Published in final edited form as:

Clin Lymphoma Myeloma Leuk. 2017 May ; 17(5): 296–304.e2. doi:10.1016/j.clml.2017.02.025.

Clinical and serologic responses following a two dose series of high-dose influenza vaccine in plasma cell disorders: a prospective, single-arm trial

Andrew R Branagan, MD^{1,2}, Eamon Duffy², Randy A Albrecht, PhD³, Dennis L. Cooper, MD^{1,2}, Stuart Seropian, MD^{1,2}, Terri L Parker, MD^{1,2}, Geliang Gan, PhD⁴, Fangyong Li, MPH⁴, Daniel Zeltermann, PhD⁴, Chandra Sekhar Boddupalli, PhD^{1,2}, Lin Zhang^{1,2}, Rakesh Verma, PhD^{1,2}, Thomas M Ferencz, RPh¹, and Madhav V Dhodapkar, MD^{1,2}

¹Yale Cancer Center, New Haven, CT

²Yale School of Medicine, New Haven, CT

³Icahn School of Medicine at Mt. Sinai, New York, NY

⁴Yale School of Public Health, New Haven, CT

Abstract

MicroAbstract—The goal of the present study was to evaluate a novel prospective influenza vaccination strategy in patients with plasma cell disorders. Fifty-one patients were treated with a two dose series of high-dose inactivated trivalent influenza vaccine. This vaccination strategy was well tolerated led to very high rates of seroprotection against influenza.

Background—Patients with multiple myeloma (MM) and other plasma cell disorders are highly susceptible to influenza infections, which are major causes of morbidity in this population despite routine administration of seasonal influenza vaccination. Existing data are limited by small and retrospective studies, which suggest poor seroprotection rates of less than 20% following standard influenza vaccination in MM.

Methods—Patients with plasma cell dyscrasia (N=51) were treated with a two dose series of high-dose inactivated trivalent influenza vaccine over the 2014–2015 influenza season. Laboratory-confirmed influenza infections were identified through seasonal surveillance, sera were collected for influenza hemagglutination inhibition (HAI) titer assays, and logistic regression models were used to identify clinical correlates of HAI serologic responses.

Corresponding Author: Andrew Branagan, MD, Andrew.branagan@yale.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: none

Author's contributions

ARB and MVD designed the study, ARB wrote the initial draft of the manuscript. DLC, SS, TP, and MVD enrolled patients in the study. RAA performed the influenza antibody titer assays. GG, FL, and DZ did the statistical analysis. All authors were involved in interpreting the data, reviewing and revising the manuscript, and providing final approval.

Findings—Influenza vaccine was well tolerated without any vaccine-related grade two adverse events. Only three patients (6%) experienced laboratory-confirmed influenza. Rates of HAI seroprotection against all three vaccine strains (influenza B, H1N1, and H3N2) increased from 4% at baseline to 49% and 65% following one and two doses, respectively. Risk factors associated with lower likelihood of HAI serologic response included plasma cell disorder requiring therapy, disease response assessment < partial response, or active conventional chemotherapy. Alternatively, active therapy with an immunomodulatory drug alone or with proteasome inhibitor was associated with a higher likelihood of HAI serologic response.

Conclusions—These data demonstrate that in contrast to historically poor results with standard influenza vaccination, this novel high-dose booster vaccination strategy leads to high rates of seroprotection. Randomized controlled studies are needed to compare this to standard vaccination strategy.

Keywords

multiple myeloma; monoclonal gammopathy of undetermined significance; immunity; influenza

Introduction

Multiple myeloma (MM) is a malignant plasma cell disorder, marked by neoplastic plasma cells in bone marrow that secrete monoclonal immunoglobulins¹. Notably, infections are a significant cause of morbidity and a leading cause of death in patients with MM². Influenza is a major cause of morbidity and in the US and is responsible for over 40,000 deaths per year³. Despite routine administration of seasonal influenza vaccination to patients with MM, risk of influenza infections remains high. A Swedish study of over 9,000 myeloma patients demonstrated that myeloma was associated with a 10 fold increased risk of viral infections, including a 6.1 fold risk of influenza, relative to healthy controls⁴.

Patients with MM have multiple factors that contribute to immunosuppression including suppression of normal uninvolved immune globulins as well as alteration in T cell immunity^{5,6}. Anti-cancer therapies can further impact the immune system and chemotherapy has been associated with diminished protection from influenza vaccination^{7,8}. Lastly, immunity in patients with plasma cell disorders is also impacted by age-related decline in immune function.

US Advisory Committee on Immunization Practices recommends that all adults over the age of 50 as well as immunocompromised individuals such as those with MM receive a single annual dose of inactivated influenza vaccine⁹. Inactivated influenza vaccination is associated with a robust primary serologic antibody response against hemagglutinin surface protein of each viral subtypes within the vaccine, which may be measured with hemagglutination antibody inhibition (HAI) titers. Seroconversion refers to four-fold rise in HAI titers and seroprotection refers to HAI titer level 1:40. HAI seroprotection corresponds to an estimated 50% clinical protection from influenza infections based on studies in young healthy adults^{10,11}. HAI titers are the most widely accepted serologic correlates and form the basis for approval of new influenza vaccines¹².

Existing data on influenza vaccine responses in MM patients are limited but suggest low HAI titers with seroprotection rates of less than 20% following standard flu vaccination^{8,13–15}. One potential approach to enhancing the immune efficacy of a vaccine is the administration of a booster dose. Two studies previously investigated the benefit of a 4-week booster dose of standard dose flu vaccine in MM patients. Ljungman et al randomly assigned a mix of patients with all hematologic malignancies (including 10 with MM and 4 with Waldenstrom's macroglobulinemia) to vaccination with one versus two doses of influenza vaccine and found no overall increase in serologic protection in the cohort receiving two vaccines¹⁵. Unfortunately, this study included patients with diverse hematologic malignancies that differed in terms of their capacity to respond to vaccines. Hahn et al reported a retrospective analysis of 48 MM patients over the 2013–2014 flu season at their institution. Sero-protection following one vaccine was detected in only 7 patients (14%). Twenty-four of the remaining patients received a second vaccine and 33% of these patients achieved seroprotection. Due to its retrospective nature, the effects on influenza infections were not systematically evaluated. There remains an unmet need for prospective data evaluating the clinical and serologic efficacy of influenza vaccination specifically in PCD patients and existing data suggest that only a minority of patients respond to current vaccination strategy.

One potential approach to enhance vaccine efficacy is to administer a higher dose of antigen. Based on studies reporting increased serologic protection, Fluzone® High-Dose inactivated influenza vaccine is approved in the US for adults aged 65 and older (60 µg of hemagglutinin antigen vs. 15 µg standard dose). Sadfar et al observed an improvement in vaccine-induced HAI titer responses by increasing the hemagglutinin antigen in a dose-dependent fashion up to 135 µg without increased toxicity¹⁶. Prior studies have not specifically evaluated booster strategy with high-dose influenza vaccine in MM patients.

Based on these considerations, we designed a novel influenza strategy for MM patients combining the use of high-dose inactivated influenza vaccine and a two-dose series in the present study.

Methods

Clinical trial design

We evaluated a novel influenza vaccine strategy in the Study of High-Dose Influenza Vaccine Efficacy by Repeated Dosing IN Gammopathy patients (SHIVERING) trial over the 2014–2015 influenza season. This pilot clinical trial was conducted at a single, US academic hospital, Yale Smilow Cancer Hospital. Eligibility criteria allowed any patient with a diagnosis of MM or another plasma cell disorder without any known contraindication to trivalent inactivated influenza vaccine. All participants received the study intervention of two doses of trivalent Fluzone® High-Dose influenza vaccination 30 days apart, regardless of age. Participants were stratified into two broad cohorts, PCD requiring therapy or asymptomatic gammopathy. Current analysis is focused on changes in HAI titer serologic response rates. Other endpoints included safety as well as the laboratory-confirmed influenza infection rates.

Study oversight

The trial was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and appropriate regulatory requirements. Local ethics committees or institutional review boards approved the protocol. All patients provided written informed consent. This trial was registered prior to enrollment on [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02267733), NCT02267733.

Study influenza vaccine

Fluzone® High-Dose (Sanofi Pasteur) is a trivalent inactivated vaccine that is administered intramuscularly. The vaccine contains influenza split virus antigens that are formulated to contain a total of 180 µg of influenza virus hemagglutinin (60 µg each from the three current influenza virus strains). During the 2014–2015 influenza season the vaccine contained: 1) an A/California/7/2009 (H1N1) pdm09-like virus; 2) A/Texas/50/2012 (H3N2)-like virus; 3) a B/Massachusetts/2/2012-like virus.

HAI titer measurements

Hemagglutination inhibition (HAI) assays were performed by the following standardized protocol²⁹. Briefly, sera were treated with receptor destroying enzyme, *Vibrio cholera* filtrate, (Sigma-Aldrich, St. Louis, MO), which eliminates nonspecific inhibitors that may confound assay results. Working stocks for each of the three current influenza virus strains included in the clinical trial flu vaccine are prepared by diluting the virus stock to a final HA titer of 8 HA units/50µL. 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 were then added. The assay is then incubated on ice until the red blood cells in the buffer control sample form a button, and hemagglutinate in the non-serum-containing control well. The HI titer is defined as the reciprocal of the highest dilution of serum that inhibits red blood cell hemagglutination. As defined elsewhere^{17,18}, seroprotection to the influenza virus vaccine is based on achieving an antibody titer 1:40 and seroconversion to the influenza virus vaccine is based on a 4-fold increase in antibody titers.

Influenza infection surveillance

All study participants underwent active influenza surveillance from time of enrollment until the end of the influenza season, May 31, 2015. Possible influenza infection was considered based on either 1) respiratory illness defined as the occurrence of one or more of the following: sneezing, nasal congestion or rhinorrhea, sore throat, cough, sputum production, wheezing, or difficulty breathing or 2) a protocol-defined influenza-like illness based on the current Common Terminology Criteria for Adverse Events (CTCAE v4.0) defined as a constellation of symptoms including dry cough, temperature >38°C, chills, body aches, malaise, and loss of appetite. If a subjects met criteria for possible influenza infection, staff members at the study site attempted to collect a nasopharyngeal swab within 5 days after

onset of the illness. Laboratory confirmation of influenza in nasopharyngeal swabs was made with a positive result by direct fluorescent antibody or PCR testing.

Statistical analysis

Patients' characteristics were summarized with frequency and percentage for categorical variables, median and range for continuous variables. McNemar test was used to compare the changes in seroprotection rate from baseline to post first vaccination and to second vaccination, as well as changes in seroconversion rate from post first vaccination to second vaccination. Generalized Estimating Equations (GEE) were used for correlated binary outcomes to assess the impact of individual risk factors on seroprotection and seroconversion. A secondary outcome was to compare the rate of lab-confirmed influenza in this cohort to the average expected annual infection rate (20%) using Fisher's exact test. 95% confidence interval was calculated. For all statistical tests, the significance level is set at $P < 0.05$, two sided, and all analyses are carried out using SAS 9.4.2 (SAS Institute, Cary, NC).

Results

Fifty-one patients were enrolled and all patients received both doses of Fluzone® High-dose influenza vaccine. Forty-nine patients had a diagnosis of MM and two of Waldenstrom's macroglobulinemia. Forty-one patients had disease-requiring therapy and ten patients had either monoclonal gammopathy of undetermined significance (MGUS) or asymptomatic disease. Median age was 65 years and additional baseline patient characteristics are summarized in Table 1.

Safety

No patients experienced grade 2 adverse events attributed to vaccine. The most common side effects were mild local soreness and redness around the site of injection, muscle aches, and general malaise.

HAI titer response rates

Rates of seroprotection significantly improved after one and two doses of Fluzone® High-Dose vaccine for all three influenza vaccine strains compared to baseline (Figure 1a and Suppl Table 1, $p < 0.0001$ for all). Seroprotection against influenza B improved significantly after the second dose (57% to 84%, $p < 0.0001$), whereas no significant increase was observed against individual H1N1 or H3N2 strains. The total seroprotection rate (seroprotection against all three vaccine influenza strains) increased from 4% at baseline to 47% after initial dose ($p < 0.001$), and to 65% after the second dose ($p < 0.01$).

Rates of seroconversion against H3N2, H1N1, Influenza B and all three combined strains after initial dose were 69% (35/51), 73% (37/51), 67% (34/51), and 39% (20/51), respectively. As shown in Figure 1b and Suppl Table 2, seroconversion against influenza B improved significantly after the second dose (67% to 96%, $p < 0.001$) and seroconversion against all three strains increased from 39% to 55% after second vaccination ($p = 0.02$). However, no significant increase was observed against individual H1N1 or H3N2 strains.

Clinical correlates of serologic responses

We utilized GEE logistic regression modeling¹⁹ to identify potential variables associated with total seroprotection (Figure 2a) and seroconversion (Figure 2b). Seven variables were significantly associated with overall odds of seroprotection. Receiving both doses of study vaccine, active treatment with intravenous immunoglobulin (IVIG) and a diagnosis of influenza infection in the previous flu season were associated with higher odds of seroprotection. In contrast, the following four variables were associated with lower odds of seroprotection: having a diagnosis of PCD requiring therapy (as opposed to asymptomatic disease/MGUS), active therapy with conventional chemotherapy, suppression of uninvolved immunoglobulins, and laboratory-confirmed diagnosis of viral respiratory infection other than influenza during the study period. A fifth variable of disease response status < partial response at study entry was identified as having a trend toward a lower likelihood of seroprotection ($p=0.07$). Figure 3 summarizes all five unfavorable risk factors associated with a lower likelihood of achieving total seroprotection. Both doses of study vaccine significantly improved likelihood of total seroprotection compared to baseline with an odds ratio (OR) of 21.78 (95% CI: 5.35 – 88.57, $p < 0.001$) after the initial dose and OR of 44.90 (95% CI: 10.59 – 190.44, $p < 0.001$) after the second dose. Additionally, the second dose significantly boosted total seroprotection relative to the initial dose with an OR of 2.06 (95% CI: 1.20 – 3.55, $p = 0.009$). These changes were still significant after controlling for all other variables (including, age, sex, having a diagnosis of PCD requiring therapy, active treatment with IVIG, having suppression of uninvolved immunoglobulins, diagnosed with flu 1 year prior to study and diagnosis of other respiratory viral infections during the study), with an adjusted odds ratio of 2.61 (95% CI: 1.35 – 5.07, $p = 0.005$) from 1st to 2nd dose of vaccine.

Older age was associated with a significantly lower likelihood of achieving seroconversion against all three vaccine strains. In contrast, active therapy with an immunomodulatory drug (IMiD^R), with or without combination with a proteasome inhibitor plus IMiD^R was associated with a higher likelihood of achieving seroconversion against all three vaccine strains. Second dose of vaccine significantly boosted seroconversion against all three influenza strains compared to initial dose with an odds ratio of 1.89 (95% CI: 1.12 – 3.17, $p = 0.02$). This change retained its significance after we controlled for age, sex, having a diagnosis of PCD requiring therapy, active treatment with combination proteasome inhibitor plus IMiD^R. The adjusted odds ratio was 2.19 (95% CI: 1.17 – 4.08), $p = 0.01$, indicating the odds of overall conversion was doubled after a second vaccine dose. In adjusted analysis, older age remained a significant predictor to lower likelihood of seroconversion independent to vaccination (OR of 10 years increase in age: 0.38, 95% CI: 0.20 – 0.72, $p=0.003$).

Laboratory-confirmed influenza infections

Only 3 of the 51 total patients enrolled developed laboratory-confirmed influenza infections and their individual characteristics are summarized in Suppl Table 3. The infection rate was 5.9% (95% confidence interval from 1.2% to 16.2%). The infection rate was significantly lower than the expected 20% infection rate based on CDC estimate ($p = 0.01$; Suppl Figure 1).

Discussion

This prospective pilot clinical trial suggests both safety and efficacy of a two dose series of trivalent Fluzone® High-Dose influenza vaccine in MM patients. This influenza vaccine strategy was well tolerated without any unexpected or vaccine-related serious adverse events. The observed rates of HAI titer seroprotection and seroconversion against all three vaccine influenza strains significantly improved after two doses compared to one. This is the first study to demonstrate that the generation of protective antibody titers following influenza vaccination is possible in a majority of MM patients. Further, improved clinical benefit was suggested by a laboratory-confirmed influenza infection rate of 6%, compared to an expected rate of at least 20%.

Total seroprotection after one dose of high-dose vaccine reached 47%, and significantly increased again to 65% after a second dose. These data compare favorably with previous studies suggesting seroprotection rates less than 20% with a single standard vaccine⁸ and up to 33% with two doses of standard vaccine¹⁴. Rates of seroconversion also significantly improved following both the initial dose to 39% and following the second dose to 55%. Rates of improvement for both seroprotection and seroconversion following a second dose compared to initial dose were largely driven by HAI titers against influenza B. It is unclear why HAI titers after a second dose were more robust for influenza B and whether or not this vaccination strategy is particularly important for an influenza B-specific response.

Notably, in this study we identify five clinical risk factors which are associated with an overall lower likelihood of achieving total seroconversion; 1) PCD requiring therapy (as opposed to asymptomatic disease/MGUS), 2) suppression of uninvolved immunoglobulins, 3) active treatment with conventional chemotherapy, 4) disease response status < partial response, and 5) diagnosis of respiratory infections other than influenza during the flu season. Interestingly, while seroprotection improved for all study patients as a whole following two doses of high-dose influenza vaccine, patients having three or more of these unfavorable risk factors seem to have the greatest benefit from the second dose. Our findings support the idea that infectious risk generally increases with a higher tumor burden in MM. For example, studies have demonstrated a particularly high risk of infection in the first month of diagnosis which improves with initial therapy but infectious risk again worsens following later stages of disease progression²⁰. Our data also identify active treatment of conventional chemotherapy as an unfavorable risk factor, as noted previously in general oncology patients²¹. Additionally, patients with concurrent viral respiratory infections were observed to have lower risk of seroprotection, suggesting that a subgroup of MM patients may have a state of increased overall infectious risk. Interestingly, patients with history of documented influenza infection in the preceding year had higher rates of seroprotection, indicating that understanding the immune response to natural infection may provide a template for improving vaccine strategies. Similarly, IVIG administration may provide passive immunity and correlated with higher rates of seroprotection.

With emergence of newer therapeutic options for MM, their potential effects on influenza and other routine vaccinations needs to be further explored. Patients being treated with IMiDs^R alone or in combination with a proteasome inhibitor demonstrated a significantly

increased likelihood of seroconversion to all three vaccine influenza strains. The underlying mechanism needs further study, but may be related to improved disease control or an immune benefit driven by IMiDs, as suggested previously²². Interestingly, we did not observe an adverse impact of corticosteroid therapy on serologic responses.

We chose to utilize formal surveillance to carefully measure laboratory-confirmed influenza infections as an endpoint in this study. Such clinical endpoints as laboratory-confirmed influenza infection rates are lacking from most vaccine studies involving MM patients. Similarly, correlations between HAI titer response rates and protection from clinical influenza infections in MM patients are unknown. However, our study suggests concordance between having protective HAI titers and protection from infection. For example, one patient was infected with influenza B and lacked seroprotective HAI titers to influenza B while having protective titers to the other strains. The other two patients were infected with H3N2 and although both had achieved seroprotective HAI titers to H3N2, circulating H3N2 was very poorly matched over the 2014–2015 flu season (CDC estimated >80% drifted from the vaccine strain²³).

Despite these encouraging results, we recognize the limitation of a relatively small single-arm pilot study. Larger sample sizes are needed for detailed analysis of risk factors predictive of poor response. Further, a non-randomized trial is limited to historical comparisons, which can be particularly challenging with influenza given the high season-to-season variability. Nonetheless, the seroprotection rates observed in this study, to our knowledge, have not been achieved with any prior influenza vaccination strategy in MM. As such, we are currently conducting a randomized, double-blind, clinical trial comparing this novel two dose series of Fluzone® High Dose influenza vaccine to a single age-based standard of care vaccination. Patients in the present study identified as having favorable risk factors (those without hypogammaglobulinemia, asymptomatic patients and those with > partial response to therapy) displayed high serologic responses following a single high-dose vaccination and thus the added benefit of a two dose series in these patients was not demonstrated. Conversely, subsets of patients with high risk features identified here achieve suboptimal seroprotection rates following this approach, and newer vaccine strategies should be explored in this cohort. Clinically relevant endpoints for MM patients (such as influenza infection rates, influenza-associated hospitalizations, and mortality) are crucial in better understanding the true burden of influenza in this patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Arthur Sekerak Cancer Fund for their generous support of this trial. ARB is supported in part by the National Institutes of Health (UL1 TR000142). MVD is supported in part by funds from the National Institutes of Health (CA106802, CA197603). We also thank all of the participating clinical providers and patients.

References

1. Boccadoro M, Pileri A. Plasma cell dyscrasias: classification, clinical and laboratory characteristics, and differential diagnosis. *Baillieres Clin Haematol.* 1995; 8(4):705–19. [PubMed: 8845568]
2. Nucci M, Anaissie E. Infections in patients with multiple myeloma in the era of high-dose therapy and novel agents. *Clin Infect Dis.* 2009; 49(8):1211–25. [PubMed: 19769539]
3. Centers for Disease C, Prevention. Estimates of deaths associated with seasonal influenza — United States, 1976–2007. *MMWR Morb Mortal Wkly Rep.* 2010; 59(33):1057–62. [PubMed: 20798667]
4. Blimark C, Holmberg E, Mellqvist UH, et al. Multiple myeloma and infections: a population-based study on 9253 multiple myeloma patients. *Haematologica.* 2015; 100(1):107–13. [PubMed: 25344526]
5. Mills KH, Cawley JC. Abnormal monoclonal antibody-defined helper/suppressor T-cell subpopulations in multiple myeloma: relationship to treatment and clinical stage. *Br J Haematol.* 1983; 53(2):271–5. [PubMed: 6217831]
6. Ogawara H, Handa H, Yamazaki T, et al. High Th1/Th2 ratio in patients with multiple myeloma. *Leuk Res.* 2005; 29(2):135–40. [PubMed: 15607360]
7. Rousseau B, Loulergue P, Mir O, et al. Immunogenicity and safety of the influenza A H1N1v 2009 vaccine in cancer patients treated with cytotoxic chemotherapy and/or targeted therapy: the VACANCE study. *Ann Oncol.* 2012; 23(2):450–7. [PubMed: 21576285]
8. Robertson JD, Nagesh K, Jowitt SN, et al. Immunogenicity of vaccination against influenza, *Streptococcus pneumoniae* and *Haemophilus influenzae* type B in patients with multiple myeloma. *Br J Cancer.* 2000; 82(7):1261–5. [PubMed: 10755398]
9. Grohskopf LA, Sokolow LZ, Olsen SJ, Bresee JS, Broder KR, Karron RA. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices, United States, 2015–16 Influenza Season. *MMWR Morb Mortal Wkly Rep.* 2015; 64(30):818–25. [PubMed: 26247435]
10. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg (Lond).* 1972; 70(4):767–77. [PubMed: 4509641]
11. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. *Virus Res.* 2004; 103(1–2):133–8. [PubMed: 15163501]
12. Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines. <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074794.htm> (accessed April 2016)
13. Cheuk DK, Chiang AK, Lee TL, Chan GC, Ha SY. Vaccines for prophylaxis of viral infections in patients with hematological malignancies. *Cochrane Database Syst Rev.* 2011; 3:CD006505.
14. Hahn M, Schnitzler P, Schweiger B, et al. Efficacy of single versus boost vaccination against influenza virus in patients with multiple myeloma. *Haematologica.* 2015; 100(7):e285–8. [PubMed: 25820335]
15. Ljungman P, Nahi H, Linde A. Vaccination of patients with haematological malignancies with one or two doses of influenza vaccine: a randomised study. *Br J Haematol.* 2005; 130(1):96–8. [PubMed: 15982350]
16. Safdar A, Rodriguez MA, Fayad LE, et al. Dose-related safety and immunogenicity of baculovirus-expressed trivalent influenza vaccine: a double-blind, controlled trial in adult patients with non-Hodgkin B cell lymphoma. *J Infect Dis.* 2006; 194(10):1394–7. [PubMed: 17054068]
17. Wang S, Taaffe J, Parker C, et al. Hemagglutinin (HA) proteins from H1 and H3 serotypes of influenza A viruses require different antigen designs for the induction of optimal protective antibody responses as studied by codon-optimized HA DNA vaccines. *J Virol.* 2006; 80(23):11628–37. [PubMed: 16987975]
18. Steel J, Lowen AC, Pena L, et al. Live attenuated influenza viruses containing NS1 truncations as vaccine candidates against H5N1 highly pathogenic avian influenza. *J Virol.* 2009; 83(4):1742–53. [PubMed: 19073731]

19. Duenas M, Salazar A, Ojeda B, Arana R, Failde I. Generalized Estimating Equations (GEE) to handle missing data and time-dependent variables in longitudinal studies: an application to assess the evolution of Health Related Quality of Life in coronary patients. *Epidemiol Prev.* 2016; 40(2): 116–23. [PubMed: 27290889]
20. Tete SM, Bijl M, Sahota SS, Bos NA. Immune defects in the risk of infection and response to vaccination in monoclonal gammopathy of undetermined significance and multiple myeloma. *Front Immunol.* 2014; 5:257. [PubMed: 24917865]
21. Brown RD, Pope B, Murray A, et al. Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10. *Blood.* 2001; 98(10):2992–8. [PubMed: 11698282]
22. Noonan K, Rudraraju L, Ferguson A, et al. Lenalidomide-induced immunomodulation in multiple myeloma: impact on vaccines and antitumor responses. *Clin Cancer Res.* 2012; 18(5):1426–34. [PubMed: 22241792]
23. United States Center for Disease Control. <http://www.cdc.gov/flu> (accessed April 2016)

Clinical Practice Points

- Plasma cell disorders are associated with immunoparesis, frequently involving hypogammaglobulinemia and other multifactorial defects of immune function.
- Infections are a major source of both morbidity and mortality in patients with plasma cell disorders.
- Patients with plasma cell disorders are highly susceptible to influenza infections, despite widespread standard vaccination practices.
- The present novel, prospective trial suggest that two doses of high-dose influenza vaccine separated by 30 days is associated with high rates of seroprotection in patients with plasma cell disorders, however, larger, randomized trials with clinical endpoints are are needed to confirm efficacy of this novel vaccination strategy.

Figure 1. Serologic Response Rates

(A) Bar graph showing proportion of patients who achieved seroprotection against influenza B, H1N1, H3N2, and all 3 vaccine strains combined. First or second vaccination significantly increased rate of seroprotection against all 3 combined strains among patient group compared to baseline, $p < 0.001$ (***). Second vaccination significantly boosted the rate of seroprotection compared with first vaccination, $p < 0.01$ (*).

(B) Bar graph showing proportion of patients who achieved seroconversion against influenza B, H1N1, H3N2, and all 3 vaccine strains combined. Second vaccination significantly boosted the rate of seroconversion compared with first vaccination, $p=0.02$ (*). Abbreviations: All 3, All three influenza strains included in the study vaccine; FluB, influenza B.

Figure 2. Correlates of Serologic Response

Forest plot illustrating effect of a group of risk factors on achieving (A) seroprotection (B) seroconversion among all patients or those with a plasma cell disorder requiring therapy (as opposed to asymptomatic/monoclonal gammopathy of undetermined significance). The x-axis is on log scale with base at 2. The filled black dot represents the estimates of the odds ratio regarding relevant risk factors. 95% confidence intervals are represented with the horizontal lines and vertical short lines at each end. P values associated with each risk factor are listed to the far right, $p < 0.05$ (*) and $p < 0.005$ (***)

Abbreviations: plasma cell disorder, PCD; monoclonal gammopathy of undetermined significance, MGUS; intravenous immunoglobulin, IVIG; partial response, PR; immunoglobulins, Igs; immunomodulatory drug, IMiD; odds Ratio, OR; lower 95% confidence interval, LCI; upper 95% confidence interval, UCI; p value, Prob.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3. Clinical Characteristics Associated with Lower Rates of Seroprotection

Bar graphs comparing rate of developing seroprotection against all 3 influenza vaccine strains between (A) Plasma cell disorder (PCD) patients requiring therapy versus those with asymptomatic/monoclonal gammopathy of undetermined significance; (B) patients with versus without suppression of uninvolved immunoglobulins; (C) PCD patients who were actively receiving conventional chemotherapy versus those who were not; (D) PCD patients with a disease response status less than partial response versus those with a partial response or better; and (E) patients who developed laboratory-confirmed respiratory viral infections

other than influenza during the study period versus those who did not. Statistically significant comparisons are bracketed, $p < 0.001$ (***), $p < 0.01$ (**), or $p < 0.05$ (*). Abbreviations: monoclonal gammopathy of undetermined significance, MGUS.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1**Patient Demographics**

All Patients	(N=51) % (n)
Median Age = 65 years	
Male Sex	61% (31)
Immunoglobulin Type	
IgA	16% (8)
IgG	59% (30)
IgM	4% (2)
Light Chain Only	21% (11)
Light Chain Type	
Kappa	80% (41)
Lambda	20% (10)
Suppression of Uninvolved Immunoglobulins	80% (41)
Received Influenza Vaccine Previous Influenza Season	76% (39)
Laboratory-Confirmed Influenza Diagnosis During Previous Influenza Season	6% (3)
Actively Receiving Intravenous Immunoglobulin	8% (4)
Plasma Cell Disorder Diagnosis	
Asymptomatic Multiple Myeloma	8% (4)
Asymptomatic Waldenstrom's Macroglobulinemia	2% (1)
Monoclonal Gammopathy of Undetermined Significance	10% (5)
Multiple Myeloma	78% (40)
Waldenstrom's Macroglobulinemia	2% (1)
Patients with a Plasma Cell Disorder Requiring Therapy	(n=41)
Disease Response Assessment at Study Entry	
Progressive Disease	17% (7)
Partial Response	17% (7)
Stable Disease	7% (3)
Very Good Partial Response	20% (8)
Complete Response	39% (16)
Receiving Active Treatment	85% (35)
Active Treatment with Proteasome Inhibitor	39% (16)
Active Treatment with Immunomodulatory Drug	54% (22)
Active Treatment with Conventional Chemotherapy [oral cyclophosphamide (n=7), fluorouracil and oxaliplatin for concurrent colorectal cancer (n=1)]	20% (8)
Active Treatment with Corticosteroids	56% (23)
Active Treatment with Proteasome Inhibitor and Immunomodulatory Drug	15% (6)
Not Receiving Active Treatment	15% (6)