs.

There are several key strengths of this work and several weaknesses that must be recognized. The primary strength is the methodology of SHIVERING 2. A randomized trial allows us to avoid selection bias, a placebo-controlled trials allows us to avoid bias from confounding factors, and the double-blinded set up avoided interpretation bias. Following the pilot trial of SHIVERING 1, which studied the safety and preliminary results of the new regimen, the randomized trial was the next step and represents the gold standard for determining superiority of a new regimen and is the cornerstone of evidenced-based medicine.[33] Additionally, the novel regimen as used in this unique patient population represents a key strength of this study and why this work will be of immediate interest to both patients and providers in the hematology community. Finally, the strength is in the data. Each patient in both trials was tracked closely throughout the trial periods, interviewed by a member of the trial team at each trial visit, and all data collected from the patient was cross-checked with their electronic medical record.

The primary weakness of this study is its size. It was difficult to enroll more than five or six patients in a day given the need to process each sample immediately. Furthermore, many potential trial participants were deemed ineligible after receiving their

flu vaccines immediately upon their availability and before hearing about the trial. It will take a multi-center, and multi-year study that further supports these results to elevate them to the height of clinical practice. Additionally, some might criticize our decision to randomize in a 2:1 distribution rather than a 1:1 distribution. This was done to increase the number of patients receiving the experimental regimen, but could be interpreted as evidence that the study team deemed our regimen to be superior from the start. However, these concerns represent the general systematic realities of designing a specified trial which required the collection of a large number of patients in a very limited period of time. In sum, this work represents a very strong effort and an interesting addition to the literature of plasma cell dyscrasias and vaccine efficacy. I hope it will trigger a passion for both fields in those who read it, as it has for the writer, and one day impact this vulnerable and fascinating patient population.

APPENDIX

1. **PBMC Protocol** – per Lin Zang of the Dhodapkar Lab

1. Peripheral blood (sodium heparin tube). Store blood tube(s) at room temperature if they cannot be processed immediately.
2. Prepare 50 ml tubes with 10 ml of Ficoll-Hypaque. Gently layer approx. 15 - 20 ml blood over F-H. Do not dilute blood to collect the plasma later.
3. Centrifuge the tubes at 2000 rpm for 25 min at RT with brake off.
4. Mononuclear leukocytes (MNL) should band in the middle of the tube. Using a 10ml pipette, gently collect the supernatant (Plasma) to within 0.5 cm of the cell layer to a 15ml tube. Collect cells into new 50 ml tube adding RPMI Medium to a total volume of 40-50ml.
5. Centrifuge at 1500 rpm, 10 min at 4 ˚C or RT.
6. Discard sup, gently re-suspend pellet with 2-5 ml cell culture medium (5% PHS/RPMI). Count cells with Trypan blue (10ul cell suspension + 10ul Trypan Blue).
7. Adding medium to the cell suspension tube to total of 20-25ml. Centrifuge at 1300 rpm for 6 min. at 4 ˚C.
8. If cells are to be used fresh: Re-suspend the cells as per protocol.
9. If PBMCs are to be cryopreserved: Prepare fresh reagent: 10% DMSO/FBS for cryopreservation while tubes are centrifuging.
10. Re-suspend PBMCs with the cryopreserving solution at 5 x 106 /ml
11. Immediately dispense cells into labeled cryovials.
12. Place the cryovials in a freezing container that has been filled with 70 % isopropanol according to the manufacturer's instructions. Store the freezing container at –80oC Freezer up to a week.
13. Transfer the cryopreservation of PBMCs into Liquid N2 Tank for long. term storage.
14. Store the collected Plasma in –80oC Freeze.

2. **HAI Titer Protocol** (as described in our published paper): sera were treated with a receptor destroying enzyme, Vibrio cholera filtrate (Sigma-Aldrich, St. Louis, MO), which eliminates nonspecific inhibitors that could confound the assay results. Working stocks for each of the 3 current influenza virus strains included in the clinical trial influenza vaccine were prepared by diluting the virus stock to a final HA titer of 8 HA units per 50 mL. Two-fold dilutions of the receptor-destroying enzyme- treated sera in buffer were then mixed with the working stock of each influenza virus strain. The serum virus samples were then incubated at room temperature for 30 minutes to allow any HA-specific antibodies present in the serum to neutralize the influenza virus. To each well, a 0.5% suspension of red blood cells was then added. The assay was then incubated on ice until the red blood cells in the buffer control sample formed a button and had agglutinated in the nonserum-containing control well. The HI titer is defined as the reciprocal of the highest dilution of serum that inhibits red blood cell agglutination. As defined previously,18 seroprotection to the influenza virus vaccine is based on achieving an antibody titer of 1:40, and seroconversion to the influenza virus vaccine is based on a fourfold increase in antibody titers.

3. **Flu morbidity screen questionnaire**: the following questions were asked to each patient at each study visit and at the end of the study, either in person or over the phone.

   Flu Morbidity Screen Questions

   □ During the study period, did the patient have any flu-like symptoms (fever, fatigue, headache, bodyache, cough, sore throat, fatigue)?

   ☐If yes, was the patient evaluated and tested for influenza?

   ☐Did the patient have a documented influenza infection during the study period?

   ☐Was the patient hospitalized as a result of an influenza infection?

   ☐Did the patient die as a result of an influenza infection?

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