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Trial Designs

High-dose influenza vaccine to reduce clinical outcomes in high-risk cardiovascular patients: Rationale and design of the INVESTED trial

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ABSTRACT

Background: Influenza leads to significant cardiopulmonary morbidity and mortality—particularly in patients with cardiovascular disease—that may be prevented with a standard influenza vaccine. However, patients with cardiovascular conditions have a reduced immune response to influenza vaccine, potentially resulting in reduced effectiveness for preventing clinical events. High-dose vaccine augments immune response in cardiac patients, suggesting that a high-dose influenza vaccination strategy may further reduce morbidity and mortality. Alternatively, broader coverage with an influenza vaccine containing an increased number of viral strains is an alternative strategy without direct evaluation.

Research design and methods: INfluenza Vaccine to Effectively Stop Cardio Thoracic Events and Decompensated heart failure (INVESTED) is a pragmatic, randomized, double-blind, parallel-group, active-controlled trial comparing the effectiveness of an annual vaccination strategy of high-dose trivalent versus standard-dose quadrivalent influenza vaccine in patients with a history of recent heart failure or myocardial infarction hospitalization. The trial will enroll approximately 9,300 patients over 4 influenza seasons. The primary hypothesis is that high-dose influenza vaccine will reduce the composite outcome of all-cause mortality and hospitalization from a cardiovascular or pulmonary cause compared with standard-dose influenza vaccine within each enrolling season. Approximately 1,300 primary outcome events will provide >90% power to detect an 18% relative risk reduction at a 2-sided α level of .05.

Conclusion: INVESTED is the largest and longest study to assess whether high-dose influenza vaccine is superior to standard-dose influenza vaccine in reducing cardiopulmonary events in a high-risk cardiovascular population (ClinicalTrials.gov Identifier: NCT02787044).

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Influenza leads to significant morbidity and mortality, particularly in patients with cardiovascular disease.[1] Influenza infection has been temporally associated with acute cardiovascular events, such as acute coronary syndrome and acute heart failure (HF).[2-4] Because of the increased risk for influenza-related complications, annual influenza immunization is recommended by the Centers for Disease Control and Prevention (CDC) and cardiovascular professional societies.[5-7] Moreover, influenza vaccination has been associated with reduced cardiac-related hospital admissions, acute exacerbations of HF, and winter mortality.[8,9] In a meta-analysis of clinical trials testing the efficacy of influenza vaccination in patients at cardiovascular risk, annual vaccination reduced the risk for major adverse cardiovascular events by 36%.[10] Numerous vaccine formulations are available, differing on number and dose of viral antigens, preparation (egg-based versus recombinant), and presence of adjuvant. Vaccine antigen composition changes annually in an effort to harmonize with circulating strains, and each year, virulence of influenza varies, as does the match between vaccine antigens and circulating strains.

Several lines of evidence suggest that a strategy of using high-dose influenza vaccine in high-risk cardiovascular patients might reduce more morbidity and mortality than the standard-dose vaccine. Immune response to influenza vaccine varies with age and concomitant medical conditions and is referred to as *immunosenescence*. Immunosenescence is present in patients with HF as evidenced by lower antibody titers after standard influenza vaccination compared with healthy controls.[11] In a randomized trial, we demonstrated that antibody responses in patients with HF were augmented by a higher dose of influenza vaccine.[12] A large randomized trial of high-dose versus standard-dose influenza vaccine in medically stable patients older than 65 years showed a 24% risk reduction in laboratory-confirmed influenza and influenza-like illness (ILI) without substantial adverse events.[13] Additionally, results supported the potential for reduced cardiopulmonary hospitalizations among those assigned to high-dose vaccine.[14] Incorporating these results with a meta-analysis of trials comparing high-dose and standard-dose influenza vaccine, higher-dose influenza vaccination was associated with a 27% reduced risk for major adverse cardiovascular events compared to standard-dose vaccine.[10] High-dose influenza vaccine is Food and Drug Administration–approved for prevention of influenza in medically stable adults older than 65 years but is not currently indicated for patients younger than 65 years, and there are limited data in those with unstable, high-risk medical conditions. On the other hand, high-dose vaccine is only currently available in trivalent (3 viral strains) presentation, whereas standard-dose vaccine is also offered as a quadrivalent (4 viral strains, containing an additional B-lineage strain) presentation. The Advisory Committee on Immunization Practices, which informs the CDC vaccine guidelines, does not preferentially recommend one influenza vaccine formulation over another, and the vaccine formulation that offers the most clinical protection in these high-risk patients is unknown.[15]

The high morbidity and health care costs among patients with high-risk cardiovascular disease along with the reduced immune responses to standard-dose influenza vaccines in patients with heart disease provide a compelling rationale to investigate alternative influenza vaccination strategies in this group. Accordingly, we designed an outcomes study to test the hypothesis that in patients with recent acute myocardial infarction (AMI) or HF hospitalization, a trivalent influenza vaccine with 4 times the dose of hemagglutinin antigen will reduce major cardiovascular and pulmonary-related morbidity and mortality compared with standard-dose quadrivalent vaccine. The comparative efficacy and safety of these influenza vaccines will be assessed over the course of 4 individual seasons in an amalgamated fashion, as well as each year independently, to accommodate for seasonal variation in influenza virulence and vaccine effectiveness.

Trial design and methods

INVESTED is a randomized, double-blind, parallel-group, active-controlled, 2-arm study comparing the effectiveness of high-dose versus standard-dose influenza vaccine in reducing all-cause mortality or cardiopulmonary hospitalizations in high-risk cardiovascular patients. The trial was designed by members of the Executive and Steering Committees in collaboration with the National Heart, Lung, and Blood Institute (NHLBI). The trial has been registered on ClinicalTrials.gov (NCT02787044).

Patients

The eligibility criteria are summarized in Table I. Briefly, eligible patients are 18 years of age or older with a documented history of either a hospitalization for spontaneous (type 1) or secondary (type 2) MI within a year of the study baseline visit, or a history of hospitalization for HF within 2 years of the baseline visit. In addition, patients need to fulfill at least 1 additional enrichment criterion (Table I). Enrichment criteria were selected in consideration that they select for patients at high risk for the primary end point as well as for immunosenescence with standard-dose influenza vaccine. (See Fig. 1.)

Table I
Eligibility criteria

Inclusion criteria
1. Willing to give written informed consent and able and willing to adhere to follow-up schedules
2. At least 18 y of age
3. Documented history of at least 1 of the below CV events:
a. Hospitalization for spontaneous MI (type 1 or type 2 event) (within 1 y of baseline visit)
b. Hospitalization for HF (within 2 y of baseline visit) but not currently acutely decompensated.
4. Fulfills at least 1 of the following additional risk factors:
a. Prior MI hospitalization (for participants qualifying on HF hospitalization or a second MI hospitalization for those qualifying based on MI)
b. Prior HF hospitalization (for participants qualifying based on MI hospitalization or a second HF hospitalization for those qualifying based on HF)
c. Age ≥65 y
d. Current or historical LVEF <40%
e. Documented diagnosis (via *ICD-9* code) of type 1 or type 2 diabetes mellitus (laboratory findings, eg, elevated A1C, FPG, plasma glucose in the absence of a clinical diagnosis is not sufficient)
f. Current BMI ≥30
g. Documented history of renal impairment (eGFR ≤60 for at least 2 readings in the past year)
h. Documented history of ischemic stroke
i. Documented history of peripheral artery disease
j. Current tobacco smoker (smokes 1 or more cigarettes daily)
Exclusion criteria
1. Known allergy, hypersensitivity (anaphylaxis), or Guillain-Barré syndrome within 6 wk after influenza vaccine, or severe allergy to egg protein
2. Any noncardiac condition that, in the opinion of the investigator, would lead to life expectancy <9 m
3. Receipt of influenza vaccine during current influenza season
4. Any acute infection requiring antibiotics within 14 d of influenza vaccination (prophylactic antibiotics prior to dental or other procedures, or scheduled use of antibiotics for other types of prophylaxis does not exclude the subject). If an acute course of antibiotics is required, the patient may still participate in INVESTED 14 d after completing antibiotics.
5. Known fever over 100°F or 38°C within 7 d of influenza vaccination
6. Women who are pregnant or breast-feeding
7. Not suitable for study participation due to other reasons at the discretion of the investigator

ICD-9, *International Classification of Diseases, Ninth Revision*; *FPG*, fasting plasma glucose.

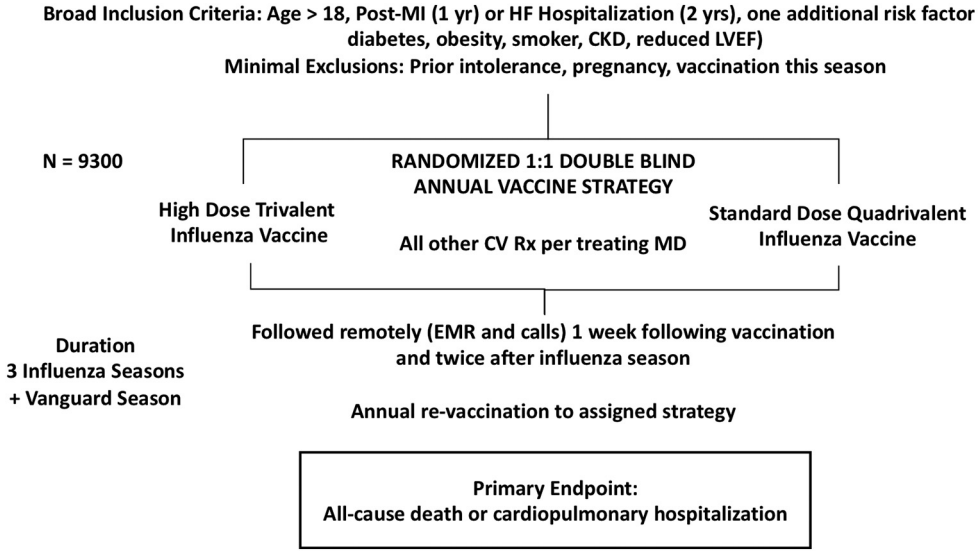

Figure 1. Study schematic.

Key exclusion criteria (Table I) include known allergy or hypersensitivity to influenza vaccine, history of serious adverse reaction to influenza vaccine, any condition that would lead to life expectancy of <9 months, prior receipt of influenza vaccine for the upcoming influenza season, infection requiring antibiotics in the 14 days prior to randomization, known fever within 7 days of randomization, pregnancy, or lactation. Enrollment in INVESTED began on September 21, 2016, following protocol approval by the study's Protocol Review Committee and an Institutional Review Board affiliated with each investigative site. The study will include approximately 200 sites in the United States and Canada. The study is being conducted in accordance with Good Clinical Practice and the Declaration of Helsinki 2002.

Study objectives

The primary objective of this study is to compare high-dose trivalent inactivated influenza vaccine (IIV3-HD) with standard-dose quadrivalent inactivated influenza vaccine (IIV4) on time to first occurrence of death or cardiopulmonary hospitalization within each enrolling season (Table II). Secondary objectives are to compare the effect of high-dose influenza vaccine versus standard-dose vaccine on total (first and recurrent) cardiopulmonary hospitalizations or death, time to first occurrence of cardiovascular death or cardiovascular hospitalization within each enrolling season, time to first occurrence of death or cardiopulmonary hospitalization across all enrolling seasons, and the individual

Table II
Study objectives

Primary objective
To compare the effects of high-dose influenza trivalent vaccine to standard-dose quadrivalent vaccine on time to first occurrence of death or cardiopulmonary hospitalization within each enrolling season
Secondary objectives
To compare the effects of high-dose influenza trivalent vaccine to standard-dose quadrivalent vaccine on:
• Total (first and recurrent) cardiopulmonary hospitalizations or death
• Time to first occurrence of cardiovascular death or cardiovascular hospitalization within each enrolling season
• Time to first occurrence of death or cardiopulmonary hospitalization across all enrolled influenza seasons
• Time to first occurrence of the individual components of the primary end point
Exploratory objectives
To compare the effects of high-dose influenza trivalent vaccine to standard-dose quadrivalent vaccine on:
• Time to first occurrence of all-cause death or cardiopulmonary hospitalization according to effectiveness of vaccine relative to virulence of influenza strain and the quality of the match between influenza strain and vaccine within individual seasons
• Time to first occurrence of cardiovascular death or HF hospitalization\
• Time to first occurrence of cardiovascular death, nonfatal MI, or nonfatal stroke
• Time to first occurrence of all-cause death and cardiopulmonary hospitalizations

components of the primary end point. Exploratory objectives are listed in Table II.

Study design

Identification of patients and enrollment

The study is comprised of several networks of performance sites: a Canadian network, a network of Veterans Administration sites, a network of other US non–Veterans Administration sites, and a network of sites from PCORnet (the National Patient-Centered Clinical Research Network). Several recruitment strategies will be used, and sites within each network may use a combination of methods depending on their capabilities. Networks and sites with electronic health record abilities may query electronic health records based on study enrolment criteria and create screening lists for individual site principal investigators, which will be forwarded to site research personnel in the early summer months prior to each enrolling season, and may include electronic contact of potential participants. Participants can be enrolled prior to discharge from a hospitalization for acute HF or myocardial infarction once no longer acutely decompensated. Screening can also occur anytime as part of an inpatient assessment or outpatient visit in a cardiology or primary care clinic, cardiac rehabilitation visit, or other clinical setting. Enrollment and randomization nevertheless will be timed to coincide with study vaccine availability with confirmation of participant eligibility at the baseline visit. Individual sites may use additional strategies for which IRB approval will be obtained prior to implementation.

Participants can be enrolled for up to 3 influenza seasons and will be vaccinated with the same vaccine strategy (high dose or standard dose) to which they were randomized during their first enrollment season using each year's World Health Organization–recommended composition of viral antigens.

Vaccination and randomized double-blind treatment period

Participants will be assigned to receive 1 of 2 formulations of influenza vaccine: IIV3-HD or IIV4. IIV3-HD is currently the only available higher-dose formulation. Nevertheless, we chose to use IIV4 as the comparator because this vaccine was projected to potentially become standard of care in the regions the trial was being conducted and because of the potential theoretical advantages of the additional B-lineage coverage in the quadrivalent vaccine. Thus, an IIV-4 represented a comparator for which there remained equipoise to determine which strategy was superior. Vaccine will be administered intramuscularly once at randomization and yearly thereafter. Both vaccines are licensed in the United States and in Canada. IIV4 is indicated for active immunization of persons 6 months of age and older against influenza disease caused by influenza virus subtypes A and type B. A single injectable sterile suspension 0.5-mL dose contains 15 μg of hemagglutinin from each of 4 viral strains for a total of 60 μg in 1 dose. IIV3-HD is indicated for active immunization of persons 65 years of age and older against influenza disease caused by influenza virus subtypes A and type B. A single injectable sterile suspension 0.5-mL dose contains 60 μg of hemagglutinin from 3 viral strains for a total of 180 μg in 1 dose. Both inactivated influenza vaccines are prepared from influenza viruses propagated in embryonated chicken eggs.

Sanofi Pasteur provides both formulations of vaccine as 0.5-mL single-dose, prefilled syringes. Vaccine syringes are subsequently blinded and labeled by a third-party vendor and shipped to investigator sites with a temperature-monitoring device to verify maintenance of the cold chain during transit.

Monitoring for safety

Following vaccine administration, participants will be monitored by site personnel for acute vaccine-related adverse events for at least 20 minutes. Participants will be provided a symptom diary to track vaccine-related events at home for 7 days. One week postvaccination, participants will be contacted by a member of the study team by phone to assess potential vaccine-related local and systemic adverse events, including allergic reactions. (see Table III)

Monitoring for cardiopulmonary events

Surveillance for hospitalization or death will include 1 telephone call completed by site personnel during influenza season and another phone call during the summer following influenza season. Participants will also be asked to inform local site personnel of hospitalizations at any time they occur.

Biomarkers, immune response, and genetic analyses

Blood will be collected in a subset of up to 3,000 consenting participants and banked for future studies. Analyses will examine associations of biomarkers that reflect immunity, inflammation, thrombosis, metabolism, and vascular or hemodynamic risk with influenza vaccine response and cardiovascular disease. One planned substudy examines postvaccination hemagglutination inhibition antibody titers in response to influenza vaccine antigens, which will be measured in participants who consent to blood draws as described above. Blood will be collected during the vaccination visit (baseline) and again 4 weeks postvaccination to test the hypothesis that a higher influenza vaccine dose will result in a more pronounced humoral immune response, evidenced by higher geometric mean titers postvaccination and greater antibody titer increases from baseline, and to test the hypothesis that higher antibody titers are associated with a reduced rate of the composite of all-cause death and cardiopulmonary hospitalization. Other key objectives include exploring the effects of each vaccination strategy on circulating biomarker levels over time and assessing the utility of incorporating biomarker levels into risk prediction models that identify patients that particularly benefit from high-dose influenza vaccine. Blood will be stored for future investigations of genetic contributors to cardiopulmonary risk and patient responsiveness to influenza vaccine.

Measures to minimize biases

Randomization

After informed consent is obtained and eligibility assessed, participants are randomized in a 1:1 ratio to IIV3-HD or IIV4 using permuted blocks of random block sizes, stratified naturally by influenza season, but no other stratification factors. Patients will receive the same dose for subsequent influenza seasons.

Table III
Schedule of time and events

Measurement	Screening* visit	Baseline visit (August-January)	Week 1 phone call (±4 d)	Week 2-4 visit (±4 d)‡	During influenza season phone call	Summer phone call	Years 2 & 3 baseline§ (August-December)
Informed consent	X						
Demographics & history†	X	X					X
Inclusion/exclusion	X	X					X
Current medications	X	X					X
Blood draw†		X		X			
Vaccine administration		X					X
Assessment of vaccine-related reactions		X	X				X
Cardiopulmonary event assessment				X	X	X	
Year 2 & 3 visit scheduling						X	

* Screening and baseline procedures may be completed at 1 visit, followed by randomization and vaccine administration.
† History includes previous vaccinations.
‡ Baseline and week 2-4 blood draw for immune end points (eg, geometric mean titers postvaccination, change in antibody titers at 4 weeks postvaccination, seroconversion, seroprotection, and B-type vaccine antigens 2-4 weeks postvaccination), biomarkers, and genetic markers will be assessed in a subset of up to 3,000 participants at participating sites [will be implemented after the Vanguard year], including an end point assessment at week 4.
§ Years 2 and 3 follow year 1 procedures after baseline visit.

Masking

In an effort to minimize crossover related to perceived benefit of one vaccine formulation over another, participants, site investigators, study personnel, persons performing follow-up surveillance, and study statisticians will remain masked to the identity of the treatment from the time of randomization until database lock, except for the statisticians supporting the Data and Safety Monitoring Board.

Study management and committees

INVESTED is conducted under a cooperative agreement to the Clinical Coordinating Center and the Data Coordinating Center from the NHLBI, under the guidance and leadership of the Executive Committee which is comprised of academic members and the NHLBI project officer. An academic steering committee also advises the Executive Committee regularly. An independent, external Data and Safety Monitoring Board appointed by the NHLBI oversees the safety of the patients in the trial and reviews the results of the interim efficacy analysis. A Clinical Endpoints Committee is responsible for classifying all deaths and for adjudicating all nonfatal events.

Statistical considerations

The primary efficacy analysis will be performed according to a modified intention-to-treat principle for the primary end point of the time to first occurrence of all-cause death or cardiopulmonary hospitalization during each enrolling season, defined as beginning 2 weeks following receipt of influenza vaccine and continuing until July 31 of the following calendar year using standard survival analysis methods. As such, participants can contribute primary end point events during multiple enrolling seasons. The primary efficacy analysis will be based on a 2-sided log-rank test at a significance level of .05, stratified by influenza season.[16] The Kaplan-Meier method will be used to estimate the survival distribution for the time to first occurrence of all-cause death or cardiopulmonary hospitalization within each enrolling season.[17] An unadjusted estimate of the hazard ratio and CI will be obtained using a Cox proportional-hazards model with only treatment as a model term, stratified by influenza season.[18]

To test the hypothesis that a strategy of high-dose influenza vaccine over multiple seasons will be superior to standard-dose vaccine, one of the secondary analyses will be a standard ITT analysis from the time from randomization until final subject censoring, which will occur following the final season the subject receives study vaccination.

A sensitivity analysis is planned to account for potential differential survivorship bias and bias due to differential dropout after the initial randomization, during which we will use principal stratification, matching based on propensity score, or inverse probability of treatment weighting for adjusted Kaplan-Meier estimator and log-rank test.[19-21]

Prespecified subgroups will be analyzed using Cox proportional-hazards models with age (<65 or ≥65 years old), baseline cardiovascular risk group (AMI or HF), and treatment (high-dose or standard-dose influenza vaccination) as model terms, stratified by influenza season, to obtain an adjusted hazard ratio with CIs, while adjusting for the following covariates: past vaccination history to adjust for the theoretical possibility of interference between successive vaccinations, and match between vaccine and circulating influenza strains, and the interaction between treatment and match for circulating B (Victoria)-lineage that is included only in the standard-dose IIV4 (binary), based on influenza typing and subtyping data from Canada and the United States to account for the differences in B vaccine antigens present only in the IIV4.

A secondary "in season" analysis will also be undertaken, limited to an evaluation of efficacy during the formally delineated influenza season with start and end of season defined according to the CDC and Public Health Agency of Canada surveillance system. For example, we will use information provided in the CDC's Flu View Report which is updated on a weekly basis (http://www.cdc.gov/flu/weekly/). For each US state, we will use the point at which influenza transitions from "sporadic" to "local" on the graphic "Geographic Spread of Influenza as Assessed by State and Territorial Epidemiologists" or by using the point of transition from "minimal" to "low" activity on the "ILINet State Activity Indicator Map." We will adopt a similar approach for each Fluwatch region in Canada (https://www.canada.ca/en/public-health/services/diseases/flu-influenza/influenza-surveillance/weekly-influenza-reports.html) using the transition from "sporadic" to "local" on the map of ILI activity for each region.

To assess the independence of the primary end points from year to year in individuals receiving influenza vaccines more than once, the frailty model version of the Cox proportional-hazards regression will be evaluated.[22] In case the independence assumption is not tenable, we will estimate intrasubject correlation from year to year using the method of Prentice and Cai.[23]

Analysis of secondary end points

Secondary end points consist of total (first and recurrent) cardiopulmonary hospitalizations or all-cause death during the subject's entire study participation duration, the composite of cardiovascular death or cardiopulmonary hospitalization within each enrolling season, the composite of all-cause death or cardiopulmonary hospitalization across all enrolling seasons, and individual components of the primary end point, including time to all-cause death and time to first occurrence of cardiopulmonary hospitalization. Time to composite end points and times to individual components of the composite end points will be analyzed similarly as the primary end point with individual components of

the composite end points that are nonterminating events analyzed using methods for competing risks.[24] Recurrent events analysis will be performed for recurrent nonterminating events across all enrolling seasons.[25-27] For all analyses, 2-sided *P* values <.05 will be considered statistically significant. In addition, the rate of cardiopulmonary hospitalization with death as competing risk will be analyzed using nonparametric and semiparametric analyses based on the *mean frequency function* defined as the marginal mean of the cumulative number of cardiopulmonary hospitalizations over time subject to a terminal event of death.[28,29]

Sample size and power

The enrollment target is approximately 4,650 participants per treatment arm, for a total of 9,300 participants. This is based on an estimated treatment effect size of IIV3-HD versus IIV4 of 18% risk reduction, that is, a hazard ratio of 0.82, in all-cause death or cardiovascular hospitalizations, with an anticipated similar magnitude of benefit for all-cause death or cardiopulmonary hospitalizations. This estimate is derived from our meta-analysis of randomized trials of relatively healthy outpatients comparing these 2 active vaccination treatments, using an estimated risk reduction of 27% for the composite end point, reduced by 35% for dilution of the treatment effect among those with active heart disease. Based on data from contemporary clinical trials of patients with coronary heart disease or HF, the event rate for the primary end point is estimated to be 9% during the subject's first enrolling season following randomization for each subject, 8% during the second enrolling season, and 7% during her third enrolling season after vaccination. The primary composite end point events are assumed to be 30% deaths and 70% cardiopulmonary hospitalizations. Considering a follow-up to the end of enrolling season (before the next influenza season) and a conservative 30% rate of not being vaccinated in a subsequent influenza season, a trial of 9,300 participants over a pilot season (n~500) during 2016/2017 and 3 subsequent influenza seasons in 2017-2018, 2018-2019, and 2019-2020 is projected to result in 45, 291, 440, and 519 primary end point events by the end of the 2019-2020 enrolling season, for a total of 1,296 events, with each patient possibly contributing primary end point events over multiple seasons. Assuming 2 interim analyses for efficacy using the O'Brien-Fleming group sequential method at the end of 2017-2018 and 2018-2019 enrolling seasons,[30] the trial will have power of 0.94 to detect an 18% risk reduction at a 2-sided significance level of .05.

Discussion

Influenza infection is associated with substantial morbidity and mortality in patients with cardiovascular disease. Although influenza vaccination is recommended in patients with cardiovascular conditions, the effectiveness may be limited because of relative immunosenescence in patients with cardiovascular conditions, and data from several trials and a meta-analysis suggest that a more effective vaccination strategy could potentially mitigate the reduced immune response. INVESTED will directly test the hypothesis that high-dose influenza vaccine reduces all-cause mortality and cardiopulmonary hospitalizations in high-risk cardiovascular patients compared with standard-dose vaccine. This trial has the potential to inform guidelines and public policy regarding use of influenza vaccine in high-risk patients.

Several elements in the design of INVESTED are worthy of consideration. We are using an active control rather than placebo because influenza vaccination is considered standard of care for influenza prevention in the United States and Canada, although a significant proportion of patients with heart disease may not get vaccinated.[31] INVESTED is enrolling participants over multiple consecutive influenza seasons. This strategy allows for accrued evaluation of efficacy and safety in the context of the unpredictable nature of variability in influenza severity and vaccine effectiveness due to influenza's mutagenicity. Whereas in other trials subject recruitment can be accomplished during all months of a given year, recruitment for influenza vaccine studies is truncated to just a few months, corresponding to the timing of seasonal vaccination. A passive recruitment approach of waiting to encounter potentially eligible participants is inadequate, as ideally participants are engaged prior to receipt of their standard-of-care influenza vaccine. This strategy requires identification of potential participants in the months prior to influenza vaccine becoming commercially available, which may be as early as August. At that time, patients may seek early vaccination in accordance with CDC and Health Canada recommendations to receive vaccine once it is available. However, this challenge also presents an opportunity to explore pragmatic approaches to participant recruitment, including use of a computable phenotype based on enrolment criteria and *International Classification of Diseases, 9th/10th Revisions,* codes to identify potential participants, and using electronic health record systems to invite potentially eligible participants to participate. Lastly, INVESTED has few exclusion criteria, coinciding with known safety of influenza vaccine in adults, allowing this to select patients highly representative of the intended cardiac population.

Many known or suspected respiratory virus infections have been associated with acute onset of MI and other cardiovascular events.[2,4] However, influenza A and influenza B have shown the most consistent association[4] and have a safe vaccine option for prevention, albeit incomplete and inconsistent year to year. In recent years, when vaccine effectiveness rates were reported at 10%-30%, notable disappointment resulted by public health officials and the lay public. However, evidence-based cardiovascular therapies offers comparable relative risk reductions for hard end points.[32,33] Thus, from the perspective of cardioprotection, we consider a 10%-30% risk reduction with influenza vaccination of high clinical value. As a safe and cost-effective intervention, vaccination is a worthwhile strategy for cardiopulmonary illness prevention.

A number of proposed mechanisms support a potential causal association between influenza infection and cardiovascular risk, either indirectly or directly. Indirect mechanisms include increased metabolic demand in the setting of influenza infection. When complemented by hypoxemia, influenza may exacerbate underlying cardiovascular disease because of increased sympathetic tone, potential volume overload, increased risk for plaque rupture, and arrhythmia.[3] Influenza infection predisposes patients to develop opportunistic infections such as pneumonia, which in itself is associated with increased cardiovascular events.[34,35] More directly, influenza infection has also been associated with myocardial depression,[36] which has been ascribed to an increase in proinflammatory cytokines,[37,38] and autopsy series have documented histologic evidence of myocardial injury, myocarditis, and myocyte necrosis following influenza-related deaths.[39] Moreover, influenza can stimulate a potent acute inflammatory response, which is a known trigger of acute plaque rupture. This mechanism is supported by observational data showing a temporal relationship between influenza infection spikes and myocardial infarctions.[2,4]

A potential limitation of the INVESTED trial is that we are not ascertaining symptoms of ILI nor are we pursuing confirmatory diagnoses of influenza infection. Symptoms of ILI have been temporally linked to influenza infection when measured in close proximity to the event. However, because we are ascertaining events at the end of influenza season, it could be months after the respiratory infection, in which case the recall bias for ILI would be substantial and unlikely to provide information relevant to the trial's hypothesis. As INVESTED is a large, simple trial, it is logistically difficult and costly to collect specimens from individuals with respiratory illnesses in real time to confirm and subtype influenza. To account for the effect of antigen match on vaccine effectiveness, we will interpret results in the context of the match between vaccine and annually changing circulating influenza strains by using prospectively collected influenza typing and subtyping data from the CDC and Public Health Agency of Canada. Another noteworthy challenge for this influenza vaccine trial is the use of a surrogate end point for vaccine effectiveness, which can dilute the impact of vaccine, particularly during seasons when activity of viruses other than influenza, such as

respiratory syncytial virus, parainfluenza virus, and human metapneumovirus, is high. INVESTED is comparing 2 vaccination strategies without a placebo control group; therefore, we cannot definitively determine the benefit of either strategy of influenza vaccination for cardioprotection over no vaccination. Although in the United States and Canada there is no longer equipoise to address this hypothesis in a randomized trial, our study will determine whether further cardioprotection can be realized from a more effective vaccine strategy, similar to rigorously tested intensive strategies of lipid-lowering therapy. Moreover, there are at least 2 ongoing international placebo-controlled trials testing the cardioprotective efficacy of standard influenza vaccination in patients with either MI (IAMI; NCT02831608)[40] or HF (RCT-IVVE; NCT02762851) with which we can indirectly compare results via network meta-analysis. Lastly, it is possible that differences between vaccine doses may vary based on the index enrollment event of MI or HF. We have prespecified examining results by enrollment subgroup; however, we are limited in power for the interaction test of index event by treatment; as such, any potential response differences will be interpreted with caution. It is also possible that the benefit of one vaccine strategy over another may be driven by pulmonary events, which are a component of our primary end point, over cardiac events.

In summary, INVESTED will examine whether high-dose compared with standard-dose influenza vaccine will reduce all-cause mortality and cardiopulmonary hospitalizations in high-risk cardiovascular patients who are particularly vulnerable to influenza and may derive inadequate immunity from standard-dose vaccination. INVESTED is the largest and longest study to assess whether vaccination is effective for secondary prevention in patients following recent presentation with HF or myocardial infarction.

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Disclaimer

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References

1. Reichert TA, Simonsen L, Sharma A, et al. Influenza and the winter increase in mortality in the United States, 1959-1999. Am J Epidemiol 2004;160(5):492-502.
2. Smeeth L, Thomas SL, Hall AJ, et al. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 2004;351(25):2611-8.
3. Corrales-Medina VF, Madjid M, Musher DM. Role of acute infection in triggering acute coronary syndromes. Lancet Infect Dis 2010;10(2):83-92.
4. Kwong JC, Schwartz KL, Campitelli MA, et al. Acute myocardial infarction after laboratory-confirmed influenza infection. N Engl J Med 2018;378(4):345-53.
5. Yancy CW, Jessup M, Bozkurt B, et al. ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 2013:2013.
6. Davis MM, Taubert K, Benin AL, et al. Influenza vaccination as secondary prevention for cardiovascular disease: a science advisory from the American Heart Association/American College of Cardiology. Circulation 2006;114(14):1549-53.
7. Piepoli MF, Hoes AW, Agewall S, et al. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2016;37(29):2315-81.
8. Nichol KL, Nordin J, Mullooly J, et al. Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. N Engl J Med 2003;348(14):1322-32.
9. de Diego C, Vila-Corcoles A, Ochoa O, et al. Effects of annual influenza vaccination on winter mortality in elderly people with chronic heart disease. Eur Heart J 2009;30(2):209-16.
10. Udell JA, Zawi R, Bhatt DL, et al. Association between influenza vaccination and cardiovascular outcomes in high-risk patients: a meta-analysis. JAMA 2013;310(16):1711-20.
11. Vardeny O, Sweitzer NK, Detry MA, et al. Decreased immune responses to influenza vaccination in patients with heart failure. J Card Fail 2009;15(4):368-73.
12. Van Ermen A, Hermanson MP, Moran JM, et al. Double dose vs. standard dose influenza vaccination in patients with heart failure: a pilot study. Eur J Heart Fail 2013;15(5):560-4.
13. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. N Engl J Med 2014;371(7):635-45.
14. DiazGranados CA, Robertson CA, Talbot HK, et al. Prevention of serious events in adults 65 years of age or older: a comparison between high-dose and standard-dose inactivated influenza vaccines. Vaccine 2015;33(38):4988-93.
15. Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices —United States, 2017-18 influenza season. MMWR Recomm Rep 2017;66(2):1-20.
16. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50(3):163-70.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. 1958;282(457-481).
18. Cox DR. Regression models and life-tables. J R Stat Soc 1972;34(2):187-220.
19. Frangakis CE, Rubin DB. Principal stratification in causal inference. Biometrics 2002;58(1):21-9.
20. Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. Biometrika 1983;70:41-55.
21. Xie J, Liu C. Adjusted Kaplan-Meier estimator and log-rank test with inverse probability of treatment weighting for survival data. Stat Med 2005;24(20):3089-110.
22. Oakes D. In: Klein JP, Goel PK, eds. Frailty models for multiple event times. Netherlands: eSASotAK; 1992.
23. Prentice RL, Cai J. Covariance and survivor function estimation using censored multivariate failure time data. Biometrika 1992;79:495-512.
24. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-509.
25. Anderson PK, Gill RD. Cox's regression model for counting processes: a large sample study. Ann Stat 1982;10:1100-20.
26. Prentice RL, Williams B, Peterson AV. On the regression analysis of multivariate failure time data. Biometrika 1981;68:373-9.
27. Wei LJ, Lin DY. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. J Am Stat Assoc 1989;84:1065-73.
28. Ghosh D, Lin DY. Nonparametric analysis of recurrent events and death. Biometrics 2000;56(2):554-62.
29. Ghosh D, Lin DY. Semiparametric analysis of recurrent events data in the presence of dependent censoring. Biometrics 2003;59(4):877-85.
30. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35(3):549-56.
31. Vardeny O, Claggett B, Udell JA, et al. Influenza vaccination in patients with chronic heart failure: the PARADIGM-HF trial. JACC Heart Fail 2016;4(2):152-8.
32. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC guideline for the management of patients with non–ST-elevation acute coronary syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014;130(25):e344-426.
33. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014;129(25 Suppl 2):S1-45.
34. Yende S, D'Angelo G, Mayr F, et al. Elevated hemostasis markers after pneumonia increases one-year risk of all-cause and cardiovascular deaths. PLoS One 2011;6(8), e22847.
35. Corrales-Medina VF, Alvarez KN, Weissfeld LA, et al. Association between hospitalization for pneumonia and subsequent risk of cardiovascular disease. JAMA 2015;313(3):264-74.
36. Fagnoul D, Pasquier P, Bodson L, et al. Myocardial dysfunction during H1N1 influenza infection. J Crit Care 2013;28(4):321-7.
37. Takano T, Tajiri H, Kashiwagi Y, et al. Cytokine and chemokine response in children with the 2009 pandemic influenza A (H1N1) virus infection. Eur J Clin Microbiol Infect Dis 2011;30(1):117-20.
38. Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, et al. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. Crit Care 2009;13(6):R201.
39. Paddock CD, Liu L, Denison AM, et al. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. J Infect Dis 2012;205(6):895-905.
40. Frobert O, Gotberg M, Angeras O, et al. Design and rationale for the Influenza vaccination After Myocardial Infarction (IAMI) trial. A registry-based randomized clinical trial. Am Heart J 2017;189:94-102.