Influenza Vaccination after Myocardial Infarction:

a randomized, double-blind, placebo-controlled, multicenter trial

Data Supplement

Contents

Appendices

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Eligibility Criteria

Inclusion criteria

- Patients with a diagnosis of ST-segment elevation myocardial infarction as defined by chest pain suggestive of myocardial ischemia for at least 30 minutes prior to hospital admission, time from onset of symptoms of less than 24 hours, and an ECG with new ST-segment elevation in two or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads or a probable new-onset left bundle branch block.

Or:

- Patients with a diagnosis of non-ST segment elevation myocardial infarction defined by a combination of: onset of symptoms such as central chest pain or aggravated angina pectoris, with or without an ECG change with ST-segment lowering or an inverted T-wave and at least two values with levels of troponin-T or troponin-I above the established margin of an AMI.

Or:

- Patients with a diagnosis of stable coronary artery disease ≥75 years of age undergoing angiography/PCI AND with at least one additional risk criterion: previous myocardial infarction, previous PCI, previous CABG, diabetes mellitus, current smoking, or an estimated glomerular filtration rate (eGFR) <40.

And:

- A finalized coronary angiography/PCI (not an inclusion criterion at Bangladeshi sites).
- Male or female subjects ≥18 years.
- Written informed consent.

Exclusion criteria

- Influenza vaccination during the current influenza season or intent to be vaccinated during the current influenza season.

- Indication for influenza vaccination because of condition other than myocardial infarction.

- Severe allergy to eggs or previous allergic reaction to influenza vaccine.

- Suspicion of febrile illness or acute, ongoing infection.

- Hypersensitivity to the active substances or ingredients of Vaxigrip or to any residues such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde, and octoxinol.

- Endogenic or iatrogenic immunosuppression that may result in a reduced immunization response.

- Inability to provide informed consent.

- Age below 18 years.

- Previous randomization in the IAMI trial.

Supplemental Table I. Vaccines and Seasons

Vaccines and seasons		Strain A	Strain A	Strain B	Strain B (Vaxigrip Tetra®/FluQuadri®)
NH 2016 (Vaxigrip®)	Reference strains recommended by WHO	A/California/7/2009 (H1N1)pdm09-like virus	A/Hong Kong/4801/2014 (H3N2)-like virus	B/Brisbane/60/2008-like virus	-
NH 2017 (Vaxigrip Tetra®)	Reference strains recommended by WHO	A/Michigan/45/2015 (H1N1)pdm09-like virus	A/Hong Kong/4801/2014 (H3N2)-like virus	B/Brisbane/60/2008-like virus	B/Phuket/3073/2013-like virus
NH 2018 (Vaxigrip Tetra®)	Reference strains recommended by WHO	A/Michigan/45/2015 (H1N1)pdm09-like virus	A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus	B/Colorado/06/2017-like virus [B/Victoria/2/87 lineage]	B/Phuket/3073/2013-like virus [B/Yamagata/16/88 lineage]
NH 2019 (Vaxigrip Tetra®)	Reference strains recommended by WHO	A/Brisbane/02/2018 (H1N1)pdm09-like virus	A/Kansas/14/2017 (H3N2)-like virus	B/Colorado/06/2017-like virus [B/Victoria/2/87 lineage]	B/Phuket/3073/2013-like virus [B/Yamagata/16/88 lineage]
SH 2018 (FluQuadri®)	Reference strains recommended by WHO	A/Michigan/45/2015 (H1N1)pdm09-like virus	A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus	B/Phuket/3073/2013-like virus	B/Brisbane/60/2008-like virus
SH 2019 (Vaxigrip Tetra® / FluQuadri®)	Reference strains recommended by WHO	A/Michigan/45/2015 (H1N1)pdm09-like virus	A/Switzerland/8060/2017 (H3N2)-like virus	B/Colorado/06/2017-like virus [B/Victoria/2/87 lineage]	B/Phuket/3073/2013-like virus [B/Yamagata/16/88 lineage]

NH: Northern Hemisphere; SH: Southern Hemisphere; WHO: World Health Organization

Supplemental Table II. Number of Patients Randomised by Country and Influenza Season

Country	Influenza Season			
	2016-17	2017-18	2018-19	2019-20
Australia	0	0	26	21
Bangladesh	0	0	0	620
Czech Republic	0	19	61	30
Denmark	88	206	162	116
Latvia	0	23	15	0
Norway	0	7	12	2
Sweden	204	284	268	209
United Kingdom	0	59	49	51

Supplemental Table III. Baseline Procedure Characteristics and Discharge Medication

	Vaccine (N=1272)	Placebo (N=1260)
Treated with PCI – no. (%)	939/1264 (74.3)	929/1250 (74.3)
Number of stents – no. (%)		
0	38/928 (4.1)	49/911 (5.4)
1	562/928 (60.6)	531/911 (58.3)
2	219/928 (23.6)	224/911 (24.6)
3	109/928 (11.7)	107/911 (11.7)
Drug eluting stent – no. (%)	879/894 (98.3)	854/873 (97.8)
Drug eluting balloon – no. (%)	48/916 (5.2)	42/893 (4.7)
Number of treated vessels – no. (%)		
0	8/923 (0.9)	5/907 (0.6)
1	739/923 (80.1)	757/907 (83.5)
2	154/923 (16.7)	130/907 (14.3)
3	22/923 (2.4)	15/907 (1.7)
RCA – no. (%)	358/937 (38.2)	342/926 (36.9)
LAD – no. (%)	455/937 (48.6)	473/926 (51.1)
LCx – no. (%)	265/937 (28.3)	215/926 (23.2)
LM – no. (%)	26/937 (2.8)	21/926 (2.3)
Bypass graft – no. (%)	2/725 (0.3)	9/714 (1.3)
Procedural success – no. (%)	921/927 (99.4)	895/908 (98.6)
Complete revascularization – no. (%)	708/907 (78.1)	694/888 (78.2)
Treated with CABG – no. (%)	28/1259 (2.2)	22/1249 (1.8)
Medical Treatment only – no. (%)	292/1259 (23.2)	295/1247 (23.7)
LVEF at discharge – no. (%)		
Normal (≥50%)	570/956 (59.6)	578/943 (61.3)
Slightly reduced (40-49%)	271/956 (28.3)	251/943 (26.6)
Moderately reduced (30-39%)	94/956 (9.8)	94/943 (10.0)
Severely reduced (<30%)	21/956 (2.2)	20/943 (2.1)
Discharge medication – no. (%)		
Aspirin	1206/1232 (97.9)	1186/1209 (98.1)
P2Y12-inhibitor	1186/1226 (96.7)	1176/1203 (97.8)
Beta-blocker	964/1226 (78.6)	932/1205 (77.3)
ACEi or ARB	865/1224 (70.7)	836/1201 (69.6)
Statin	1207/1230 (98.1)	1185/1208 (98.1)

Numbers in table are frequency (percentage); percentages are calculated out of all non-missing values. ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin II receptor blocker; LVEF=left ventricular ejection fraction; LAD=left anterior descending artery; LCx=left circumflex artery; LMCA=left main coronary artery; PCI=percutaneous coronary intervention; RCA=right coronary artery.

Supplemental Table IV. Cause of Death

Cause of Death, no. (%)	Vaccine (N=1264)	Placebo (N=1255)
Cardiovascular Death	**34 (2.7)**	**56 (4.5)**
Sudden Cardiac Death	3 (0.2)	6 (0.5)
Death due to Acute Myocardial Infarction	5 (0.4)	12 (1.0)
Death due to Heart Failure or Cardiogenic Shock	2 (0.2)	2 (0.2)
Death due to Cerebrovascular Event	1 (0.1)	2 (0.2)
Death due to other Cardiovascular Causes	1 (0.1)	4 (0.3)
Presumed Cardiovascular Death	22 (1.7)	30 (2.4)
Non Cardiovascular Death	**3 (0.2)**	**5 (0.4)**
Pneumonia	1 (0.1)	2 (0.2)
Septic Shock	0 (0.0)	1 (0.1)
Bacterial Pericarditis/Septic Shock	1 (0.1)	0 (0.0)
Pancreatic Cancer	0 (0.0)	1 (0.1)
Lung Cancer	0 (0.0)	1 (0.1)
Suicide	1 (0.1)	0 (0.0)

Percentages are calculated out of all patients with known vital status at 1 year. All deaths not attributed to the categories of cardiovascular death and not clearly attributed to a non-cardiovascular cause, are presumed cardiovascular deaths.

Supplemental Table V. Serious Adverse Events

System Organ Class/Preferred Term-no. (%)	Vaccine (N=1272)	Placebo (N=1260)	P-value
Cardiac disorders	**4 (0.3)**	**3 (0.2)**	1.000
Atrial fibrillation	1 (0.1)	0	1.000
Cardiac tamponade	0	1 (0.1)	0.498
Palpitations	1 (0.1)	0	1.000
Pericarditis	1 (0.1)	0	1.000
Unstable angina	1 (0.1)	1 (0.1)	1.000
Ventricular fibrillation	0	1 (0.1)	0.498
Gastrointestinal	**1 (0.1)**	**5 (0.4)**	0.123
Abdominal pain	0	2 (0.2)	0.248
Cholecystitis	0	2 (0.2)	0.248
Diarrhea	1 (0.1)	1 (0.1)	1.000
Loss of appetite	0	1 (0.1)	0.498
General disorders	**0**	**3 (0.2)**	0.123
Cold extremities	0	1 (0.1)	0.498
Sweating	0	1 (0.1)	0.498
Tremor	0	1 (0.1)	0.498
Infections and infestations	**5 (0.4)**	**3 (0.2)**	0.726
Fever	3 (0.2)	2 (0.2)	1.000
Pneumonia	2 (0.2)	1 (0.1)	1.000
Investigations	**0**	**1 (0.1)**	0.498
Hemoglobin decreased	0	1 (0.1)	0.498
Musculoskeletal	**4 (0.3)**	**3 (0.2)**	1.000
Chest pain	2 (0.2)	2 (0.2)	1.000
Myalgia	2 (0.2)	1 (0.1)	1.000
Neoplasms	**0**	**1 (0.1)**	0.498
Pancreatic cancer	0	1 (0.1)	0.498
Nervous system	**5 (0.4)**	**2 (0.2)**	0.453
Blurry vision	1 (0.1)	0	1.000
Dizziness	4 (0.3)	0	0.125
Headache	2 (0.2)	2 (0.2)	1.000
Renal and urinary disorders	**0**	**1 (0.1)**	0.498
Urinary retention	0	1 (0.1)	0.498
Respiratory	**4 (0.3)**	**2 (0.2)**	0.687
Cough	2 (0.2)	0	0.500
Dyspnea	2 (0.2)	0	0.500
Epistaxis	0	1 (0.1)	0.498
Sore throat	0	1 (0.1)	0.498
Skin and administration site	**11 (0.9)**	**5 (0.4)**	0.209
Angioedema	0	1 (0.1)	0.498
Pruritus	2 (0.2)	2 (0.2)	1.000
Sore injection site	5 (0.4)	1 (0.1)	0.218
Urticaria	4 (0.3)	1 (0.1)	0.375

Numbers in table are frequency (percentage) of patients with an adverse event within 12 months of randomisation; p-value from Fisher's exact test

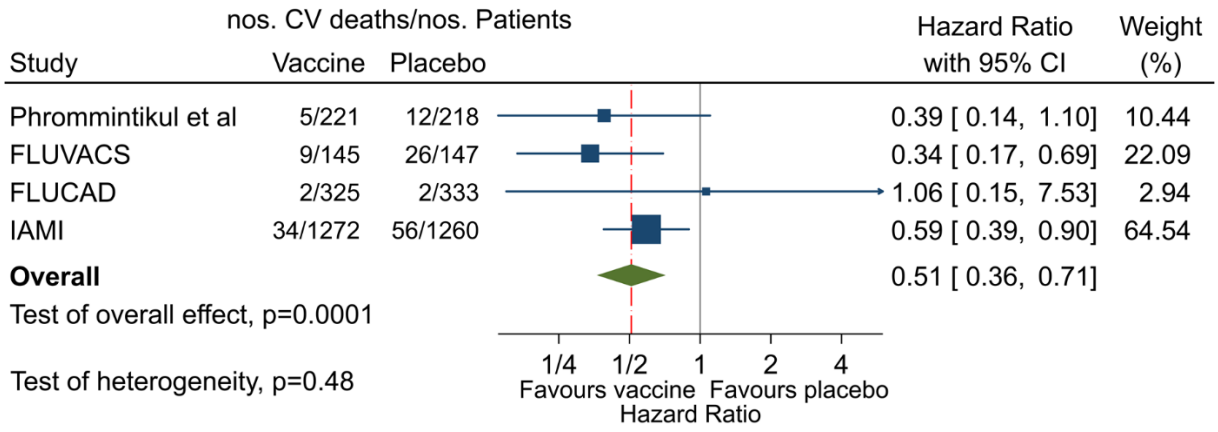
Supplemental Table VI. Patient-reported Reactions, Vaccinations and Acute Respiratory Illness

Reaction	Vaccine (N=1272)	Placebo (N=1260)	P-value
Patients returning 7-day questionnaire - no.(%)	1066 (83.8)	1046 (83.0)	
Systemic reaction - no. (%)			
Shivering	63 (5.9)	56 (5.4)	0.579
Fever	101 (9.5)	93 (8.9)	0.642
Headache	80 (7.5)	87 (8.3)	0.484
Muscle ache	75 (7.0)	60 (5.7)	0.222
Disturbed sleep	55 (5.2)	66 (6.3)	0.255
Feeling of general discomfort	65 (6.1)	57 (5.4)	0.523
Other	101 (9.5)	99 (9.5)	0.994
Injection site reaction - no. (%)			
Pain of any intensity	129 (12.1)	68 (6.5)	<0.0001
Severe or extreme pain	3 (0.3)	6 (0.6)	0.338
Redness	72 (6.8)	23 (2.2)	<0.0001
Severe or extreme redness	7 (0.7)	4 (0.4)	0.548
Swelling	67 (6.3)	16 (1.5)	<0.0001
Severe or extreme swelling	6 (0.6)	0 (0.0)	0.031
Itching	38 (3.6)	24 (2.3)	0.084
Severe or extreme itching	7 (0.7)	7 (0.7)	1.000
Hardening	77 (7.2)	19 (1.8)	<0.0001
Severe or extreme hardening	12 (1.1)	0 (0.0)	0.0005
Bruising	48 (4.5)	44 (4.2)	0.739
Severe or extreme bruising	9 (0.8)	10 (1.0)	0.822
1-year self-reported vaccinations and acute respiratory illnesses - no./total no. (%)			
Influenza vaccination	193/1264 (15.3)	165/1254 (13.2)	0.129
Pneumococcal vaccination	21/1036 (2.0)	23/1040 (2.2)	0.770
Acute respiratory illnesses during last 12 months	76/1264 (6.0)	76/1254 (6.1)	0.960

P values from chi squared test or Fisher's exact test as appropriate. Numbers in table are frequency (%).

Supplemental Figure I. Meta-Analysis of Cardiovascular Mortality in Influenza Vaccine Trials

Post hoc meta-analysis for the key secondary end point of cardiovascular death at one year combining our results with those from three published clinical trials which had investigated the effect of influenza vaccination in patients with cardiovascular disease.[8-10] Estimates of the log hazard ratio and its standard error were obtained from the reported hazard ratios and 95% confidence intervals (CI) and a pooled estimate was obtained using a fixed-effect model with weights calculated using the inverse variance method.



Research Protocol, June 2016

Influenza vaccination After Myocardial Infarction (IAMI trial)

A multicenter, prospective, randomized controlled clinical trial based on the Swedish angiography and angioplasty registry (SCAAR) platform

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Title: Influenza vaccination After Myocardial Infarction (IAMI trial). A multicenter, prospective, randomized controlled clinical trial based on the Swedish angiography and angioplasty registry (SCAAR) platform

Index

Study synopsis

Name of investigational treatment Influenza vaccine (Vaxigrip, Sanofi Pasteur MSD).
Title of study Influenza vaccination After Myocardial Infarction (IAMI trial).
Coordinating Principal Investigator and Sponsor Adjunct Professor Ole Fröbert MD, PhD, Dept. of Cardiology, Örebro University Hospital, Örebro, Sweden.
Study centers Up to 35 invasive centers in Sweden, Denmark, Finland and Iceland.
Planned study period 2016 – 2019 from October 1 to March 1 (influenza season). Long-term follow up to 2023 via registries.
Phase of development Phase IV.
Objectives In a multicenter, prospective, randomized registry-based controlled clinical trial based on the SCAAR and SWEDEHEART platforms and other national registries in the participating countries to compare influenza vaccination and placebo in reducing future major adverse cardiac and cerebrovascular events in patients with myocardial infarction.
Methodology Following informed consent patients are randomized in a 1:1 fashion to influenza vaccination or placebo from 24 hours prior to coronary angiography/PCI (NSTEMI patients) to 48 hours following coronary angiography/PCI (NSTEMI and STEMI patients).
Number of subjects 4 400
Inclusion criteria - Patients with a diagnosis of ST-elevation myocardial infarction (STEMI) or - Patients with a diagnosis of non-STEMI and - A finalized coronary angiography/PCI. - Male or female subjects ≥18 years. - Written informed consent.
Exclusion criteria - Influenza vaccination within 12 months prior to inclusion. - Indication for influenza vaccination for some indication other than myocardial infarction. - Severe allergy to eggs or previous allergic reaction to influence vaccine. - Suspicion of febrile illness or acute, ongoing infection. - Hypersensitivity to the active substances or ingredients of Vaxigrip or against any residues, such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde and octoxinol. - Subjects with endogenic or iatrogenic immunosuppression that may result in reduced immunization response. - Inability to provide informed consent. - Age below 18 years. - Previous randomization in the IAMI trial.
Primary endpoint Time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year. These data will be obtained from national health registries.
Secondary endpoints - Time to all-cause death till 1 year - Time to stent thrombosis till 1 year - Time to revascularization till 1 year

- Time to myocardial infarction till 1 year - Time to stroke till 1 year - Time to hospitalization for heart failure. - Length of hospital stay
Follow up by telephone and registry information The follow up for endpoints will be performed using the SCAAR registry. At 7 days after the vaccination patients will be requested to return a standard questionnaire to asses if any adverse event has occurred following vaccination. Follow up of primary and secondary endpoints will also be performed by telephone contacts with the patients or first degree relatives by a nurse phone call after 350±10 days.

1. Abbreviations

ACS	Acute coronary syndrome
AE	Adverse events
AMI	Acute myocardial infarction
AE	Adverse events
CRF	Case report form
IEC	Independent endpoint committee
NSTEMI	Non-ST-segment elevation myocardial infarction
PCI	Percutaneous coronary intervention
PI	Principal investigator
RRCT	Registry-based randomized clinical trial
SAE	Serious adverse event
SCAAR	Swedish Coronary Angiography and Angioplasty Registry
STEMI	ST-segment elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reaction
SWEDEHEART	National Swedish registry on heart disease integrating information from four different registries: RIKS-HIA (registry on cardiac intensive care units), SEPHIA (secondary prevention of heart disease registry), the Swedish heart surgery registry and SCAAR
UCR	Uppsala Clinical Research Center

2. Study rationale

2.1 Background

Regardless of the progress in medical and invasive treatment strategies cardiovascular disease remains the leading cause of death globally. Inflammation is assumed to play a central role in the atherosclerotic process from initiation of atherosclerosis to progression and rupture of atherosclerotic plaques [1]. Despite documentation of *Chlamydia* species, *Helicobacter pylori* and *Cytomegalovirus* in atherosclerotic lesions antibiotic and antiviral treatments have failed to reduce cardiovascular events [2].

A relation between influenza and cardiovascular events was described in an early study of influenza epidemics from 1915 to 1929 including the 1918-1920 pandemic [3]. The author concludes that: 'In the case of organic heart diseases there was a peak, corresponding in time with the influenza peak, for practically every epidemic.' Accumulating observational studies have subsequently documented similar associations. In a study of more than 22 000 patients in a self-controlled case series analysis the risk for acute myocardial infarction (AMI) the first three days after consultation for acute respiratory infection was significantly increased (incidence ratio, 4.19 (95% confidence interval (CI), 3.18-5.53) [4].

A post hoc analysis of the ONTARGET/TRANSCEND trials (examining the effects of angiotensin receptor blocker and angiotensin-converting enzyme inhibitor therapy in subjects with known vascular disease or diabetes mellitus with documented end-organ damage) enrolling 31 546 participants found a beneficial effect of influenza vaccination on subsequent risks of major adverse vascular events but the authors concluded that 'sensitivity analyses revealed that risk of bias remained. A randomized trial is needed to definitively address this question' (vaccination) [5]. In a case-control study of more than 11 000 cases of AMI and an equal number of matched controls the adjusted odds ratio for AMI risk in the 7 days following respiratory infection was 2.10 (95% CI, 1.38-3.21) [6]. In the same study also the risk of stroke following infection was doubled.

In a recent Australian study of 275 cases of inpatients with AMI and outpatient controls without AMI, influenza was an unrecognized comorbidity in more cases than controls but after adjustment for background factors influenza was not a predictor of AMI [7]. However, influenza vaccination was found to be significantly protective against AMI (odds ratio 0.55, 95% CI, 0.15-0.65).

Some prospective randomized clinical trials of influenza vaccination to patients with an acute coronary syndrome (ACS) have been conducted. The FLUVACS study randomized 301 patients (200 with AMI and 101 for whom percutaneous coronary intervention (PCI) was scheduled) to either influenza vaccine or a control group [8]. Follow-up till 2 years showed a significantly reduced risk of death due to cardiovascular causes in the intervention group that was reduced over time. In the FLUCAD study 658 patients with angiographic evidence of coronary

artery disease were randomized to receive either influenza vaccination or placebo. A significant protective effect of influenza vaccination was seen against coronary ischemic events (hazard ratio 0.54, 95% CI 0.29–0.99, p=0·047) after a median follow-up of 298 days [9]. In a recent prospective randomized open with blinded endpoints trial 442 patients with acute coronary syndrome were randomized to influenza vaccination or no treatment [10]. The primary combined endpoint of major cardiovascular events, including death, hospitalization from ACS, hospitalization from heart failure, and hospitalization from stroke, occurred less frequently in the vaccine group than the control group (9.5 vs. 19.3%, unadjusted hazard ratio 0.70 (0.57–0.86), P = 0.004).

The pathophysiological background for a putative benefit of influenza vaccination in ACS may comprise shielding effects from inflammation, coagulation and other factors. It is conceivable that influenza may precipitate plaque rupture [11], increase cytokines with central roles in plaque destabilization [12] and trigger the coagulation cascade [13]. B-cells may play a role in atherogenesis [14] and the humoral response following an influenza vaccination stimulus involves multiple B cell subsets generating a multifaceted humoral response that provides protective antibodies [15] which might contribute to explain the possible protection against ACS.

A science advisory from the American Heart Association and the American College of Cardiology endorses influenza vaccination in patients with cardiovascular disease and states that beneficial effects pertains to both a reduction in ACS but also to reduced mortality from influenza per se [16]. Despite the lack of prospective randomized trials within the area influenza vaccination carries a Class I, level of evidence B recommendation. The European Society of Cardiology recommends annual influenza vaccinations for patients with established cardiovascular disease but do not provide a class of recommendation or a level of evidence [17]. The scientific community strongly advocates that a sufficiently powered prospective randomized clinical trial on influenza vaccination as secondary prevention in cardiovascular disease is carried out [2, 4, 8, 9, 18]. The need for such a study was highlighted in a Cochrane review published in May 2015 concluding that additional higher-quality evidence is necessary to confirm whether influenza vaccination is effective in preventing cardiovascular disease [19].

In the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) detailed information on all patients treated by PCI in Sweden is registered. While registry and database information by nature is retrospective, we will in the present study use the SCAAR and national registries in the other Nordic countries (NORIC [Norway], Western Denmark Heart Registry (WDHR) and the WEBPATS [Denmark]), as prospective platforms for conducting a randomized clinical multicenter trial. The rationale being that with standardized and validated information coupled to health care registries by social security number almost complete follow-up can be assured with limited extra work related to conducting a trial. Another important advantage by using registries as platforms for randomization is the opportunity to include a large number of patients

over a relatively short time period, thus allowing investigation of hard endpoints such as death, revascularization, myocardial infarction, stroke and stent thrombosis. Stent thrombosis, although a very rare condition is one of the most devastating complications to a coronary intervention [20]. Stent thrombosis has more or less only two clinical presentations: 1) death (registered as death in this trial) or 2) a new myocardial infarction leading to a new coronary angiography where a stent thrombosis is clearly identifiable and Swedish data on this condition is international reference material due to total national database coverage in invasive cardiology [21, 22].

The concept of a registry-based randomized clinical trial (RRCT) was recently introduced [23, 24] and carried out with success in Sweden, Iceland and Denmark in the 7244 patients TASTE trial on thrombus aspiration in ST-segment elevation myocardial infarction (STEMI) [25]. This novel trial model has been designated a possible shift of paradigm in clinical medicine [26].

2.2 Purpose of the study

The primary objective is to study the effect of influenza vaccine (Vaxigrip, Sanofi Pasteur MSD) compared to placebo, on major adverse cardiac events i.e. all-cause death, myocardial infarction and stent thrombosis (first occurring) till 1 year in patients with STEMI or non-ST-segment elevation myocardial infarction (NSTEMI) undergoing coronary angiography/PCI.

Secondary objectives are time to all-cause death, time to stent thrombosis, time to revascularization, time to myocardial infarction, time to stroke or time to rehospitalization for heart failure till 1 year. Also length of hospital stay is a secondary objective.

2.3 Rationale

In this trial we test the hypothesis that influenza vaccination is superior to no influenza vaccination in reducing time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) at one year in patients with STEMI or NSTEMI (primary end point). Secondary endpoints are each of the endpoints in the composite primary endpoint evaluated separately and time to revascularization, stroke, rehospitalization for heart failure and length of hospital stay.

2.4 Clinical relevance

STEMI and NSTEMI remain two of the leading causes of death globally. Thrombolysis was a major step forward in the treatment of STEMI [27-29] and further progress was made when primary PCI was established as a golden therapeutic standard [30] as was the case for NSTEMI [31]. Treatment has been further optimized with pre, peri- and post procedure platelet inhibition, statins, angiotensin converting enzyme inhibitors and beta adrenoreceptor blockade. Despite these improvements in care, cardiovascular disease is the leading cause of death globally. Thus, a simple, cheap treatment to prevent recurrent cardiovascular events is highly warranted.

3. Study design

3.1 Patients

A total of 4400 patients will be included in the study.

3.1.1 Patient inclusion

Individuals for inclusion will be recruited among the patients referred to the participating centers for coronary angiography/PCI because of STEMI or NSTEMI (Figure 1). Patients will be recruited during the influenza season only (from October 1 till March 1). The patients will not receive any honorarium for participation.

3.1.2 Inclusion criteria

- Patients with a diagnosis of STEMI as defined by chest pain suggestive for myocardial ischemia for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours, and an ECG with new ST-segment elevation in two or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads or a probable new-onset left bundle branch block

Or:

- Patients with a diagnosis of NSTEMI defined by a combination of: onset of symptoms such as central chest pain or an aggravated angina pectoris, with or without an ECG change with ST-segment lowering or an inverted T-wave, and at least two values with levels of troponin-T or troponin-I above the established margin of an AMI.

And:

- A finalized coronary angiography/PCI.
- Male or female subjects ≥18 years.
- Written informed consent.

3.1.3 Exclusion criteria

- Influenza vaccination within 12 months prior to inclusion
- Indication for influenza vaccination for some indication other than Myocardial Infarction
- Severe allergy to eggs or previous allergic reaction to influence vaccine.
- Suspicion of febrile illness or acute, ongoing infection.
- Hypersensitivity to the active substances or ingredients of Vaxigrip or against any residues, such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde and octoxinol.
- Subjects with endogenic or iatrogenic immunosuppression that may result in reduced immunisation response.
- Inability to provide informed consent.
- Age below 18 years.
- Previous randomization in the IAMI trial.

3.2 Consort patient flow chart

Before study start, each of the hospitals entering data in SCAAR (Sweden) and the other national registries has to decide whether or not to participate in the trial. The understanding will be that all PCI operators in the participating hospitals will actively attempt to include all eligible patients in the study period.

In the SCAAR and the other national registries here is a prospective registration of all patients with STEMI and NSTEMI. Reasons for not including particular patients will be documented on an electronic consort patient flow chart.

3.3 Baseline demographic data and follow-up

Demographic data and procedure-related data are entered into the national PCI registries which are coupled to health quality national registries via personal identification numbers. Data entered at study inclusion will be used for analysis. Validation of SWEDEHEART source data against electronic health records is performed periodically in all hospitals by comparing 50 entered variables in 30-40 randomly selected patients per hospital and year with an overall agreement of 95% [32].

Patients in the study will not attend any follow-up visits. The endpoints will be monitored using national registries, the SCAAR database and national PCI registries and a 12 month telephone interview.

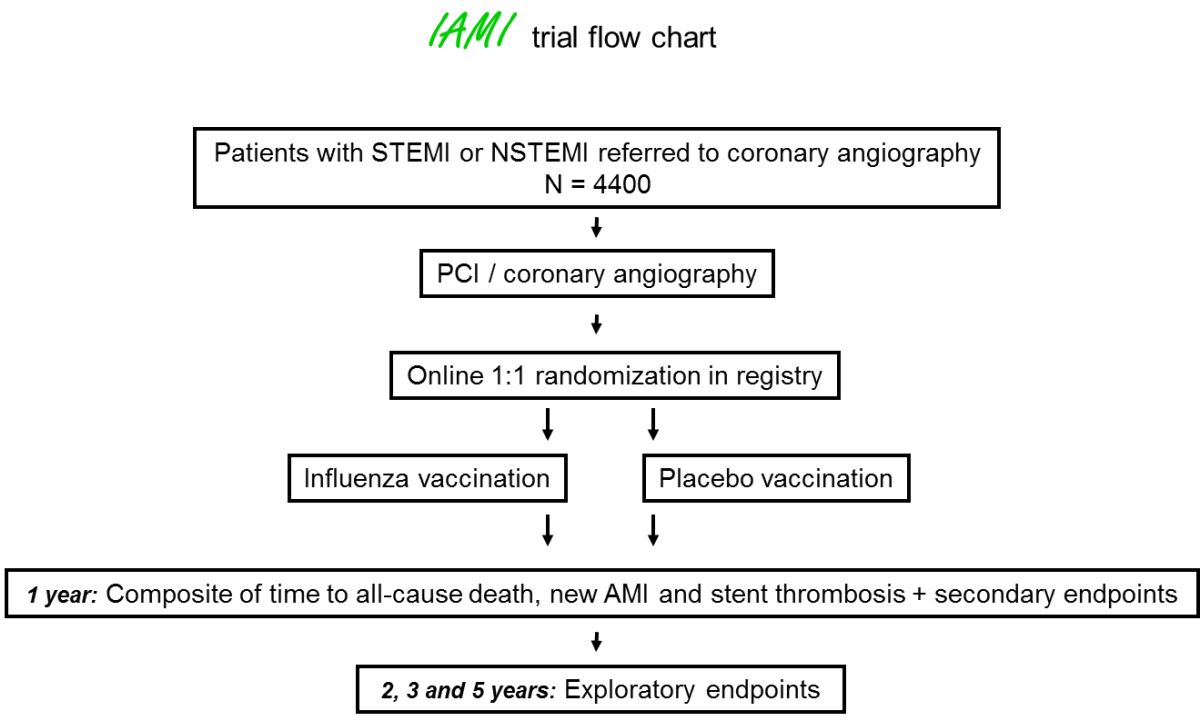

Figure 1. **Flow chart of study design**

AMI: Acute myocardial infarction; NSTEMI: non-ST-segment elevation myocardial infarction. PCI: percutaneous coronary intervention; SCAAR: Swedish Coronary Angiography and Angioplasty Registry; STEMI: ST-segment elevation myocardial infarction.

At 7 days after the vaccination patients will be requested to return a postage paid standard questionnaire to asses if any adverse event has occurred following vaccination.

Follow up of primary and secondary endpoints will also be performed by telephone contacts with the patients or first degree relatives by a nurse phone call after 350±10 days. The nurses will also accumulate hospital record information on these endpoints. A central adjudication will be performed for all reported primary endpoints for the 350 days follow up. Every site will prepare source documents for the event for central adjudication by an independent committee.

3.4 Treatment strategies

3.4.1 Influenza vaccination and placebo

Following informed consent one of the investigators (PCI physician) will randomize the patient in the SCAAR database. An unblinded study nurse at each center, not otherwise involved or participating in the study, will prepare the study medication (Vaxigrip/placebo).

According to randomization, Vaxigrip is administered in a pre-filled syringe or the same volume of placebo (0.5 ml Sodium Chloride) is drawn up in a small syringe just before the vaccination. A list of information regarding what has been given to each patient (Vaxigrip/placebo) and when (date and time) will be prepared, signed and kept by the unblinded study nurse. To ascertain blinding, the nurse will lay a piece of foil around the syringe to ensure that the patient cannot see what is administered during the vaccination. The influenza vaccination, or placebo, is given as a deep subcutaneous injection from 24 hours prior to coronary angiography/PCI (NSTEMI patients) to 48 hours following coronary angiography/PCI (NSTEMI and STEMI patients, Figure 2). Patients will be observed for 20 minutes after vaccination/placebo to monitor, and potentially treat, side effects .This strategy is chosen to optimize compliance with randomization and ensure simplicity.

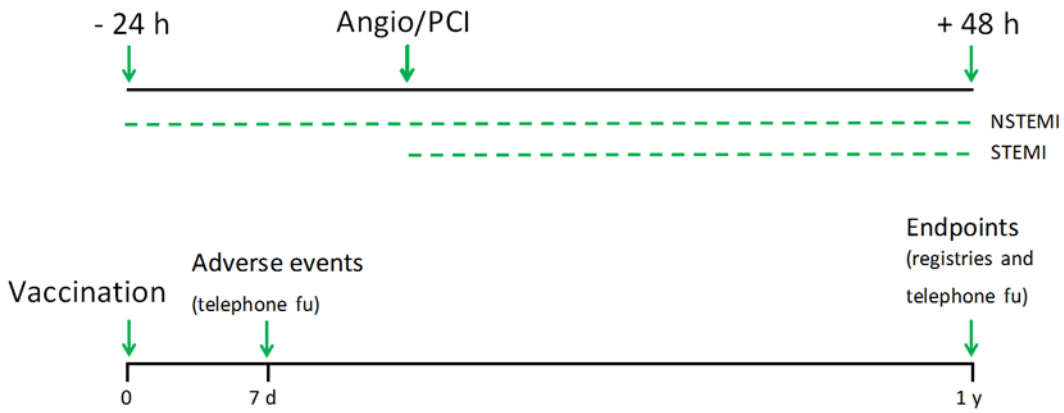

Figure 2. Timing of vaccination (upper panel, dotted line) and follow-up (fu) till 12 months (lower panel).

The chosen type of influenza vaccination (Vaxigrip, Sanofi Pasteur MSD - suspension for injection in pre-filled syringe) may, in contrast to other vaccines given via the intramuscular route, be administered as a deep subcutaneous injection and is chosen to minimize the risk of bleeding. For patients in the placebo group, sodium chloride will be used.

Study products (Vaxigrip/placebo) will be ordered according to each participating units ordinary requisition routines and will not be marked with any study specific information.

3.4.2 Post-procedure platelet inhibition

After the index PCI, lifelong acetylsalicylic acid is encouraged but will be according to national and local clinical routine**. Also,** duration of glycoprotein 2b/3a inhibitor treatment, ticagrelor, clopidogrel or other P2Y12 inhibitor is left to the discretion of the treating physician.

3.5 Endpoints

3.5.1 Primary endpoint

- The primary endpoint is time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year. These data will be obtained from national health registries. All primary endpoints up to 350 days will be adjudicated by a central adjudication committee.

3.5.2 Secondary endpoints

- Time to all-cause death till 1 year.
- Time to stent thrombosis till 1 year.
- Time to revascularization till 1 year.
- Time to myocardial infarction till 1 year.
- Time to stroke till 1 year.
- Time to hospitalization for heart failure.
- Length of hospital stay.

Data on stroke are according to reports in the Swedish national patient registry and registries in the other participating countries. From a hypothesis generating perspective we reserve the possibility of following up patients through registries beyond 1 year. Because influenza may precipitate plaque rupture [11] it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques [33]. Endpoints beyond 1 year will be regarded as exploratory. In that case such endpoints will be investigated at 2 years and repeated each year till a maximum of 5 years of follow-up until survival curves of the primary endpoint and/or secondary endpoints merge.

3.5.3 Endpoint definition

Death: All reasons for death, i.e. cardiac, non-cardiac or unknown. Myocardial infarction: ICD codes I21, I21.4 and I22, heart failure as I50 and stroke as I63.9. New PCIs and stent thromboses are followed in SCAAR and the other national PCI registries.

4. Statistics and data management

The data management work and statistical analyses will be performed at Örebro University Hospital in collaboration with the accredited Swedish clinical research organization, Lytics, which will be in charge of external web-randomization (http://lytics.ai/company).

4.1 Statistical analysis

The results will be analyzed according to the intention-to-treat principle. Differences between groups in time-to-event endpoints will be assessed with the log-rank test. For the primary endpoint, patients will be censored at 1 year; analyses at other time points will be handled in a

similar way. Survival probabilities will be displayed and calculated using Kaplan-Meier methodology. Hazard ratios (HR) with 95% confidence intervals between study groups will be calculated using Cox proportional hazard model, if violation to proportional hazard assumption time-dependent HR will be calculated and adjustment will be made for stratification variables, center and STEMI/NSTEMI.

Differences between study groups will be assessed with unpaired t-tests on original scale or log scale as appropriate. Ordinal variables will be assessed with chi-2 test for trend or Mann-Whitney U test and Pearson's chi-square test or Fisher's exact test will be used to test differences between proportions. Two-sided statistical significance levels of 5% will be used and estimates will be presented with 95% confidence intervals.

Subgroup analyses will first and foremost be carried out for the primary endpoint and its components. All subgroup analyses of event data will be performed using a proportional hazards model with factors treatment, subgroup, and treatment-subgroup interaction, and will be presented with within-group hazard ratios with 95% confidence intervals and the interaction p-value. The primary subgroup analyses will focus on the STEMI and NSTEMI populations and the effect of intervention in each of the three influenza seasons, with the purpose of evaluating effect in each subgroup.

4.2 Interim Safety Analysis

A maximum of 3 months following inclusion of the first 1000 patients an independent endpoint committee (IEC) will monitor study endpoints. Variables to be assessed are all-cause death, a new myocardial infarction and stent thrombosis. Premature termination of the study will be mandated in the event that one of the treatment strategies shows statistically significance at the 0.001 alpha level for the composite of time to all-cause death, a new myocardial infarction or stent thrombosis.

4.3 Analysis population

The results will be analyzed according to the intention-to-treat principle, i.e. patients randomized to a certain group will be followed and assessed irrespectively of the actual treatment. Protocol violations will be monitored continuously and the responsible centers notified. Data collected during the study will be coded so that no subjects can be identified.

4.4 Sample size calculations

Sample size is calculated on the basis of three smaller randomized studies [8-10], demographic data from annual SCAAR reports (accessible at http://www.ucr.uu.se/swedeheart/) and from the TASTE trial in which the number of high risk patients included was lower than expected [25].

The combined 1-year primary endpoint of all-cause death, a new AMI or stent thrombosis is estimated at 10.0% (expected survival probability of 0.9) for individuals randomized to placebo. With a 5% two-sided significance level we calculated that 386 events would be needed to have a 80% statistical power to detect a 25% reduction of the primary endpoint in the influenza vaccination group, corresponding to a hazard ratio of 0.75 [34]. With this estimation 2186 patients are needed per study group, power calculation utilized with STATA release 11 (College Station, TX, USA). In order to control for dropouts and crossing from one group to the other (both were negligible in TASTE), 4400 patients will be included.

4.5 Randomization procedure

An external web-page for randomization coupled to relevant national registries in the participating countries will be constructed (Figure 3). Following written informed consent randomization is stratified by center and diagnosis (STEMI/NSTEMI) with a 1:1 allocation within each stratum using predefined block sizes. Block randomization is by a computer generated random number list prepared by Lytics, the clinical research organization in charge of external web-randomization (http://lytics.ai/company). The patient, investigators and all other medical staff are kept blinded to the allocation.

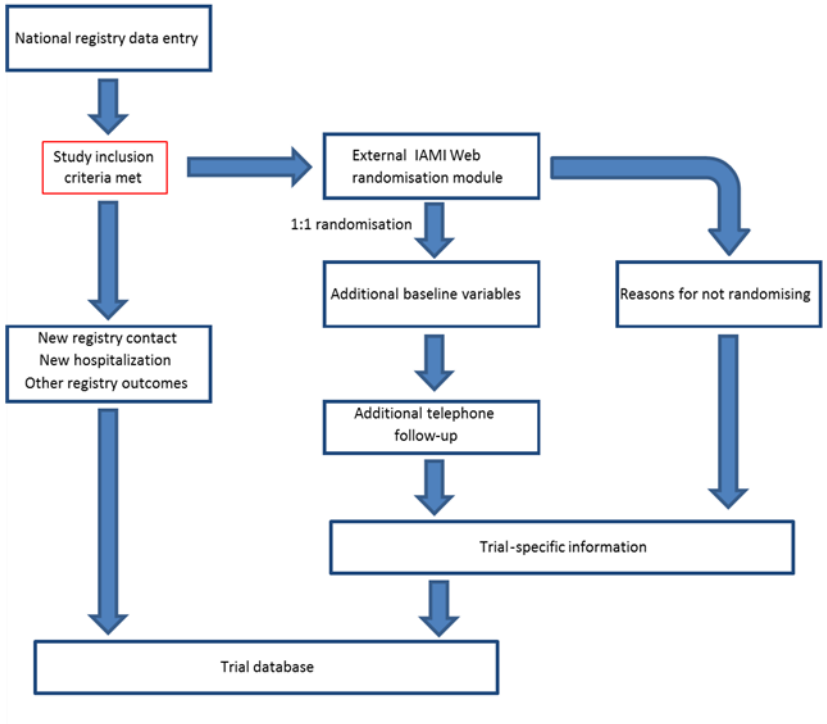

Figure 3. External Web-based randomization and relation to a national clinical registry.

4.6 Database and Case Report Form

A study data base with all patients included in the study will be generated based on the ordinary national registry process and a study specific randomization module. An electronic case report form (CRF) will be generated automatically based on the ordinary registration form and stored at Lytics for each patient included. The patient's identity will always be confidential. Study data will be entered directly in the national registries and stored in each national registry

The investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the CRFs. All data from the 7 day follow up questionnaire and telephone calls after 350±10 days and SUSARs will be imported into the study data base.

4.7 Documentation and data collection

Criteria for inclusion, informed consent and the decision to include the patient will be documented in the patients' hospital record. Also the randomization number will be reported in the hospital record. Follow up data will be registered in the national databases.

5. Monitoring

The study will be monitored using the ordinary SCAAR monitoring system and registry monitoring systems in each participating country by independent professionals. Before starting the clinical trial all centers will have a telephone/web-based start meeting with presentation of the study, study procedures and documentation. The first visit at site will be when the center has included some patients into the study.

During the study period, monitors will have regular contact with the participating departments to ensure that the trial is conducted in compliance with the protocol and applicable regulatory requirements. The monitors will also provide information and support to the investigator(s).

The number of monitoring visits will be limited and unless no specific problems occur the main part of the monitoring will be centralized by regular checks of the data quality in the database. Moreover logs of signed informed consents and AE forms will be faxed to the sponsor for follow-up. The monitors will review source documents for verification of consistency with the study data recorded in CRF according to risk based monitoring. Investigators and other responsible personnel must be available during the monitoring visits, possible audits and inspections and should devote sufficient time to these processes.

6. Administration

6.1 Organization

Swedish, Danish, Finnish, Norwegian and Icelandic PCI centers with interest in the trial and willingness to randomize all eligible STEMI and NSTEMI patients during the study period can participate in the study.

There will be a local investigator for each center. The investigators will be responsible for the study in the respective centers. Further, there will be national principal investigators (PIs) and key investigators who will also be members of the steering committee (please refer to protocol front page for names and affiliations) of the study, and in charge of the study.

6.2 Insurance

The patients in the study are covered by the Swedish/Danish/Finnish/Norwegian/Icelandic patient insurance and drug insurance.

6.3 Economy

The IAMI trial is an academic study conceived and conducted by cardiovascular interventionalists in the respective countries. The study is independent of commercial interests. Study logistics, handling of data and statistical assessments will be financed by the Department of Cardiology, Örebro University Hospital, Sweden. The steering committee will apply for grants from public funds and from the manufacturer of the influenza vaccine used in the study. Possible external sponsors will have no influence on the conduct of the study.

7. Ethical considerations

The study will be conducted in accordance with the protocol, applicable regulatory requirements such as and the ethical principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly in Helsinki, Finland, in 1964 and subsequent versions. The study will be initiated, when the Medical Ethical Committee of Uppsala, Sweden and the Swedish Medical Products Agency (Läkemedelsverket) have approved the protocol. Significant additions or changes to the protocol may be conducted after the application for amendment is approved by the Regulatory Authority and the Ethics Committee.

7.1 Standard care and current guidelines

7.1.1 Influenza vaccination is not part of standard STEMI and NSTEMI acute care

In this study we compare influenza vaccination and placebo. Because influenza vaccination is not part of standard of care in hospitalized patients with STEMI and NSTEMI we do not foresee severe ethical concerns in this part of trial.

The active arm, influenza vaccination, is recommended routinely in other parts of the health care system for selected risk groups and is openly accessible for patients in Sweden, Denmark, Finland, and Iceland. A 1:1 randomization in the present study will most likely increase vaccination coverage in STEMI and NSTEMI patients (see below) and in the view of the steering committee does not confer any special ethical considerations either.

7.1.2 Influenza vaccination is a guideline recommendation for high risk groups

The World Health Organization recommends seasonal influenza vaccination to people with chronic medical conditions ranked as priority group 3 (after nursing-home residents and elderly individuals) (http://www.who.int/mediacentre/factsheets/fs211/en/). Conducting a randomized clinical trial where half of the patients will receive placebo could thus be considered unethical. We argue that it is not. Rather it is unethical not to conduct a randomized clinical trial to aid in establishing evidence:

- Influenza vaccination for patients with ischemic heart disease is primarily carried out in primary care. Yet annual influenza vaccination coverage for Sweden approaches only roughly 50% of target populations [35].
  Because the present clinical trial only intends to include patients not previously vaccinated and not considering being vaccinated during the current influenza season the trial will *increase* vaccination coverage in the target population.

- The evidence for influenza vaccination to patients with ischemic heart disease is based on underpowered clinical trials, registries and expert opinion and there is a widespread appeal for adequately powered clinical trials [2, 4, 8, 9, 18] [5]. An example of a similar recommendation gone wrong, also based on registries and expert opinion, was the recommendation for hormone substitution therapy in post-menopausal women based on a report from the U.S. Nurses' Health Study [36]. From this registry-based information the leading U.S. scientific societies in heart disease and gynecology recommended in the 1990'es middle-aged women to take estrogen treatment to reduce the risk of myocardial infarction [37].

  However, this recommendation did not adhere to the classical criteria of causality between frequency and consistency in epidemiology put forward by Sir Austin Bradford Hill [38]. A stark warning that the recommendation was unsound came in 2002, with the Heart and Estrogen/progestin Replacement Study including 2,763 women who already had heart disease [39]. Half of the women received hormone therapy and the other half received placebo. The results showed that estrogen treatment *increased* the risk of cardiovascular

disease and therefore did not have the protective effect indicated in the Nurses' Health Study. A recent Cochrane review concluded that hormone replacement therapy in post-menopausal women for either primary or secondary prevention of cardiovascular disease is not effective, and causes an increase in the risk of stroke, and venous thromboembolic events.[40].

7.2 Timing of informed consent

Patients may be enrolled from 24 hours prior to coronary angiography/PCI (NSTEMI patients) to 48 hours following coronary angiography/PCI (NSTEMI and STEMI patients). This time window should allow patients sufficient time to read and consider the patient information and decide whether to participate in the trial or not.

7.3 Risks, side-effects, advantages and disadvantages in participation

Patients randomized to placebo will be treated according to standard clinical praxis. We expect that patients in the influenza vaccination arm of the study will benefit from fewer cardiovascular events (the study hypothesis) although this cannot be guaranteed.

The most common side effects to influenza vaccination are soreness, redness, or swelling where the shot was given, low grade fever, aches. Life-threatening allergic reactions are very rare. Signs of serious allergic reaction can include breathing problems, hoarseness or wheezing, hives, paleness, weakness, a fast heartbeat, or dizziness. If they do occur, it is typically within a few minutes after the shot and anti-allergic medication is readily available in all cardiological and medical wards. These reactions are more likely to occur among persons with a severe allergy to eggs (an exclusion criterion of this trial), because the viruses used in most influenza vaccines are grown in hens' eggs. Side effect will be registered according to 7b World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (https://www.aaaai.org/Aaaai/media/MediaLibrary/PDF%20Documents/Immunotherapy%20Forms/7b-World-Allergy-Organization-Systemic-Reaction-Grading-systemx.pdf).

The timing of vaccination, from 24 hours prior to 48 hours after coronary angiography/PCI, may give rise to concerns about a potential harm – inducing an additional immunologic stimulus during the inflammatory state associated with an acute coronary syndrome.

However, vaccination per se induces only a mild inflammatory reaction and while this may affect endothelial vascular function, aspirin, a drug routinely given to all ACS patients, prevents endothelial dysfunction [41]. The reasons for this strategy are the following:

- If influenza vaccination in relation to coronary angiography/PCI can be shown to reduce the risk for future cardiovascular events such a strategy can easily be implemented in future care of patients with an acute coronary syndrome as opposed to the current

influenza vaccination coverage where only half of target populations meet guideline recommendations [35].

- Patient compliance and investigator adherence to the protocol will be higher using a simple vaccination scheme as in this protocol. Postponing vaccination to a later stage will reduce recruitment rate and endanger trial completion.

Influenza vaccination shorty following PCI was tested in the FLUVACS study where the majority of patients had a recent STEMI or NSTEMI (N=200) and vaccination was carried out within 72 hours from symptom onset without any vaccine-related adverse events (AE's) being reported [8]. A large case-series of more than 20 000 persons with a first myocardial infarction and 19 000 persons with a first stroke who received influenza vaccine found no increase in the risk of myocardial infarction or stroke in the first three months after influenza vaccination [42].

Following study inclusion some patients could be anticipated to decide to accept influenza vaccination at a later stage during the same influenza season. For patients who received active vaccination as part of the study additional vaccination does not impose a health risk (http://www.fass.se/LIF/product?10&userType=0&nplId=19980417000092&docType=6 accessed March 3, 2014).

The study is conducted on an intention to treat basis and for patients randomized to placebo vaccination at a later stage will per definition be overcrossing – a deviation which cannot be monitored in health registries but is checked in the 350 days telephone follow-up.

Historically there is a small possibility that influenza vaccine could be associated with Guillain-Barré syndrome although this could not be confirmed in a recent study [43].

The overall risk/benefit assessment of the study is positive due to the low risk of an approved treatment in combination with extensive clinical experience and significant potential benefits in this population.

7.4 Biological material

Biological material will not be collected or stored in the study.

7.5 Guidelines for obtaining informed consent

Patients will enter the study after signing the informed consent form. Candidate participants will receive written information of the study, and they will receive oral information by medical doctors participating in the study. The patients will be given time to think through the study participation and to ask questions

7.6 Withdrawal

A patient can be withdrawn from the study at any time, if it is the wish of the patient, or if it is medically indicated, as judged by the investigator. A patient's participation in the study will be discontinued, if any of the following criteria applies: a) the patient's general condition contraindicates continuing the study, b) non-eligible patient, c) protocol violation. Data collected up to the end of follow-up will be used in the final analysis of the study. If a patient wants to discontinue the study participation, data collected until that time point will be analyzed in the study.

8. Safety assessments

8.1 Safety parameters

The following listed safety parameters will be monitored during the study treatment administration: Vital signs, allergic reactions, bleeding, arrhythmia and consciousness. If indicated, basic blood chemistry analyses and blood gases will be examined.

8.2 Adverse Events – AE

Registration of adverse events will start after informed consent and when treatment with study medication has been given and continue until the patient leaves the hospital after the coronary angiography/PCI procedure up to a minimum of 7 days following influenza vaccination. The same time limit will be used in both treatment groups. The patients will be informed to contact the investigator or study nurse if any adverse event should occur during this timeframe.
At 7 days after the vaccination patients will be requested to return a postage paid standard questionnaire to asses if any adverse event has occurred following vaccination.

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal investigational product.

Medical occurrences that are symptoms of existing disease, and that do represent an exacerbation of that disease, or the PCI procedure are not defined as AE's in this clinical trial. Also elective hospitalisations for pre-treatment conditions are not AE's nor expected reactions to vaccination, such as but not limited to, redness, swelling, pain, fever and chills. AEs not to be reported are also those defined as study endpoints, see chapter 3.5. IEC will evaluate for safety after 1000 patients.

8.3 Serious Adverse Event – SAE

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing inpatients' hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth effect,
- other important medical event

Hospitalisation or prolongation for existing inpatient hospitalisation disease and that do represent an exacerbation of that disease and the coronary angiography/PCI procedure as well as other events non-related to the study medication will not be reported as an SAE.

8.4 Suspected Unexpected Serious Adverse Reaction – SUSAR

All serious adverse events (SAE) must be evaluated unexpected and drug related or not. The definition of an unexpected adverse reaction is an adverse event, which has not been documented or reported earlier.

If the responsible investigator judges the SAE as being drug related and unexpected it must be promptly reported to the sponsor, who is responsible for reporting SUSARs to the Regulatory Authorities and the Ethics Committee. Whether the reaction is expected or not will be assessed against the SPC.

8.5 Definitions of severity and relationship

8.5.1 Assessment of severity

For all adverse events, serious as well as non-serious, the investigator must make an assessment of severity. Relationship should be classified according to the following definitions.

- **Mild**: Awareness of sign or symptom, but easily tolerated and cause no interference with daily activities.
- **Moderate**: Discomfort enough to cause interference with daily activities.
- **Severe:** Inability to perform normal daily activities.

8.5.2 Relationship to study drug

The investigator will judge whether or not, in his/her opinion, the adverse event is associated with the study treatment. Relationship should be classified according to the following definitions:

Probable: An adverse event, which might be due to the use of the drug. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely, e.g. concomitant drug(s), concomitant disease(s).

Possible: An adverse event, which might be due to the use of the drug. An alternative explanation, e.g. concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Unlikely: An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

8.6 Reporting procedures for Adverse Events and Serious Adverse Events

Only adverse events and serious adverse events that are not considered as signs and symptoms expected and related to STEMI or NSTEMI or known side effects from the study drug will be reported in this study. Events defined as endpoints in the study (e.g. all-cause death, a new myocardial infarction or stent thrombosis) will not be reported as adverse events. This means that other clinical signs and symptoms, which are reported by the patient and observed by the investigator, and in the opinion of the investigator are unexpected in relation to actual diagnosis, will be reported up to 7 days post vaccination**.**

8.7 SUSAR reporting procedure

If the responsible investigator judges the SAE as being drug-related and unexpected the event must be reported to the sponsor within one working day. The documentation will be on a CIOMS form (http://www.cioms.ch/index.php/cioms-form-i). The sponsor is then responsible for reporting SUSAR to the regulatory authorities and ethics committee. The sponsor is also responsible for information to all involved investigators in the study.

- A SUSAR resulting in death or judged as life threatening must be reported to regulatory authorities and the ethics committee within 7 days after the sponsor has been notified about the event. A full report has to be sent to the authorities within 15 days.
- A SUSAR which is not resulting in death or is life threatening has to be reported to regulatory authorities and ethics committee within 15 days after the sponsor has been

notified about the event. A full report has to be sent to the authorities as soon as possible.

8.8 Annual report

A safety report, including assessment of overall safety and all reported SUSARs will be submitted yearly to the Regulatory Authorities and if requested to the Ethics Committee.

9. Publication

Results, positive as well as negative, will be published in an international cardiovascular journal. Publication and author issues will be decided by the steering committee on basis of general involvement in the study (drafting of protocol, core laboratory. function, endpoint committee membership, etc.) and on number of included patients. The sequence of additional authors will be determined by the inclusion rates of the participating centers.

10. Sub-studies

Initiation of sub-studies are encouraged, but should be accepted by the steering committee. No sub-studies are part of the primary application for ethical approval of the IAMI study.

11. Study report

Study results will be summarized and submitted to the Regulatory Authority and the Ethics Committee within 12 months after completion of the trial.

12. End of trial and archiving

The study will end when the last follow-up has been performed for the last subject. Data collected during the study will be archived for at least 10 years after the study has been completed

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Signature page

I have read this protocol and it contains all necessary details for carrying out this study. I will conduct this study as outlined herein and according to ICH Good Clinical Practice, the Declaration of Helsinki and the regulations governing the conduct of clinical studies.

I will provide this protocol and all pertinent information to all persons who will assist me in conducting this study correctly. I am aware of my responsibility to keep these persons adequately informed and trained.

……
Signature of PI Date

……
Name of PI

……..…….....
Department of Cardiology Hospital

Research Protocol, ~~May~~ September 2018

Influenza vaccination After Myocardial Infarction (IAMI trial)

A multicenter, prospective, randomized controlled clinical trial based on national angiography and angioplasty registries

Ole Fröbert, MD, PhD [1] (Sponsor, PI), Matthias Götberg, MD, PhD [2] (co-PI), John Pernow, MD, PhD [8] (Chariman)

For complete list of investigators and centers – please refer to appendix 1

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ClinicalTrials.gov number, NCT02831608. Swedish ethical committee approval number, 2014 / 264.

Index

Study synopsis

Name of investigational treatment Influenza vaccine (Vaxigrip Tetra Sanofi Pasteur Europe).
Title of study Influenza vaccination After Myocardial Infarction (IAMI trial).
Coordinating Principal Investigator and Sponsor Adjunct Professor Ole Fröbert MD, PhD, Dept. of Cardiology, Örebro University Hospital, Örebro, Sweden.
Study centers Up to 35 invasive centers in Sweden, Denmark, Norway, Scotland, Latvia, Czech Republic and ~~Hungary~~ Australia.
Planned study period 2016 – ~~2019~~ 2021 from September 1 to March 1 (influenza season). Long-term follow up to 2023 via registries.
Phase of development Phase IV.
Objectives In a multicenter, prospective, randomized registry-based controlled clinical trial based on the SCAAR and SWEDEHEART platforms and other national registries in the participating countries to compare influenza vaccination and placebo in reducing future major adverse cardiac and cerebrovascular events in patients with myocardial infarction.
Methodology Following informed consent patients are randomized in a 1:1 fashion to influenza vaccination or placebo up to 72 hours following coronary angiography/PCI (NSTEMI and STEMI patients).
Number of subjects 4 400
Inclusion criteria - Patients with a diagnosis of ST-elevation myocardial infarction (STEMI) or - Patients with a diagnosis of non-STEMI or - Patients with stable coronary artery disease ≥75 years of age undergoing angiography/PCI AND with at least one additional risk criterion and - A finalized coronary angiography/PCI. - Male or female subjects ≥18 years. - Written informed consent.
Exclusion criteria - Influenza vaccination during the current influenza season or the subject anticipating to be vaccinated during the current influenza season - Indication for influenza vaccination for some indication other than myocardial infarction. - Severe allergy to eggs or previous allergic reaction to influence vaccine. - Suspicion of febrile illness or acute, ongoing infection. - Hypersensitivity to the active substances or ingredients of Vaxigrip or against any residues, such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde and octoxinol. - Subjects with endogenic or iatrogenic immunosuppression that may result in reduced immunization response. - Inability to provide informed consent. - Age below 18 years. - Previous randomization in the IAMI trial.
Primary endpoint Time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year. These data will be obtained from national health registries, telephone interviews and hospital records.

Secondary endpoints

- Time to all-cause death till 1 year.
- Time to cardiovascular death till 1 year.
- Time to stent thrombosis till 1 year.
- Time to revascularization till 1 year.
- Time to myocardial infarction till 1 year.
- Time to cardiovascular death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year.
- Time to stroke, including TIA till 1 year
- Time to hospitalization for heart failure till 1 year
- Length of hospital stay

Exploratory endpoints

- From a hypothesis generating perspective we aim to follow up patients through registries beyond 1 year and up to 5 years.

Follow up by telephone and registry information

The follow up for endpoints will be performed using the Swedish SCAAR registry and other national registries in the participating countries. At 7 days after the vaccination patients will be requested to return a standard questionnaire to asses if any adverse event has occurred following vaccination. Follow up of primary and secondary endpoints will also be performed by telephone contacts with the patients or first degree relatives by a nurse phone call after 350±10 days.

1. Abbreviations

ACS	Acute coronary syndrome
AE	Adverse events
AMI	Acute myocardial infarction
AE	Adverse events
CRF	Case report form
IEC	Independent endpoint committee
NSTEMI	Non-ST-segment elevation myocardial infarction
PCI	Percutaneous coronary intervention
PI	Principal investigator
RRCT	Registry-based randomized clinical trial
SAE	Serious adverse event
SCAAR	Swedish Coronary Angiography and Angioplasty Registry
STEMI	ST-segment elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reaction
SWEDEHEART	National Swedish registry on heart disease integrating information from four different registries: RIKS-HIA (registry on cardiac intensive care units), SEPHIA (secondary prevention of heart disease registry), the Swedish heart surgery registry and SCAAR

2. Study rationale

2.1 Background

Regardless of the progress in medical and invasive treatment strategies cardiovascular disease remains the leading cause of death globally. Inflammation is assumed to play a central role in the atherosclerotic process from initiation of atherosclerosis to progression and rupture of atherosclerotic plaques [1]. Despite documentation of *Chlamydia* species, *Helicobacter pylori* and *Cytomegalovirus* in atherosclerotic lesions antibiotic and antiviral treatments have failed to reduce cardiovascular events [2].

A relation between influenza and cardiovascular events was described in an early study of influenza epidemics from 1915 to 1929 including the 1918-1920 pandemic [3]. The author concludes that: 'In the case of organic heart diseases there was a peak, corresponding in time with the influenza peak, for practically every epidemic.' Accumulating observational studies have subsequently documented similar associations. In a study of more than 22 000 patients in a self-controlled case series analysis the risk for acute myocardial infarction (AMI) the first three days after consultation for acute respiratory infection was significantly increased (incidence ratio, 4.19 (95% confidence interval (CI), 3.18-5.53) [4].

A post hoc analysis of the ONTARGET/TRANSCEND trials (examining the effects of angiotensin receptor blocker and angiotensin-converting enzyme inhibitor therapy in subjects with known vascular disease or diabetes mellitus with documented end-organ damage) enrolling 31 546 participants found a beneficial effect of influenza vaccination on subsequent risks of major adverse vascular events but the authors concluded that 'sensitivity analyses revealed that risk of bias remained. A randomized trial is needed to definitively address this question' (vaccination) [5]. In a case-control study of more than 11 000 cases of AMI and an equal number of matched controls the adjusted odds ratio for AMI risk in the 7 days following respiratory infection was 2.10 (95% CI, 1.38-3.21) [6]. In the same study also the risk of stroke following infection was doubled.

In a recent Australian study of 275 cases of inpatients with AMI and outpatient controls without AMI, influenza was an unrecognized comorbidity in more cases than controls but after adjustment for background factors influenza was not a predictor of AMI [7]. However, influenza vaccination was found to be significantly protective against AMI (odds ratio 0.55, 95% CI, 0.15-0.65).

Some prospective randomized clinical trials of influenza vaccination to patients with an acute coronary syndrome (ACS) have been conducted. The FLUVACS study randomized 301 patients (200 with AMI and 101 for whom percutaneous coronary intervention (PCI) was scheduled) to either influenza vaccine or a control group [8]. Follow-up till 2 years showed a

significantly reduced risk of death due to cardiovascular causes in the intervention group that was reduced over time.

In the FLUCAD study 658 patients with angiographic evidence of coronary artery disease were randomized to receive either influenza vaccination or placebo. A significant protective effect of influenza vaccination was seen against coronary ischemic events (hazard ratio 0.54, 95% CI 0.29–0.99, p=0·047) after a median follow-up of 298 days [9]. In a recent prospective randomized open with blinded endpoints trial 442 patients with acute coronary syndrome were randomized to influenza vaccination or no treatment [10]. The primary combined endpoint of major cardiovascular events, including death, hospitalization from ACS, hospitalization from heart failure, and hospitalization from stroke, occurred less frequently in the vaccine group than the control group (9.5 vs. 19.3%, unadjusted hazard ratio 0.70 (0.57–0.86), P = 0.004).

The pathophysiological background for a putative benefit of influenza vaccination in ACS may comprise shielding effects from inflammation, coagulation and other factors. It is conceivable that influenza may precipitate plaque rupture [11], increase cytokines with central roles in plaque destabilization [12] and trigger the coagulation cascade [13]. B-cells may play a role in atherogenesis [14] and the humoral response following an influenza vaccination stimulus involves multiple B cell subsets generating a multifaceted humoral response that provides protective antibodies [15] which might contribute to explain the possible protection against ACS.

A science advisory from the American Heart Association and the American College of Cardiology endorses influenza vaccination in patients with cardiovascular disease and states that beneficial effects pertains to both a reduction in ACS but also to reduced mortality from influenza per se [16]. Despite the lack of prospective randomized trials within the area influenza vaccination carries a Class I, level of evidence B recommendation. The European Society of Cardiology recommends annual influenza vaccinations for patients with established cardiovascular disease but do not provide a class of recommendation or a level of evidence [17]. The scientific community strongly advocates that a sufficiently powered prospective randomized clinical trial on influenza vaccination as secondary prevention in cardiovascular disease is carried out [2, 4, 8, 9, 18]. The need for such a study was highlighted in a Cochrane review published in May 2015 concluding that additional higher-quality evidence is necessary to confirm whether influenza vaccination is effective in preventing cardiovascular disease [19].

In the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) detailed information on all patients treated by PCI in Sweden is registered. While registry and database information by nature is retrospective, we will in the present study use the SCAAR and national registries in the other Nordic countries (NORIC [Norway], Western Denmark Heart Registry

(WDHR) and the WEBPATS [Denmark]), as prospective platforms for conducting a randomized clinical multicenter trial.

The rationale being that with standardized and validated information coupled to health care registries by social security number almost complete follow-up can be assured with limited extra work related to conducting a trial. Another important advantage by using registries as platforms for randomization is the opportunity to include a large number of patients over a relatively short time period, thus allowing investigation of hard endpoints such as death, revascularization, myocardial infarction, stroke and stent thrombosis. Stent thrombosis, although a very rare condition is one of the most devastating complications to a coronary intervention [20]. Stent thrombosis has more or less only two clinical presentations: 1) death (registered as death in this trial) or 2) a new myocardial infarction leading to a new coronary angiography where a stent thrombosis is clearly identifiable and Swedish data on this condition is international reference material due to total national database coverage in invasive cardiology [21, 22].

The concept of a registry-based randomized clinical trial (RRCT) was recently introduced [23, 24] and carried out with success in Sweden, Iceland and Denmark in the 7244 patients TASTE trial on thrombus aspiration in ST-segment elevation myocardial infarction (STEMI) [25]. This novel trial model has been designated a possible shift of paradigm in clinical medicine [26].

2.2 Purpose of the study

The primary objective is to study the effect of influenza vaccine (Vaxigrip Tetra, Sanofi Pasteur Europe) compared to placebo, on major adverse cardiac events i.e. all-cause death, myocardial infarction and stent thrombosis (first occurring) till 1 year in patients with STEMI or non-ST-segment elevation myocardial infarction (NSTEMI) or stable coronary artery disease and an increased risk of future cardiovascular events undergoing coronary angiography/PCI.

Secondary objectives are time to all-cause death, time to stent thrombosis, time to revascularization, time to myocardial infarction, time to stroke or time to rehospitalization for heart failure till 1 year. Also length of hospital stay is a secondary objective.

2.3 Rationale

In this trial we test the hypothesis that influenza vaccination is superior to no influenza vaccination in reducing time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) at one year in patients with STEMI or NSTEMI or stable coronary artery disease (primary end point). Secondary endpoints are each of the endpoints in the composite primary endpoint evaluated separately and time to revascularization, stroke, rehospitalization for heart failure and length of hospital stay.

2.4 Clinical relevance

STEMI and NSTEMI remain two of the leading causes of death globally. Thrombolysis was a major step forward in the treatment of STEMI [27-29] and further progress was made when primary PCI was established as a golden therapeutic standard [30] as was the case for NSTEMI [31]. Treatment has been further optimized with pre, peri- and post procedure platelet inhibition, statins, angiotensin converting enzyme inhibitors and beta adrenoreceptor blockade. Despite these improvements in care, cardiovascular disease is the leading cause of death globally. Thus, a simple, cheap treatment to prevent recurrent cardiovascular events is highly warranted.

3. Study design

3.1 Patients

A total of 4400 patients will be included in the study.

3.1.1 Patient inclusion

Individuals for inclusion will be recruited among the patients referred to the participating centers for coronary angiography/PCI because of STEMI or NSTEMI or stable coronary artery disease and an increased risk of future cardiovascular events (Figure 1). Patients will be recruited during the influenza season only (from September 1 till March 1). The patients will not receive any honorarium for participation.

3.1.2 Inclusion criteria

- Patients with a diagnosis of STEMI as defined by chest pain suggestive for myocardial ischemia for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours, and an ECG with new ST-segment elevation in two or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads or a probable new-onset left bundle branch block

Or:

- Patients with a diagnosis of NSTEMI defined by a combination of: onset of symptoms such as central chest pain or an aggravated angina pectoris, with or without an ECG change with ST-segment lowering or an inverted T-wave, and at least two values with levels of troponin-T or troponin-I above the established margin of an AMI.

Or:

- Patients with a diagnosis of stable coronary artery disease ≥75 years of age undergoing angiography/PCI AND with at least one additional risk criterion - previous myocardial infarction,

previous PCI, previous CABG, diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40.

And:

- A finalized coronary angiography/PCI.
- Male or female subjects ≥18 years.
- Written informed consent.

3.1.3 Exclusion criteria

- Influenza vaccination during the current influenza season or the subject anticipating to be vaccinated during the current influenza season.
- Indication for influenza vaccination for some indication other than Myocardial Infarction
- Severe allergy to eggs or previous allergic reaction to influence vaccine.
- Suspicion of febrile illness or acute, ongoing infection.
- Hypersensitivity to the active substances or ingredients of Vaxigrip or against any residues, such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde and octoxinol.
- Subjects with endogenic or iatrogenic immunosuppression that may result in reduced immunisation response.
- Inability to provide informed consent.
- Age below 18 years.
- Previous randomization in the IAMI trial.

3.2 Consort patient flow chart

Before study start, each of the hospitals entering data in SCAAR (Sweden) and the other national registries has to decide whether or not to participate in the trial. The understanding will be that all PCI operators in the participating hospitals will actively attempt to include all eligible patients in the study period.

In the SCAAR and the other national registries there is a prospective registration of all patients with STEMI and NSTEMI and stable coronary artery disease. Reasons for not including particular patients will be documented on a consort patient flow chart.

3.3 Baseline demographic data and follow-up

Demographic data and procedure-related data are entered into the national PCI registries which are coupled to health quality national registries via personal identification numbers. Data entered at study inclusion will be used for analysis. Validation of SWEDEHEART source data against electronic health records is performed periodically in all hospitals by comparing 50

entered variables in 30-40 randomly selected patients per hospital and year with an overall agreement of 95% [32].

Patients in the study will not attend any follow-up visits. The endpoints will be monitored using national registries, the SCAAR database and national PCI registries and a 12 month telephone interview.

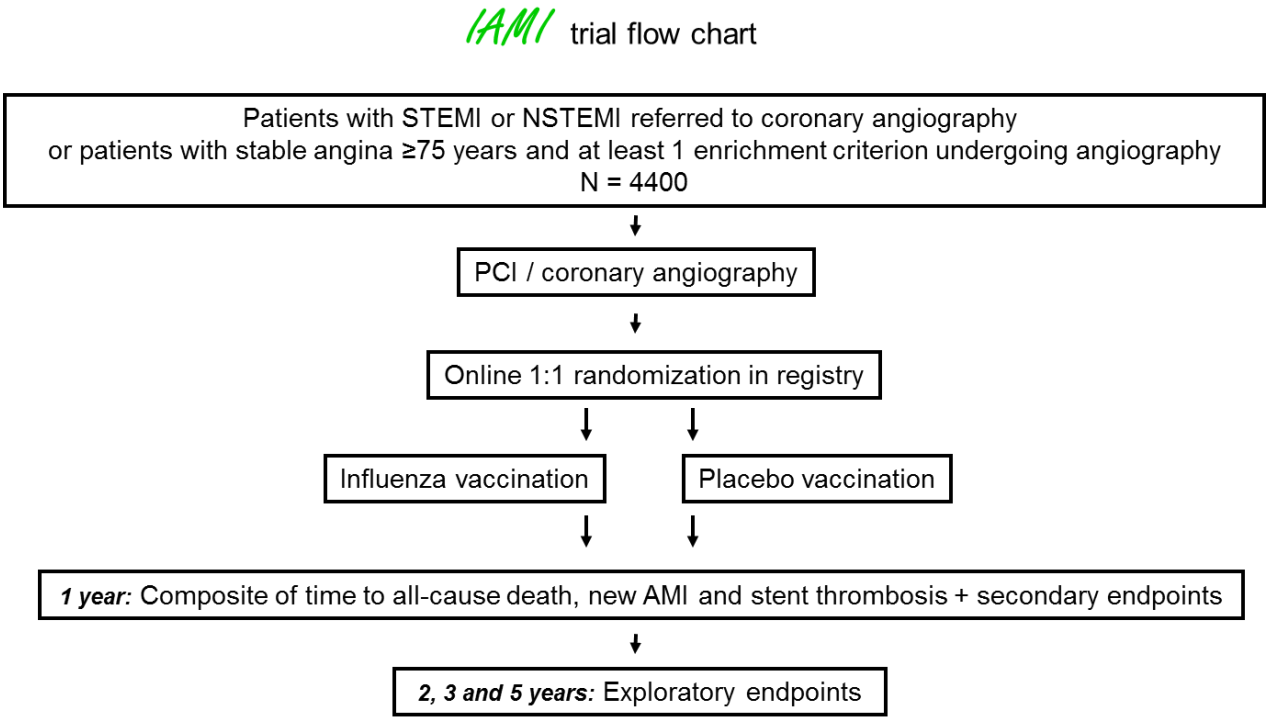

Figure 1. **Flow chart of study design**

AMI: Acute myocardial infarction; NSTEMI: non-ST-segment elevation myocardial infarction. PCI: percutaneous coronary intervention; SCAAR: Swedish Coronary Angiography and Angioplasty Registry; STEMI: ST-segment elevation myocardial infarction.

At 7 days after the vaccination patients will be requested to return a postage paid standard questionnaire to asses if any adverse event has occurred following vaccination.

Follow up of primary and secondary endpoints will also be performed by telephone contacts with the patients or first degree relatives by a nurse phone call after 350±10 days. The nurses will also accumulate hospital record information on these endpoints. A central adjudication will be performed for all reported primary endpoints for the 350 days follow up. Every site will prepare source documents for the event for central adjudication by an independent committee.

3.4 Treatment strategies

3.4.1 Influenza vaccination and placebo

Following informed consent the patient will be randomized via the SCAAR database or exclusively in the study-specific online Web-system for non-Swedish centers. An unblinded study nurse at each center, not otherwise involved or participating in the study, will prepare the study medication (VaxigripTetra/placebo). VaxigripTetra will be delivered to each participating center by the pharmaceutical distributor Tamro AB. Placebo will be obtained from each center's ordinary medical supply.

According to randomization, VaxigripTetra is administered in a pre-filled syringe or the same volume of placebo (0.5 ml Sodium Chloride) is drawn up in a small syringe just before the vaccination. A list of information regarding what has been given to each patient (VaxigripTetra/placebo) and when (date and time) will be prepared, signed and kept by the unblinded study nurse. To ascertain blinding, the nurse can lay a piece of foil around the syringe to ensure that the patient cannot see what is administered during the vaccination. The influenza vaccination, or placebo, is given as a deep subcutaneous injection up to 72 hours following coronary angiography/PCI (NSTEMI, STEMI and stable angina patients, Figure 2). Patients will be observed for 20 minutes after vaccination/placebo to monitor, and potentially treat, side effects. This strategy is chosen to optimize compliance with randomization and ensure simplicity. According to ICH GCP 4.3.1 the investigator is responsible for all medical decisions regarding the study. Thus, if deemed necessary for serious and unexpected adverse experiences that are associated with the use of the drug the investigator will be able to unblind the study drug immediately, without restrictions and without prior contact to the sponsor or the monitor.

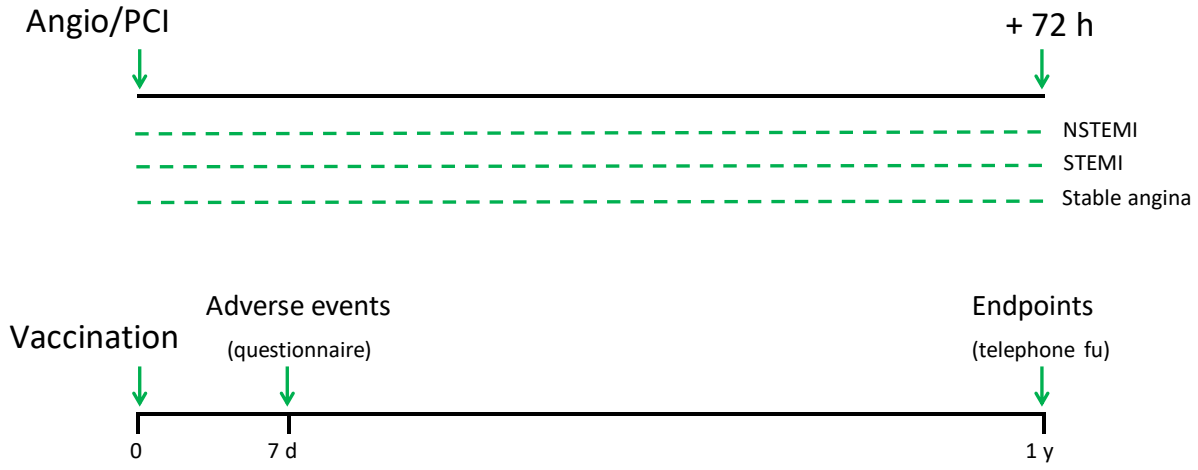

Figure 2. *Timing of vaccination (upper panel, dotted line) and follow-up (fu) till 12 months (lower panel).*

The chosen type of influenza vaccination (VaxigripTetra, Sanofi Pasteur Europe - suspension for injection in pre-filled syringe) may, in contrast to other vaccines given via the intramuscular route, be administered as a deep subcutaneous injection and is chosen to minimize the risk of bleeding. For patients in the placebo group, sodium chloride will be used.

VaxigripTetra will be labeled for the study by Tamro AB. Each center will order VaxigripTetra from Tamro AB and the vaccine will be delivered to the centers as a refrigerated temperature controlled transport (+2 to +8°C). Placebo will be ordered according to each participating units ordinary requisition routines and will not be marked with any study specific information

3.4.2 Post-procedure platelet inhibition

After the index PCI, lifelong acetylsalicylic acid is encouraged but will be according to national and local clinical routine**. Also,** duration of glycoprotein 2b/3a inhibitor treatment, ticagrelor, clopidogrel or other P2Y12 inhibitor is left to the discretion of the treating physician.

3.5 Endpoints

3.5.1 Primary endpoint

- The primary endpoint is time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year. These data will be obtained from national health registries. All primary endpoints up to 350 days will be adjudicated by a central adjudication committee.

3.5.2 Secondary endpoints

- Time to all-cause death till 1 year.
- Time to cardiovascular death till 1 year.
- Time to stent thrombosis till 1 year.
- Time to revascularization till 1 year.
- Time to myocardial infarction till 1 year.
- Time to cardiovascular death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year.
- Time to stroke including transient ischemic attack (TIA) till 1 year.
- Time to hospitalization for heart failure.
- Length of hospital stay (if information is available).

Data on stroke are according to reports in the Swedish national patient registry~~, and~~ registries in the other participating countries and from follow-up telephone interviews of patients or

relatives. From a hypothesis generating perspective we aim to follow up patients through registries beyond 1 year and up to 5 years. Because influenza may precipitate plaque rupture [11] it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques [33]. Endpoints beyond 1 year will be regarded as exploratory.

3.5.3 Endpoint definition

Death: All reasons for death, i.e. cardiac, non-cardiac or unknown. Myocardial infarction: ICD codes I21, I21.4 and I22, heart failure as I50 and stroke as I63.9. New PCIs and stent thromboses are followed in SCAAR and the other national PCI registries. All endpoints will be adjudicated according to a separate Adjudication Charter.

4. Statistics and data management

The data will be passed on from the participating centers to Örebro University Hospital where data management work and statistical analyses will be performed in collaboration with the accredited Swedish clinical research organization, Lytics, which will be in charge of external web-randomization (http://lytics.ai/company).

4.1 Statistical analysis

The results will be analyzed according to the intention-to-treat principle. Differences between groups in time-to-event endpoints will be assessed with the log-rank test. For the primary endpoint, patients will be censored at 1 year; analyses at other time points will be handled in a similar way. Survival probabilities will be displayed and calculated using Kaplan-Meier methodology.

Hazard ratios (HR) with 95% confidence intervals between study groups will be calculated using Cox proportional hazard model, if violation to proportional hazard assumption time-dependent HR will be calculated and adjustment will be made for stratification variables, center and STEMI/NSTEMI/stable angina.

Differences between study groups will be assessed with unpaired t-tests on original scale or log scale as appropriate. Ordinal variables will be assessed with chi-2 test for trend or Mann-Whitney U test and Pearson’s chi-square test or Fisher's exact test will be used to test differences between proportions. Two-sided statistical significance levels of 5% will be used and estimates will be presented with 95% confidence intervals.

Subgroup analyses will first and foremost be carried out for the primary endpoint and its components. All subgroup analyses of event data will be performed using a proportional hazards model with factors treatment, subgroup, and treatment-subgroup interaction, and will be

presented with within-group hazard ratios with 95% confidence intervals and the interaction p-value. The primary subgroup analyses will focus on the STEMI and NSTEMI populations and the effect of intervention in each of the three influenza seasons, with the purpose of evaluating effect in each subgroup.

4.2 Interim Safety Analysis

A maximum of 3 months following inclusion of the first 1000 patients an independent endpoint committee (IEC) will monitor study endpoints. Variables to be assessed are all-cause death, a new myocardial infarction and stent thrombosis.

Premature termination of the study will be mandated in the event that one of the treatment strategies shows statistically significance at the 0.001 alpha level for the composite of time to all-cause death, a new myocardial infarction or stent thrombosis.

4.3 Analysis population

The results will be analyzed according to the intention-to-treat principle, i.e. patients randomized to a certain group will be followed and assessed irrespectively of the actual treatment. Protocol violations will be monitored continuously and the responsible centers notified. Data collected during the study will be coded so that no subjects can be identified.

4.4 Sample size calculations

Sample size is calculated on the basis of three smaller randomized studies [8-10], demographic data from annual SCAAR reports (accessible at http://www.ucr.uu.se/swedeheart/) and from the TASTE trial in which the number of high risk patients included was lower than expected [25].

The combined 1-year primary endpoint of all-cause death, a new AMI or stent thrombosis is estimated at 10.0% (expected survival probability of 0.9) for individuals randomized to placebo. For patients with stable coronary artery disease we performed an analysis of data from SCAAR of 11761 individuals from the Total-AMI cohort [34] and identified a subgroup of patients with the same 1-year risk of cardiovascular events (death or MI) as for patients with NSTEMI or STEMI. After applying enrichment criteria for individuals ≥75 years of age undergoing coronary angiography/PCI and with at least one additional risk criterion - previous myocardial infarction, previous PCI (in addition to a current PCI), previous CABG, diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40 ml/min the risk for the primary composite endpoint was calculated to be on par with patients with STEMI and NSTEMI (9.3% for death and AMI and assuming the risk for stent thrombosis till 1 year to be 0.2% totaling a 9.5% risk for the primary composite endpoint). With a 5% two-sided significance level we calculated that 386 events would be needed to have a 80% statistical power to detect a 25% reduction of the primary endpoint in the influenza vaccination group, corresponding to a hazard ratio of 0.75 [35].

With this estimation 2186 patients are needed per study group, power calculation utilized with STATA release 11 (College Station, TX, USA). In order to control for dropouts and crossing from one group to the other (both were negligible in TASTE), 4400 patients will be included.

4.5 Randomization procedure

An external web-page for randomization coupled to relevant national registries in the participating countries will be constructed (Figure 3). Following written informed consent randomization is stratified by center with a 1:1 allocation within each stratum using predefined block sizes. Block randomization is by a computer generated random number list prepared by Lytics, the clinical research organization in charge of external web-randomization (http://lytics.ai/company). The patient, investigators and all other medical staff are kept blinded to the allocation.

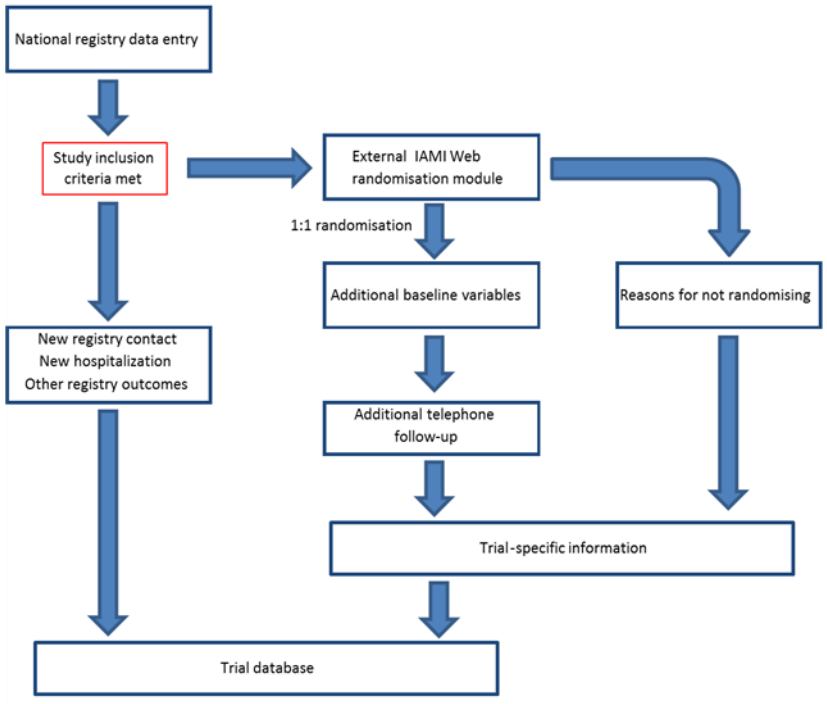

Figure 3. External Web-based randomization and relation to a national clinical registry.

4.6 Database and Case Report Form

A study data base with all patients included in the study will be generated based on the ordinary national registry process and a study specific randomization module. An electronic case report form (CRF) will be generated automatically based on the ordinary registration form and stored at Lytics for each patient included. The patient's identity will always be confidential. Study data will be entered directly in the national registries and stored in each national registry

The investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the CRFs. All data from the 7 day follow up questionnaire and telephone calls after 350±10 days and SUSARs will be imported into the study data base.

4.7 Documentation and data collection

Criteria for inclusion, informed consent and the decision to include the patient will be documented in the patients' hospital record. Also the randomization number will be reported in the hospital record. Follow up data will be registered in the national databases.

5. Monitoring

The study will be monitored using the ordinary SCAAR monitoring system and registry monitoring systems in each participating country by independent professionals. Before starting the clinical trial all centers will have a telephone/web-based start meeting with presentation of the study, study procedures and documentation. The first visit at site will be when the center has included some patients into the study.

During the study period, monitors will have regular contact with the participating departments to ensure that the trial is conducted in compliance with the protocol and applicable regulatory requirements. The monitors will also provide information and support to the investigator(s).

The number of monitoring visits will be limited and unless no specific problems occur the main part of the monitoring will be centralized by regular checks of the data quality in the database. The monitors will review source documents for verification of consistency with the study data recorded in CRF according to risk based monitoring. Investigators and other responsible personnel must be available during the monitoring visits, possible audits and inspections and should devote sufficient time to these processes.

6. Administration

6.1 Organization

Swedish, Danish, Norwegian, Scottish, Latvian, Czech and Australian PCI centers with interest in the trial and willingness to randomize all eligible STEMI and NSTEMI and stable angina patients during the study period can participate in the study.

There will be a local investigator for each center. The investigators will be responsible for the study in the respective centers. Further, there will be national principal investigators (PIs) and key investigators who will also be members of the steering committee

(please refer to protocol front page for names and affiliations) of the study, and in charge of the study.

6.2 Insurance

The patients in the study are covered by the Swedish/Danish/Norwegian patient insurances and drug insurances. For other countries specific insurance coverage will be obtained.

6.3 Economy

The IAMI trial is an academic study conceived and conducted by cardiovascular interventionalists in the respective countries. The study is independent of commercial interests. (although an unrestricted grant from Sanofi Pasteur covers part of the study costs). Study logistics, handling of data and statistical assessments will be financed by the Department of Cardiology, Örebro University Hospital, Sweden. The steering committee will apply for grants from public funds and from the manufacturer of the influenza vaccine used in the study. Possible external sponsors will have no influence on the conduct of the study.

7. Ethical considerations

The study will be conducted in accordance with the protocol, applicable regulatory requirements such as and the ethical principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly in Helsinki, Finland, in 1964 and subsequent versions. The study will be initiated, when the Medical Ethical Committee of Uppsala, Sweden and the Swedish Medical Products Agency (Läkemedelsverket) have approved the protocol. Significant additions or changes to the protocol may be conducted after the application for amendment is approved by the Regulatory Authority and the Ethics Committee.

7.1 Standard care and current guidelines

7.1.1 Influenza vaccination is not part of standard STEMI and NSTEMI acute care

In this study we compare influenza vaccination and placebo. Because influenza vaccination is not part of standard of care in hospitalized patients with STEMI and NSTEMI or stable coronary artery disease we do not foresee severe ethical concerns in this part of trial.

The active arm, influenza vaccination, is recommended routinely in other parts of the health care system for selected risk groups and is openly accessible for patients in Europe. A 1:1 randomization in the present study will most likely increase vaccination coverage in STEMI and NSTEMI and stable coronary artery disease patients (see below) and in the view of the steering committee does not confer any special ethical considerations either.

7.1.2 Influenza vaccination is a guideline recommendation for high risk groups

The World Health Organization recommends seasonal influenza vaccination to people with chronic medical conditions ranked as priority group 3 (after nursing-home residents and elderly individuals) (http://www.who.int/mediacentre/factsheets/fs211/en/). Conducting a randomized clinical trial where half of the patients will receive placebo could thus be considered unethical. We argue that it is not. Rather it is unethical not to conduct a randomized clinical trial to aid in establishing evidence:

- Influenza vaccination for patients with ischemic heart disease is primarily carried out in primary care. Yet annual influenza vaccination coverage for Sweden approaches only roughly 50% of target populations [36].
  Because the present clinical trial only intends to include patients not previously vaccinated and not considering being vaccinated during the current influenza season the trial will *increase* vaccination coverage in the target population.

- The evidence for influenza vaccination to patients with ischemic heart disease is based on underpowered clinical trials, registries and expert opinion and there is a widespread appeal for adequately powered clinical trials [2, 4, 8, 9, 18 5]. An example of a similar recommendation gone wrong, also based on registries and expert opinion, was the recommendation for hormone substitution therapy in post-menopausal women based on a report from the U.S. Nurses' Health Study [37]. From this registry-based information the leading U.S. scientific societies in heart disease and gynecology recommended in the 1990'es middle-aged women to take estrogen treatment to reduce the risk of myocardial infarction [38].

  However, this recommendation did not adhere to the classical criteria of causality between frequency and consistency in epidemiology put forward by Sir Austin Bradford Hill [39]. A stark warning that the recommendation was unsound came in 2002, with the Heart and Estrogen/progestin Replacement Study including 2,763 women who already had heart disease [40]. Half of the women received hormone therapy and the other half received placebo. The results showed that estrogen treatment *increased* the risk of cardiovascular disease and therefore did not have the protective effect indicated in the Nurses' Health Study. A recent Cochrane review concluded that hormone replacement therapy in post-menopausal women for either primary or secondary prevention of cardiovascular disease is not effective, and causes an increase in the risk of stroke, and venous thromboembolic events.[41].

7.2 Timing of informed consent

Patients may be enrolled up to 72 hours following coronary angiography/PCI (NSTEMI, STEMI and stable coronary artery disease patients). This time window should allow patients sufficient time to read and consider the patient information and decide whether to participate in the trial or not.

7.3 Risks, side-effects, advantages and disadvantages in participation

Patients randomized to placebo will be treated according to standard clinical praxis. We expect that patients in the influenza vaccination arm of the study will benefit from fewer cardiovascular events (the study hypothesis) although this cannot be guaranteed.

The most common side effects to influenza vaccination are soreness, redness, or swelling where the shot was given, low grade fever, aches. Life-threatening allergic reactions are very rare. Signs of serious allergic reaction can include breathing problems, hoarseness or wheezing, hives, paleness, weakness, a fast heartbeat, or dizziness. If they do occur, it is typically within a few minutes after the shot and anti-allergic medication is readily available in all cardiological and medical wards. These reactions are more likely to occur among persons with a severe allergy to eggs (an exclusion criterion of this trial), because the viruses used in most influenza vaccines are grown in hens' eggs. Side effect will be registered according to 7b World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (https://www.aaaai.org/Aaaai/media/MediaLibrary/PDF%20Documents/Immunotherapy%20Forms/7b-World-Allergy-Organization-Systemic-Reaction-Grading-systemx.pdf).

The timing of vaccination, up to 72 hours after coronary angiography/PCI, may give rise to concerns about a potential harm – inducing an additional immunologic stimulus during the inflammatory state associated with an acute coronary syndrome.

However, vaccination per se induces only a mild inflammatory reaction and while this may affect endothelial vascular function, aspirin, a drug routinely given to all ACS patients, prevents endothelial dysfunction [42]. The reasons for this strategy are the following:

- If influenza vaccination in relation to coronary angiography/PCI can be shown to reduce the risk for future cardiovascular events such a strategy can easily be implemented in future care of patients with an acute coronary syndrome as opposed to the current influenza vaccination coverage where only half of target populations meet guideline recommendations [36].
- Patient compliance and investigator adherence to the protocol will be higher using a simple vaccination scheme as in this protocol. Postponing vaccination to a later stage will reduce recruitment rate and endanger trial completion.

Influenza vaccination shorty following PCI was tested in the FLUVACS study where the majority of patients had a recent STEMI or NSTEMI (N=200) and vaccination was carried out within 72 hours from symptom onset without any vaccine-related adverse events (AE's) being reported [8]. A large case-series of more than 20 000 persons with a first myocardial infarction and 19 000 persons with a first stroke who received influenza vaccine found no increase in the risk of myocardial infarction or stroke in the first three months after influenza vaccination [43].

Following study inclusion some patients could be anticipated to decide to accept influenza vaccination at a later stage during the same influenza season. For patients who received active vaccination as part of the study additional vaccination does not impose a health risk (http://www.fass.se/LIF/product?10&userType=0&nplId=19980417000092&docType=6 accessed March 3, 2014).

The study is conducted on an intention to treat basis and for patients randomized to placebo vaccination at a later stage will per definition be overcrossing – a deviation which cannot be monitored in health registries but is checked in the 350 days telephone follow-up.

Historically there is a small possibility that influenza vaccine could be associated with Guillain-Barré syndrome although this could not be confirmed in a recent study [44].

The overall risk/benefit assessment of the study is positive due to the low risk of an approved treatment in combination with extensive clinical experience and significant potential benefits in this population.

7.4 Biological material

Biological material will not be collected or stored in the study.

7.5 Guidelines for obtaining informed consent

Patients will enter the study after signing the informed consent form. Candidate participants will receive written information of the study, and they will receive oral information by medical doctors participating in the study. The patients will be given time to think through the study participation and to ask questions. Informed consent shall be obtained by a medical doctor participating in the study.

7.6 Withdrawal

A patient can be withdrawn from the study at any time, if it is the wish of the patient, or if it is medically indicated, as judged by the investigator. A patient's participation in the study will be discontinued, if any of the following criteria applies: a) the patient's general condition contraindicates continuing the study, b) non-eligible patient, c) protocol violation. Data collected up to the end of follow-up will be used in the final analysis of the study. If a patient wants to

discontinue the study participation, data collected until that time point will be analyzed in the study.

8. Safety assessments

8.1 Safety parameters

The following listed safety parameters will be monitored during the study treatment administration: Vital signs, allergic reactions, bleeding, arrhythmia and consciousness. If indicated, basic blood chemistry analyses and blood gases will be examined.

8.2 Adverse Events – AE

Registration of adverse events will start after informed consent and when treatment with study medication has been given and continue until the patient leaves the hospital after the coronary angiography/PCI procedure up to a minimum of 7 days following influenza vaccination. The same time limit will be used in both treatment groups. The patients will be informed to contact the investigator or study nurse if any adverse event should occur during this timeframe. At 7 days after the vaccination patients will be requested to return a postage paid standard questionnaire to asses if any adverse event has occurred following vaccination.

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal investigational product.

Medical occurrences that are symptoms of existing disease, and that do represent an exacerbation of that disease, or the PCI procedure are not defined as AE's in this clinical trial. Also elective hospitalisations for pre-treatment conditions are not AE's nor expected reactions to vaccination: redness, swelling, pain, fever and chills. AEs not to be reported are also those defined as study endpoints, see chapter 3.5. IEC will evaluate for safety after 1000 patients.

8.3 Serious Adverse Event – SAE

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing inpatients' hospitalisation,
- results in persistent or significant disability or incapacity,

- is a congenital anomaly or birth effect,
- other important medical event

Hospitalisation or prolongation for existing inpatient hospitalisation disease and that do represent an exacerbation of that disease and the coronary angiography/PCI procedure as well as other events non-related to the study medication will not be reported as an SAE.

8.4 Suspected Unexpected Serious Adverse Reaction – SUSAR

All serious adverse events (SAE) must be evaluated unexpected and drug related or not. The definition of an unexpected adverse reaction is an adverse event, which has not been documented or reported earlier.

If the responsible investigator judges the SAE as being drug related and unexpected it must be promptly reported to the sponsor, who is responsible for reporting SUSARs to the Regulatory Authorities and the Ethics Committee. Whether the reaction is expected or not will be assessed against the SPC.

8.5 Definitions of severity and relationship

8.5.1 Assessment of severity

For all adverse events, serious as well as non-serious, the investigator must make an assessment of severity. Relationship should be classified according to the following definitions.

- **Mild**: Awareness of sign or symptom, but easily tolerated and cause no interference with daily activities.
- **Moderate**: Discomfort enough to cause interference with daily activities.
- **Severe:** Inability to perform normal daily activities.

8.5.2 Relationship to study drug

The investigator will judge whether or not, in his/her opinion, the adverse event is associated with the study treatment. Relationship should be classified according to the following definitions:

Probable: An adverse event, which might be due to the use of the drug. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely, e.g. concomitant drug(s), concomitant disease(s).

Possible: An adverse event, which might be due to the use of the drug. An alternative explanation, e.g. concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Unlikely: An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

8.6 Reporting procedures for Adverse Events and Serious Adverse Events

Only adverse events and serious adverse events that are not considered as signs and symptoms expected and related to STEMI, NSTEMI or stable coronary artery diesease or known side effects from the study drug will be reported in this study. Events defined as endpoints in the study (e.g. all-cause death, a new myocardial infarction or stent thrombosis) will not be reported as adverse events. This means that other clinical signs and symptoms, which are reported by the patient and observed by the investigator, and in the opinion of the investigator are unexpected in relation to actual diagnosis, will be reported to a minimum 7 days post vaccination**.**

8.7 SUSAR reporting procedure

If the responsible investigator judges the SAE as being drug-related and unexpected the event must be reported to the sponsor within one working day. The documentation will be on a CIOMS form (http://www.cioms.ch/index.php/cioms-form-i). The sponsor is then responsible for reporting SUSAR to the regulatory authorities and ethics committee. The sponsor is also responsible for information to all involved investigators in the study.

- A SUSAR resulting in death or judged as life threatening must be reported to regulatory authorities and the ethics committee within 7 days after the sponsor has been notified about the event. A full report has to be sent to the authorities within 15 days.
- A SUSAR which is not resulting in death or is life threatening has to be reported to regulatory authorities and ethics committee within 15 days after the sponsor has been notified about the event. A full report has to be sent to the authorities as soon as possible.

8.8 Annual report

A safety report, including assessment of overall safety and all reported SUSARs will be submitted yearly to the Regulatory Authorities and if requested to the Ethics Committee.

9. Publication

Results, positive as well as negative or inconclusive, will be published in an international cardiovascular journal. Publication and author issues will be decided by the steering committee

on basis of general involvement in the study (drafting of protocol, core laboratory. function, endpoint committee membership, etc.) and on number of included patients. The sequence of additional authors will be determined by the inclusion rates of the participating centers.

10. Sub-studies

Initiation of sub-studies are encouraged, but should be accepted by the steering committee. No sub-studies are part of the primary application for ethical approval of the IAMI study.

11. Study report

Study results will be summarized and submitted to the Regulatory Authority and the Ethics Committee within 12 months after completion of the trial.

12. End of trial and archiving

The study will end when the last follow-up has been performed for the last subject. The sponsor reserves the right to terminate the study prematurely e.g. if study participant recruitment is too slow, if study participant retention in the study is insufficient or if undue risk related to the study intervention arises.

Data collected during the study will be archived for at least 10 years after the study has been completed

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Signature page

I have read this protocol and it contains all necessary details for carrying out this study. I will conduct this study as outlined herein and according to ICH Good Clinical Practice, the Declaration of Helsinki and the regulations governing the conduct of clinical studies.

I will provide this protocol and all pertinent information to all persons who will assist me in conducting this study correctly. I am aware of my responsibility to keep these persons adequately informed and trained.

……
Signature of PI Date

……
Name of PI

……..…….....
Department of Cardiology Hospital

Protocol updates

Date, 2016-06-01, Version 4.0
Approval MPA: Amendment withdrawn and included in amendment protocol version 2016-09-01
Approval EC: 2016-07-22

Changes and additions to protocol

- Additional sites in Sweden
- Addition of other national PCI registries apart from the SCAAR and SWEDEHEART platforms
- Clarification that patients will be observed for 20 minutes after vaccination/placebo to monitor, and potentially treat, side effects.
- 7 days follow up performed by standard questionnaire instead of telephone follow up.
- Secondary objectives will be followed till 1 year, not 2, 3 and 5 years.
- Change of endpoints so that endpoints beyond 1 year (2, 3 and 5 years) will be regarded as exploratory

Addition of text: From a hypothesis generating perspective we aim to follow up patients through registries beyond 1 year and up to 5 years. Because influenza may precipitate plaque rupture [11] it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques [33]. Endpoints beyond 1 year will be regarded as exploratory

- Endpoints will be followed up by national PCI registries and a 12 month telephone interview, previous only through SCAAR and SWEDEHEART databases.
- Addition of text:

Data on stroke are according to reports in the Swedish national patient registry and registries in the other participating countries. From a hypothesis generating perspective we reserve the possibility of following up patients through registries beyond 1 year. Because influenza may precipitate plaque rupture [11] it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques [33]. Endpoints beyond 1 year will be regarded as exploratory. Exploratory analyses will primarily be of interest if the study hypothesis is confirmed. In that case such endpoints will be investigated at 2 years and repeated each year till a maximum of 5 years of follow-up until survival curves of the primary endpoint and/or secondary endpoints merge.

- Change in data management work and statistical analyses that will be performed at Örebro University Hospital in collaboration with Lytics, a Swedish clinical research organization that also is in charge of external web-randomization instead of Uppsala Clinical Research Center (UCR), Uppsala University Hospital, Sweden.
- Change in statistical analysis:

Previous wording: *Differences between groups in time-to-event endpoints will be assessed with the log-rank test (for the primary endpoint, patients will be censored at 1 year; analyses at other time points will be handled in a similar way). Survival probabilities will be displayed and calculated using Kaplan-Meier methodology. Hazard ratios between groups will be calculated using Cox proportional hazard model. Differences between group means will be assessed with the two-tailed Student's t-test. Chi-square analysis or Fisher's exact test will be used to test differences between proportions. For the primary endpoint a two-tailed P-value <0.049 is considered statistically significant. For secondary endpoints a two-tailed P-value <0.05 is considered statistically significant.*

New wording: *The results will be analyzed according to the intention-to-treat principle. Differences between groups in time-to-event endpoints will be assessed with the log-rank test. For the primary*

endpoint, patients will be censored at 1 year; analyses at other time points will be handled in a similar way. Survival probabilities will be displayed and calculated using Kaplan-Meier methodology. Hazard ratios (HR) with 95% confidence intervals between study groups will be calculated using Cox proportional hazard model, if violation to proportional hazard assumption time-dependent HR will be calculated and adjustment will be made for stratification variables, center and STEMI/NSTEMI.

Differences between study groups will be assessed with unpaired t-tests on original scale or log scale as appropriate. Ordinal variables will be assessed with chi-2 test for trend or Mann-Whitney U test and Pearson's chi-square test or Fisher's exact test will be used to test differences between proportions. Two-sided statistical significance levels of 5% will be used and estimates will be presented with 95% confidence intervals.

Subgroup analyses will first and foremost be carried out for the primary endpoint and its components. All subgroup analyses of event data will be performed using a proportional hazards model with factors treatment, subgroup, and treatment-subgroup interaction, and will be presented with within-group hazard ratios with 95% confidence intervals and the interaction p-value. The primary subgroup analyses will focus on the STEMI and NSTEMI populations and the effect of intervention in each of the three influenza seasons, with the purpose of evaluating effect in each subgroup.

- Updated sample size calculations

Previous wording *Previous wording: Sample size is calculated on the basis of three smaller randomized studies 8-10, demographic data from annual SCAAR reports (accessible at http://www.ucr.uu.se/swedeheart/) and from the TASTE trial in which the number of highrisk patients included was lower than expected 25. The combined 1-year primary endpoint of all-cause death, a new AMI or stent thrombosis is estimated at 10.0% for individuals randomized to placebo.*

We expect that influenza vaccination will reduce the risk of the primary endpoint to 7.5% in the intervention group corresponding to a 2.5/10 = 25% relative risk reduction. The ratio of intervention to non-intervention (controls) is 1:1. If the hazard ratio (relative risk) of influenza vaccination per placebo patient is set to 0.75 we will need to study 2088 placebo patients and 2088 vaccinated patients to be able to reject the null hypothesis that the experimental and control survival curves are identical with a probability (power) of 0.80. The Type I error probability associated with testing of this null hypothesis is 0.05 (www.openepi.com). In order to control for dropouts and crossing from one group to the other, 4400 patients will be included.

New wording: *The combined 1-year primary endpoint of all-cause death, a new AMI or stent thrombosis is estimated at 10.0% (expected survival probability of 0.9) for individuals randomized to placebo. With a 5% two-sided significance level we calculated that 386 events would be needed to have a 80% statistical power to detect a 25% reduction of the primary endpoint in the influenza vaccination group, corresponding to a hazard ratio of 0.75 [34]. With this estimation 2186 patients are needed per study group, power calculation utilized with STATA release 11 (College Station, TX, USA). In order to control for dropouts and crossing from one group to the other (both were negligible in TASTE), 4400 patients will be included.*

- Updated randomization procedure

Previous wording: *Following written informed consent by the patient, the randomization procedure will be performed online in the in the web-based SCAAR database using a 1:1 ratio. There will be a stratified randomization according to center. Randomization lists will be performed by UCR and the randomization numbers inserted into the SCAAR register by programmers for the system. Randomization lists will also be distributed to the unblinded study nurse at each centre, so that she/he knows what should be given to each patient based on the randomization (Vaxigrip/placebo)*

New wording: *An external web-page for randomization coupled to relevant national registries in the participating countries will be constructed (Figure 3). Following written informed consent randomization is stratified by center and diagnosis (STEMI/NSTEMI) with a 1:1 allocation within each stratum using predefined block sizes. Block randomization is by a computer generated random number list prepared by Lytics, the clinical research organization in charge of external web-*

randomization (http://lytics.ai/company). The patient, investigators and all other medical staff are kept blinded to the allocation.

- Clarification regarding incurrences in other countries than Sweden

- **Date, 2016-09-01, Version 5.0**

Approval MPA: 2016-09-16
Approval EC: 2016-09-19

Changes and additions to protocol

The same changes/additions as in protocol date, 2016-06-0, version 4.0 with addition:

- Update IMP distribution where the vaccine will be delivered by Tamro AB. Placebo will be obtained from each center's ordinary medical supply.

- Clarification regarding expected reactions to vaccination: redness, swelling, pain, fever and chills not being AEs

- Clarification regarding publication
  Previous wording
  Results, positive as well as negative, will be published in an international cardiovascular journal

  New wording
  Results, positive as well as negative or inconclusive, will be published in an international cardiovascular journal

- Addition of exclusion criteria
  - Time to cardiovascular death till 1 year.
  - Time to cardiovascular death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year.

- Clarification regarding end of trial and archiving
  Addition of text: *The sponsor reserves the right to terminate the study prematurely e.g. if study participant recruitment is too slow, if study participant retention in the study is insufficient or if undue risk related to the study intervention arises.*

- **Date, 2016-10-10, Version 6.0**

Approval MPA 2016-12-21
Approval EC 2016-12-05

Changes and additions to protocol

- Changed timeframes for vaccination from 42 hours following coronary angiography/PCI (NSTEMI and STEMI patients) to 72 hours to optimize compliance and facilitate the implementation of the study.

- Clarification regarding unblinding

Additional text

According to ICH GCP 4.3.1 the investigator is responsible for all medical decisions regarding the study. Thus, if deemed necessary for serious and unexpected adverse experiences that are associated with the use of the drug the investigator will be able to unblind the study drug immediately, without restrictions and without prior contact to the sponsor or the monitor.

- **Date, 2017-06-20, Version 7.0**

Approval MPA 2017-09-11
Approval EC 2017-07-20

Changes and additions to protocol

- Change of study title from Swedish national registries to national registries

Influenza vaccination After Myocardial Infarction (IAMI trial). A multicenter, prospective, randomized controlled clinical trial based on ~~the Swedish~~ national angiography and angioplasty registries ~~registry (SCAAR) platform~~

- Change of vaccine from Vaxigrip to VaxigripTetra and from Sanofi Pasteur MSD to Sanofi Pasteur Europe
- Additional countries and sites
- Clarification of exclusion criteria:

Previous wording: Influenza vaccination within 12 months prior to inclusion

New wording: Influenza vaccination within 12 months prior to inclusion or the subject anticipating to be vaccinated during the current influenza season.

- Change in timeframe for enrolment and vaccination from 24 hours prior to coronary angiography/PCI (NSTEMI patients) up to 72 hours following coronary angiography/PCI for both NSTEMI and STEMI patients

- Clarification that Informed consent shall be obtained by a medical doctor participating in the study.

- Change in timeframe for vaccination (24 hours prior to coronary angiography/PCI (NSTEMI patients) since there is a risk that complications that arise after the procedure may be difficult to derive from the procedure itself or for study treatment, so no vaccination performed before coronary angiography/PCI procedure.

- **Date, 2018-05-23 Version 8.0**

Approval MPA 2018-07-10
Approval EC 2018-06-05

Changes and additions to protocol

- Change in study period

Annual inclusion start September 1st instead of October 1st

- Change in exclusion criteria

Previous wording
Influenza vaccination within 12 months prior to inclusion or the subject anticipating to be vaccinated during the current influenza season

New wording
Influenza vaccination during the current influenza season or the subject anticipating to be vaccinated during the current influenza season

- **Date, 2018-09-17, Version 9.0**

Approval MPA 2018-10-11
Approval EC 2018-09-03

Changes and additions to protocol

- Prolonged study period, inclusion to 2021 and follow up (exploratory endpoints) t0 2026
- Additional study population, patients with stable coronary artery disease and an increased risk of future cardiovascular events.
- Additional inclusion criteria; Patients with stable coronary artery disease ≥75 years of age undergoing angiography/PCI AND with at least one additional risk criterion
- Addition that primay endpoints also can be obtained by telephone interviews and hospital records not only from national health registries,
- Clarifications regarding secondary endpoints
  New wordings underlined:

- Time to stroke, <u>including TIA</u> till 1 year

- Length of hospital stay <u>(if information is available).</u>

- Clarification regarding randomization in study-specific online Web-system for non-Swedish centers.
- Addition that all endpoints will be adjudicated according to a separate Adjudication Charter.
- Addition of text regarding additional study population

For patients with stable coronary artery disease we performed an analysis of data from SCAAR of 11761 individuals from the Total-AMI cohort [34] and identified a subgroup of patients with the same 1-year risk of cardiovascular events (death or MI) as for patients with NSTEMI or STEMI. After applying enrichment criteria for individuals ≥75 years of age undergoing coronary angiography/PCI and with at least one additional risk criterion - previous myocardial infarction, previous PCI (in addition to a current PCI), previous CABG, diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40 ml/min the risk for the primary composite endpoint was calculated to be on par with patients with STEMI and NSTEMI (9.3% for death and AMI and assuming the risk for stent thrombosis till 1 year to be 0.2% totaling a 9.5% risk for the primary composite endpoint).

icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number: PR-19005** **Version No. 3.00** **Version date: 25-03-2019**	**FOR OFFICE USE ONLY**			
	RRC Approval:	☒ Yes	☐ No	27/02/2019
	ERC Approval:	☐ Yes	☒ No	
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☒ Yes	☐ No	Date:16/12/2014
	Name of External :Läkemedelsverket / Swedish Medical Products Agency			

Protocol Title:* (maximum 250 characters including space**) Effect of influenza vaccination on recurrent cardiovascular events among Myocardial Infarction (MI) patients in Bangladesh: A multicounty, randomized clinical trial**

Short Title: (maximum **100**characters including space) **Influenza vaccination after Mocardial Infarction (IAMI trial)**

Key Words:*Randomized control trial, influenza vaccine, cardiovascular events, MI, IAMI

Name of theResearch Division Hosting the Protocol:*

- ☐ Health Systems and Population Studies Division (HSPSD)
- ☐ Nutrition and Clinical Services Division (NCSD)
- ☒ Infectious Diseases Division (IDD)
- ☐ Maternal and Child Health Division (MCHD)
- ☐ Laboratory Sciences and Services Division (LSSD)
- ☐ Other (specify)

Has the Protocol been Derived from an Activity:* ☒ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2015-8):* (check all that apply**)**

- ☐ Reducing maternal and neonatal mortality
- ☒ Controlling enteric and respiratory infections
- ☐ Preventing and treating maternal and childhood malnutrition
- ☐ Detecting and controlling emerging and re-emerging infections
- ☐ Achieving universal health coverage
- ☐ Examining the health consequences of climate change
- ☒ Preventing and treating non-communicable diseases

Research Phase (4 Ds):*(check all that apply**)**

- ☒ Discovery
- ☒ Development
- ☐ Delivery
- ☐ Evaluation of Delivery

Anticipated Impact of Research:* (check all that apply**)**

- ☒ Knowledge Production
- ☐ Capacity Building
- ☒ Informing Policy
- ☐ Health and Health Sector Benefits
- ☐ Economic Benefits

Which of the Sustainable Development Goal This Protocol Relates to?:* (check all that apply**)**

- ☐ 1. End poverty in all its forms everywhere
- ☐ 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture
- ☒ 3. Ensure healthy lives and promote well-being for all at all ages
- ☐ 4. Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
- ☐ 5. Achieve gender equality and empower all women and girls
- ☐ 6. Ensure availability and sustainable management of water and sanitation for all
- ☐ 7. Ensure access to affordable, reliable, sustainable and modern energy for all
- ☐ 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- ☐ 9. Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
- ☐ 10. Reduce inequality within and among countries
- ☐ 11. Make cities and human settlements inclusive, safe, resilient and sustainable
- ☐ 12. Ensure sustainable consumption and production patterns
- ☐ 13. Take urgent action to combat climate change and its impacts
- ☐ 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development
- ☐ 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combatdesertification, and halt and reverse land degradation and halt biodiversity loss
- ☐ 16. Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels
- ☐ 17. Strengthen the means of implementation and revitalize the global partnership for sustainable development

Does this Protocol Use the Gender Framework:* (Please visit:http://www.icddrb.net.bd/jahia/Jahia/pid/684 for Gender Alanysis Tool with instructions)	☒ Yes (please complete Gender Analysis Tool) ☐ No
If 'no' is the response, its reason(s) in brief:	
Will this Research Specifically Benefit the Disadvantaged (economically, socially and/or otherwise**):**	☐ Yes ☒ No
Does this Protocol use Behaviour Change Communication:	☐ Yes ☒ No

Principal Investigator(s) – Internal: Sex ☐ Female ☒ Male

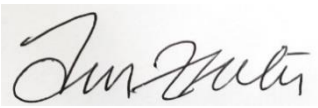
Dr. Zubair Akhtar, MPH

Senior Research Investigator, Programme for Emerging Infections, IDD, icddr,b, Phone: +880-2- 9827001-10, Ext. 2585, Cell: 01974333888, email:zakhtar@icddrb.org

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)

Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)

Primary Scientific Division of the Co-PI: IDD

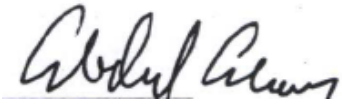


Approval of the Respective Senior Director/ Programme Head

(Signature)

Principal Investigator (External):* Sex☐ Female ☒ Male Ole FröbertMD, PhD,FESC **Adjunct Professor**, Department of Cardiology ÖrebroUniversity Hospital SödraGrevRosengatan 701 85 Örebro, Sweden Office phone: +46 19 602 54 13 Cell: +46 730 895 413 Fax: +46 19 602 54 38 E-mail: ole.frobert@regionorebrolan.se Signature or written consent of PI-External: (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional PIs] Do you have ethics certification? ☒ No ☐ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☐ Yes (please attach the certificate with CV below)	
Principal Co-Investigator(s) Internal: Sex☐ Female ☒ Male **Professor Allen G. P. Ross**| MD, PhD, DSc, FRCP Edin, FRCPath, FACTM Senior Director, Division of Infectious Diseases PO BOX 128 | Dhaka 1000 | Bangladesh Mobile: +880-2-1730380119 | Telephone: +880-2-9827001-10 Extn: 3400 Web: www.icddrb.org | Twitter: @icddr_b | Facebook: /icddr_b Signature or written consent of Co-PI: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-PIs] Do you have ethics certification? ☐ No ☐ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☐ Yes (please attach the certificate with CV below)	Primary Scientific Division/ Programme of the Co-PI: Infectious diseases division _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) – Internal Sex☐ Female ☒ Male **Prof. Dr. Mahmudur Rahman** **Address** (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): Consultant Programme for Emerging Infections Division of Infectious Diseases **Phone: +880-2-9827001-10, Ext: 3480; Cell:** +880 1711 595139**;** **email: rahman.mahmudur@icddrb.org**	**Primary Scientific Division of the Co-I: IDD** **Approval of the Respective Senior Director/ Programme Head** **(Signature)**

Co-Investigator(s) – Internal Sex☐ Female ☒ Male **Dr. Md. Khalequzzaman** **Address** (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): Emeritus Scientist Enteric and Respiratory Infections Infectious Disease Division (IDD) **Phone:** 880-2-9827001-10, Ext: 3806; Cell: +880 1713 047100 **email:** kzaman@icddrb.org	**Primary Scientific Division of the Co-I: IDD** **Approval of the Respective Senior Director/ Programme Head** **(Signature)**
Co-Investigator(s) - Internal: Sex ☒ Female ☐ Male **Dr. Firdausi Qadri PhD** Emeritus Scientist and head Enteric and Respiratory Infections Infectious Disease Division (IDD) icddr,b Cell: +8801711595367, Fax: +8802-, Email: fqadri@icddrb.org Signature or written consent of Co-I: (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is	Primary Scientific Division of the Co-I: IDD Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) – Internal Sex☒ Female ☐ Male **Dr. Sayera Banu** **Address** (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): Senior Scientist & Acting Head Programme for Emerging Infections Division of Infectious Diseases **Phone: +880-2-9827001-10, Ext: 3480; Cell:01817054481;** **email: sbanu@icddrb.org**	**Primary Scientific Division of the Co-I: IDD** **Approval of the Respective Senior Director/ Programme Head** **(Signature)**
Co-Investigator(s) – Internal Sex☒ Female ☐ Male **Dr. Fahmida Chowdhury MBBS, MPH** **Address** (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): Deputy Project Coordinator Lead Respiratory Viruses Research Group Programme for Emerging Infections Phone: +880-2-9827001-10, Ext: 2550; Cell:01817054481; **email: fahmida_chow@icddrb.org**	**Primary Scientific Division of the Co-I: IDD** **Approval of the Respective Senior Director/ Programme Head** **(Signature)**

Co- Investigator (external) (Should be icddr,b staff):* Sex ☐ Female ☒ Male **Mohammad Abdul Aleem, MBBS, MPhil, MPH** (Position, phone no, extension no, cell, and email address **):** PhD student **School of Public Health and Community Medicine (SPHCM),** **University of New South Wales (UNSW), Australia** E-mail: drmdaleem@icddrb.org; drmdaleem@gmail.com	
Co-Investigator(s) – External: Sex ☒ Female ☐ Male **Raina MacIntyreMBBS, MPH, PhD** **Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address):** **Professor and Head,** **School of Public Health and Community Medicine (SPHCM),** **University of New South Wales (UNSW), Australia** **Mobile No.: +61(2)9385 3811** **Email: r.macintyre@unsw.edu.au** **Signature or written consent of Co-I:** **(electronic signature or email or any sort of written consent)** **[if more than one, please copy and paste this row for additional Co-Is]**	
Student Investigator(s) - Internal: Sex ☐ Female ☐ Male (Position, phone no, extension no, cell, and email address **):** Signature or written consent of Student Investitor: _____ (electronic signature or email or any sort of written consent) Have ethics certificate? ☐ No ☐ Yes (If Yes, please attach to your CV below)	Students Affiliation _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Student Investigator(s) - External: Sex ☐ Female ☐ Male Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): Signature or written consent of Student Investitor: _____ (electronic signature or email or any sort of written consent)	

Collaborating Institute(s): Please provide full official address

Institution # 1

Country	Sweden
Contact person	**Ole Fröbert**
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Institution (with official address)	Örebro University Hospital SödraGrevRosengatan 701 85 Örebro, Sweden Office phone: +46 19 602 54 13 Cell: +46 730 895 413 Fax: +46 19 602 54 38 E-mail: ole.frobert@regionorebrolan.se
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 2

Country	Bangladesh
Contact person	Prof. Afzalur Rahman
Department (including Division, Centre, Unit)	National Institute of Cardiovascular Disease (NICVD)
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Directorate (in case of GoB i.e. DGHS)	Telephone: 08142806
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Institution # 3

Country	Bangladesh
Contact person	National professor brig. (rtd.) Abdul malik
Department (including Division, Centre, Unit)	Founder and President
Institution (with official address)	National Heart Foundation of Bangladesh.
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	Bangladesh
Contact person	Prof. Dr. M. A. Rashid
Department (including Division, Centre, Unit)	Cardiology Depratment
Institution (with official address)	Ibrahim cardiac hospital and research institute
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Contribution by the Members of the Scientific Team:

Members' Name	Contribution								
	Research idea/ concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing
Dr. Zubair Akhtar	☐	☒	☒	☒	☒	☒	☒	☒	☒
Ole Fröbert	☒	☒	☒	☒	☐	☒	☒	☒	☒
Prof. Dr. Mahmudur Rahman	☐	☒	☐	☒	☐	☐	☒	☒	☒
Dr. Fahmida Chowdhury	☐	☒	☒	☐	☐	☒	☒	☒	☒
Dr. Mohammad Abdul Aleem	☒	☒	☒	☐	☐	☐	☒	☒	☒
Professor Allen G. P. Ross	☐	☒	☒	☒	☒	☒	☒	☒	☒
Dr. S ayera Banu	☐	☒	☐	☐	☐	☐	☐	☒	☒
Dr. Md. Khalequzzaman	☐	☒	☐	☐	☐	☐	☐	☒	☒
Dr. Firdausi Qadri PhD	☒	☒	☒	☐	☐	☐	☐	☒	☒
Raina MacIntyre	☒	☒	☐	☐	☐	☐	☒	☒	☒

Study Population: Sex, Age, Special Group and Ethnicity

Research Subject:
- ☒ Human
- ☐ Animal
- ☐ Microorganism
- ☐ Other (specify): _____

Sex:
- ☒ Male
- ☒ Female
- ☒ Transgender

Age:
- ☐ 0 – 4 years
- ☐ 5 – 10 years
- ☐ 11 – 17 years
- ☒ 18 – 64 years
- ☒ 65 +

Special Group:
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service Providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee
- ☐ Others (specify): _____

Ethnicity:
- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Other (specify): _____

NOTE: It is icddr.b's policy to include men, women, children and transgender in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Consent Process: (Check all that apply**)**
- ☒ Written
- ☐ Oral
- ☐ Audio
- ☐ Video
- ☐ None

Language:
- ☒ Bangla
- ☐ English
- ☐ Other (specify: _____

Project/Study Site: (Check all that apply**)**

☐ Chakaria
☐ Bandarban
☐ Dhaka Hospital
☐ Kamalapur Field Site/HDSS
☐ Mirpur (Dhaka)
☐ Matlab DSS Area
☐ Matlab non-DSS Area
☐ Matlab Hospital
☐ Mirzapur
☐ Bianibazar (Sylhet)
☐ Kanaighat (Sylhet)
☐ Jakigonj (Sylhet)
☐ Other community in Dhaka
Name: _____
☒ Other sites in Bangladesh
Name: ***National Institute of Cardiovascular Diseases (NICVD).***
☒ Multi-national Study
Name of the countryAustralia, Czech, Denmark, Latvia, Norway, Sweden, United Kingdom,

Project/Study Type:(Check all that apply)

☐ Case Control Study
☒ Clinical Trial (Hospital/Clinic/Field)*
☐ Community-based Trial/Intervention
☐ Cross Sectional Survey
☐ Family Follow-up Study
☒ Longitudinal Study (cohort or follow-up)
☐ Meta-analysis
☐ Programme Evaluation
☐ Programme (Umbrella Project)
☒ Prophylactic Trial
☐ Record Review
☐ Secondary Data Analysis
Protocol No. of Data Source: _____
☐ Surveillance/Monitoring
☐ Systematic Review
☐ Other (specify):

***Note**: International Committee of Medical Journal Editors (ICMJE) defines Clinical Trial as "*Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

PI of the RRC- and ERC-approved Clinical Trials should provide necessary information to IRB Secretariat (Research Administration) for registration and uploading into relevant websites (usually at the https://register.clinicaltrials.gov/). They should also provide relevant information to the IRB Secretariat in the event of amendment/modification after their approval by RRC and ERC.

Biological Specimen:

a) Will the biological specimen be stored for future use?	☐Yes ☐No☒ Not applicable
b) If the response is 'yes', how long the specimens will be preserved?	
c) What types of tests will be carried out with the preserved specimens?	
d) Will the consent be obtained from the study participants for use of the preserved specimen for other initiative(s) unrelated to this study, without their re-consent?	☐Yes ☐No☒ Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/countries.	☐Yes ☐No☒ Not applicable Name
f) If shipped to another country, will the surplus/unused specimen be returned to icddr,b? If the response is 'no', then the surplus/unused specimen must be destroyed.	☐Yes ☐No☒ Not applicable
g) Who will be the custodian of the specimen at icddr,b?	
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	
i) Who will be the owner(s) of the specimens?	

j) Has a MoU been signed with regards to collection, storage, use and ownership of specimen? If the response is 'yes', please attach a copy of the MoU.. If the response is 'no', appropriate justification should be provided for not signing a MoU.	☐Yes ☐No☒ Not applicable

Proposed Sample Size:
Sub-group (Name of subgroup e.g. Men, Women) and Number

Name	Number	Name	Number
(1) **Vaccinated cohort** (Patients aged ≥18 years with myocardia infarction or patients aged ≥75 years with stable coronary artery disease AND a finalized coronary angiography/PCI)	500		
(2) **Non-vaccinated cohort**(Patients aged ≥18 years with myocardia infarction or patients aged ≥75 years with stable coronary artery disease AND a finalized coronary angiography/PCI)	500		
Total sample size (for Bangladesh)	1000		

Determination of Risk: Does the Research Involve (Check all that apply**)**

- ☐ Human exposure to radioactive agents?
- ☐ Foetal tissue or abortus?
- ☐ Investigational new device?
  Specify: _____
- ☐ Existing data available from Co-investigator?
- ☐Human exposure to infectious agents?
- ☐ Investigational new drug?
- ☐ Existing data available via public archives/sources?
- ☐ Pathological or diagnostic clinical specimen only?
- ☐ Observation of public behaviour?
- ☒New treatment regime?

	Yes	No
Will the information be recorded in such a manner that study participants can be identified from the information directly or through identifiers linked to the study participants?	☐	☒
Does the research deal with sensitive aspects of the study participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	☐	☒

Could information on study participants, if available to people outside of the research team:

	Yes	No
a) Place them at risk of criminal or civil liability?	☐	☒
b) Damage their financial standing, reputation or employability, or social rejection, or lead to stigma, divorce etc.?	☐	☒

Do you consider this research: (check one)

☒ Greater than minimal risk	☐ No more than minimal risk	☐ Only part of the diagnostic test

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

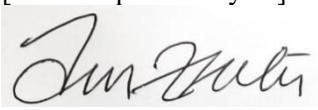
Risk Group of Infectious Agent and Use of Recombinant DNA	
a) Will specimens containing infectious agent be collected?	☐Yes ☒No☐ Not applicable
b) Will the study involve amplification by culture of infectious agents?	☐Yes ☐No☒ Not applicable

c) If response to questions (a) and/or (b) is 'yes', to which Risk Group (RG) does the agent(s) belong? (Please visit http://www.icddrb.net.bd/jahia/Jahia/pid/684 to review list of microorganism by Risk Group)	☐RG1 ☐RG2 ☐ RG3 ☐ RG4
d) Does the study involve experiments with recombinant DNA?	☐Yes ☒No☐ Not applicable

Does the study involve any biohazards materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?

☐Yes ☒No

[If the response is 'yes']



_____ _____

Signature of the Principal Investigator **Date**

Dissemination Plan: [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/ agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	☐ No	☒ Yes	We will disseminate research findings in the PEI seminar for icddr,b scientists/ staff.
Internal publication	☒ No	☐ Yes	
Working paper	☒ No	☐ Yes	
Sharing with GoB (e.g. DGHS/ Ministry, others)	☐ No	☒ Yes	
Sharing with national NGOs	☒ No	☐ Yes	
Presentation at national workshop/ seminar	☐ No	☒ Yes	
Presentation at international workshop/ conference	☐ No	☒ Yes	
Peer-reviewed publication	☐ No	☒ Yes	
Sharing with international agencies	☒ No	☐ Yes	
Sharing with donors	☐ No	☒ Yes	We will share research findings with the sponsors and collaborators: Ole Frobert, MD, PhD, Örebro University Hospital, Swedish Heart Lung Foundation, Sanofi Pasteur, a Sanofi Company, Uppsala University, Lytics
Policy brief	☒ No	☐ Yes	
Other			
Other			

Funding:

Is the protocol fully funded?	☒ Yes	☐ No
If the answer is yes, please provide sponsor(s)'s name	1. **Sanofi Pasteur, a Sanofi Company**	
	2.	
Is the protocol partially funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	

If fund has not been identified:

Is the proposal being submitted for funding?	☐ Yes	☐ No
If yes, name of the funding agency	1.	

Conflict of interest:
Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

☒ No ☐ Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Proposed Budget:

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date :01/04/19

End Date :30/09/21

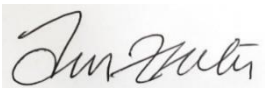
Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	74,138	13,988	88,125
Year-2	96,468	19,294	115,762
Year-3	80,095	16,294	96,114
Total	250,700	49,300	300,000

Certification by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the NAVISION if a grant is awarded as a result of this application.

I also certify that I have read icddr,b Data Policies and understand the PIs' responsibilities related to archival and sharing of research data, and will remain fully compliant to the Policies. (Note: The Data Policies can be found here: http://www.icddrb.org/who-we-are/data-policies)



Signature of PI **Date**

Approval of the Project by the Division Director of the Applicant:

The above-mentioned project has been discussed and reviewed at the Division level.

Professor Allen G. P. Ross

Name of the Division Director Signature Date of Approval

Table of Contents

☒ Check here if appendix is included

Project Summary
[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: **Zubair Akhtar**

Research Protocol Title: **Effect of influenza vaccination on recurrent cardiovascular events among Myocardial Infarction (MI) patients in Bangladesh: A multicounty, randomized clinical trial**

Proposed start date:01/04/19 Estimated end date: 30/09/21

Background (brief):

a. Burden:

Cardiovascular disease is a leading cause of death globally estimated to be responsible for about 17 million deaths annually. The health burden due to life threatening cardiovascular events like acute myocardial infarction is more prominent in low income countries like Bangladesh compared to developed regions of the world.

b. Knowledge gap:

It is assumed that, vaccinating the high-risk group of patients with myocardial infarction against influenza may prevent future adverse vascular events. However, studies examining influenza vaccination and vascular events have shown conflicting results.

c. Relevance:

If influenza vaccine is shown to reduce adverse cardiovascular events, it will represent an important change in how prevention of adverse cardiovascular events is thought about.

Hypothesis (if any):
Influenza vaccination is associated with reduced incidences of adverse cardiovascular events.

Objectives:

1. To asssess whether influenza vaccine can reduce adverse vascular events among patients aged ≥18 years with myocardial infarction or patients aged ≥75 years with stable coronary heart disease.

Methods:
The current protocol will be part of a multi-country network study. We propose to implement this study at National Institute of Cardiovascular Diseases (NICVD) in Dhaka, National Heart Foundation hospital and Ibrahim Cardiac Hospital, Bangladesh. This will be a randomized, placebo controlled study where 1000 participants will be enrolled and randomized to either influenza vaccine or normal saline placebo, either of which they will receive annually and followed up for 12 months since enrollment. These data will be obtained from telephone interviews and hospital records (prescription notes/discharge certificate notes) review by quarterly household visits.

Outcome measures/variables:

Primary endoint

1. Time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring)

Secondary endpoints

1. Time to future adverse cardiovascular event till 1 year.
2. Length of hospital stay.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis:☐ No☒ Yes (describe below)

Influenza vaccination is associated with reduced incidences of adverse cardiovascular events

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

1. To asssess whether influenza vaccine can reduce adverse vascular events among patients aged ≥18 years with myocardial infarction and patients aged ≥75 years with stable coronary heart disease.

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Cardiovascular disease is a leading cause of death globally estimated to be responsible for ~ 17 million deaths annually.(1) Heart disease and stroke account for nearly one third of all deaths and are a major cause of hospitalization.(2-8)Observational studies have established an association between influenza infection and major adverse vascular events. Mechanisms that have been postulated to explain this increased risk include the precipitation of plaque rupture(9),endothelial dysfunction(10, 11),reactivation of other latent infections leading to plaque rupture(12),fever-associated tachycardia(13),and metabolic derangements related to infection, including elevation of triglycerides and serum glucose levels(14, 15). It follows that vaccinating such a high risk group as patients with acute Myocardial infarction against influenza may prevent adverse vascular events. Studies examining influenza vaccination and vascular events however have shown conflicting results(16-27).As we describe below, we recently conducted an observational study using databases from two large clinical trials (27),indicating that influenza vaccination may be associated with a reduction of major adverse vascular events; however, because of the strong possibility of bias, these results need to be rigorously confirmed in a prospective, randomized trial. Therefore, while national guidelines endorse influenza vaccination for patients with chronic cardiac disease, actual vaccination rates remain low, and importantly, these guidelines are largely based on observational data and expert opinion, with data lacking from adequately powered, prospective, randomized trials. Clinical equipoise exists as to whether influenza vaccine in fact prevents recurrent cardiovascular events in patients with acute Myocardial infarction. Consequently, a randomized controlled trial is needed to address the question. Adverse vascular events are a global threat to health and have an enormous impact in low to middle income countries.

Using a large clinical database consisting of prospectively collected data from the ONTARGET and TRANSCEND randomized controlled trials (these include most INTER-CHF sites), we performed an analysis to determine the association between influenza vaccination and major adverse vascular events(27).Annual immunization status with trivalent influenza vaccine was determined using a self-reported questionnaire at the study enrolment visit, 2-year follow-up visit and end of study visit. There was an associated reduced risk of the primary outcome in the influenza vaccinated group when the influenza virus matched the vaccine antigen well (2004-2005 (OR 0.62, 95% CI 0.50 – 0.77), 2005-2006 (OR 0.69, 95% CI 0.53 – 0.91) and 2006-2007 (OR 0.52 95% CI 0.42 – 0.65)), but there was no association in 2003- 2004 when there was an incomplete vaccine antigen match with the circulating influenza virus (odds ratio 0.96 95% CI 0.73-1.27). The summary OR for the 4 adjusted OR from the influenza seasons was 0.65 (95% CI 0.58 – 0.74, $p<0.001$), and there was statistically

significant heterogeneity (p=0.003). Although our findings suggest an association, residual confounding cannot be excluded and it remains uncertain as to whether influenza vaccination can reduce major adverse vascular events.

There have been 3 systematic reviews of the effect of influenza vaccination on major adverse vascular events(28-30).A Cochrane review summarized the results of 2 small randomized controlled trials.(28) One of these trials, the FLUVAC study, although reported as one study, consisted of two randomized controlled trials(31),one of which randomized 200 patients with acute MI to influenza vaccine or placebo (FLUVAC MI) and the second randomized 102 patients planned to have PCI (FLUVAC PCI).The FLUCAD study was a randomized double blinded placebo controlled single centre trial where 658 patients with coronary disease were randomized to influenza vaccine or placebo. The pooled RR for cardiovascular death in these studies was 0.39 (95%CI 0.20 to 0.77) and meta- analysis led to a pooled RR of 0.85 (95% CI 0.44 to 1.62) for MI. A recent trial randomized patients with a history of ACS to either influenza vaccination or no treatment(32).Twenty-one of 221 patients in the vaccine group compared to 42 of 218 in the comparison group had major adverse vascular events (unadjusted HR 0.70; 95% CI, 0.57to 0.86). Although these findings suggest an effect, the fact that the trial was open label may have introduced bias, and an accompanying editorial to this study stressed the need for a large simple randomized controlled trial of influenza vaccination. Two trials of participants that were not enrolled on the basis of cardiovascular illness and reported a risk ratio of 0.64 (0.48 to 0.86) for adverse cardiovascular events and a risk ratio of 0.81 (95% CI 0.36 to 1.83) for cardiovascular death. The conclusion of the authors in the latest systematic review was that "A large, adequately powered, multicenter trial is warranted to address these findings and assess individual cardiovascular end points"(30).

There is uncertainty in the medical community about the benefit of influenza vaccination to prevent adverse vascular events. Observational studies cannot definitely address the question because of confounding. There is also uncertainty about the non-cardiovascular benefits of the vaccine. A recent Cochrane review of influenza vaccine in persons > 65 years, pooled 3 RCTs (2217 participants, including those from a nursing home and a psychiatric hospital) found a RR of 0. 42 (0.27, 0.66) for preventing influenza but complications were not assessed(28).However, in observational studies, vaccines were ineffective in the prevention of influenza, RR 0.19 (0.02, 2.01), influenza-like illness, RR 0.75 (0.42, 1.33), pneumonia, RR 0.88 (0.64, 1.20), or hospital admissions or deaths from any respiratory disease, RR0.88 (0.54, 1.43). Selection bias because of differential vaccine uptake has been extensively cited as the most likely explanation for these counter-intuitive results (i.e. lack of effect on influenza, influenza-like illness, pneumonia, respiratory hospital admissions but prevention of influenza and pneumonia hospitalizations and death). In fact, the reduction in death from all causes far exceeds the estimated impact of influenza vaccine on winter season mortality of 5% in an average season. A report of reduced influenza/pneumonia hospitalization (RR 0.72; 0.59, 0.89) and reduced all cause death (RR 0.39; 0.33, 0.47) when seniors were vaccinated and outcomes assessed prior to influenza season offers evidence of selection bias due to preferential vaccination of healthy seniors. Seniors with greater frailty (i.e. at increased risk for hospitalization and death) were shown to be less likely to receive influenza vaccination. The authors of the Cochrane review conclude that "to resolve the uncertainty of the role of vaccines, an adequately powered, publicly-funded, high quality placebo-controlled trial run over several seasons should be undertaken"(28).Given broad consensus among experts in the field that clinical equipoise exists on the effect of influenza vaccine in CHF patients, a large, prospective randomized trial is delivery.

Purpose of the study

The primary objective is to study the effect of influenza vaccine (Vaxigrip Tetra, Sanofi Pasteur Europe) compared to placebo, on major adverse cardiac events i.e. all-cause death, myocardial infarction and stent thrombosis (first occurring) till 1 year in patients.

Secondary objectives are time to all-cause death, time to cardiovascular death, time to stent thrombosis, time to revascularization, time to myocardial infarction, time to stroke or time to rehospitalization for heart failure till 1 year. Also, length of hospital stay is a secondary objective.

Rationale

In this trial we test the hypothesis that influenza vaccination is superior to no influenza vaccination in reducing time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) at one year in patients with STEMI or NSTEMI (primary end point). Secondary endpoints are each of the endpoints in the composite

primary endpoint evaluated separately and time to revascularization, stroke, rehospitalization for heart failure and length of hospital stay.

Clinical relevance
STEMI and NSTEMI remain two of the leading causes of death globally. Thrombolysis was a major step forward in the treatment of STEMI [27-29] and further progress was made when primary PCI was established as a golden therapeutic standard [30] as was the case for NSTEMI [31]. Treatment has been further optimized with pre, peri- and post procedure platelet inhibition, statins, angiotensin converting enzyme inhibitors and beta adrenoreceptor blockade. Despite these improvements in care, cardiovascular disease is the leading cause of death globally. Thus, a simple, cheap treatment to prevent recurrent cardiovascular events is highly warranted.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving thespecific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

Study design
This is a multi-centre (in multiple centers of multiple countries), randomized, placebo controlled, trial. Participants aged ≥18 years with myocardial infarction and participants aged ≥75 years with stable coronary heart disease will be randomized to inactivated influenza vaccine (Vaxigrip Tetra, Sanofi Pasteur Europe) or placebo and followed prospectively for 12 months. Bangladesh will be the 8th country to participate in this multi-center study. Same study desing will be followed for this study in Bangladesh.

Patients
A total of 1000 patients (500 vaccinated with inactivated influenza vaccine and 500 with placebo) will be included in the study.

Patient inclusion
Individuals for inclusion will be recruited among the patients hospitalized with myocardial infarction or stable coronary artery disease and an increased risk of future cardiovascular events (Figure 1). Patients will be recruited during the influenza season only (from April to October in Bangladesh). The patients will not receive any honorarium for participation.

Inclusion criteria
- Patients hospitalized with a diagnosis of STEMI as defined by chest pain suggestive for myocardial ischemia for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours, and an ECG with new ST-segment elevation in two or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads or a probable new-onset left bundle branch block.

Or:
- Patients hospitalized with a diagnosis of NSTEMI defined by a combination of: onset of symptoms such as central chest pain or an aggravated angina pectoris, with or without an ECG change with ST-segment lowering or an inverted T-wave, and at least two values with levels of troponin-T or troponin-I above the established margin of an AMI.

Or:
- Patients hospitalized with a diagnosis of stable coronary artery disease ≥75 years of age AND at least one additional risk criterion - previous myocardial infarction, previous PCI, previous CABG, diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40.

And:
- Male/female transgender subjects ≥18 years.
- Provided written informed consent.

Exclusion criteria

- Influenza vaccination for current season or the subject anticipating to be vaccinated during the current influenza season
- Indication for influenza vaccination for some indication other than Myocardial Infarction
- Severe allergy to eggs or previous allergic reaction to influence vaccine.
- Suspicion of febrile illness or acute, ongoing infection.
- Hypersensitivity to the active substances or ingredients of Vaxigrip or against any residues, such as eggs (ovalbumin or chicken proteins), neomycin, formaldehyde and octoxinol.
- Subjects with endogenic or iatrogenic immunosuppression that may result in reduced immunisation response.
- Inability to provide informed consent.
- Age below 18 years.
- Previous randomization in the IAMI trial.

IAMI trial flow chart

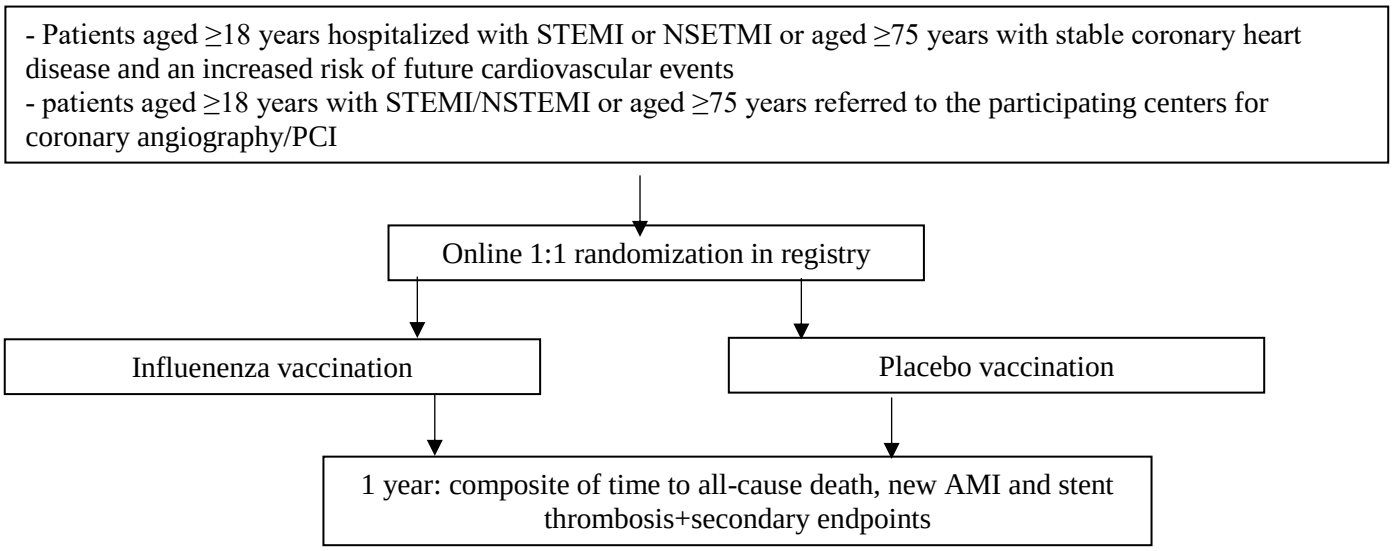


Figure 1: flow chart of study design

At 7 days after the vaccination patients will be requested to complete standard questionnaire to asses if any adverse event has occurred following vaccination by study researchers either during hospital stay or by household visits.

Follow up of primary and secondary endpoints will also be performed by telephone contacts with the patients or first-degree relatives by a medical officer/researcher phone call after 350±10 days. The study researchers will also accumulate hospital record information on these endpoints. A central adjudication will be performed for all reported primary endpoints for the 350 days follow up. Every site will prepare source documents for the event for central adjudication by an independent committee.

Treatment strategies

Influenza vaccination and placebo
Following informed consent, the patient will be randomized in the SCAAR database over realtimethorugh internet connection as local site. An unblinded study nurse at each center, not otherwise involved or participating in the study, will prepare the study medication (VaxigripTetra/placebo). VaxigripTetra (appendix VIII) will be delivered to each participating center by the pharmaceutical distributor in Bangladesh. Placebo will be obtained from each center's ordinary medical supply.

According to randomization, VaxigripTetra is administered in a pre-filled syringe or the same volume of placebo (0.5 ml 0.9% Sodium Chloride, normal saline) is drawn up in a small syringe just before the vaccination. A list of information regarding what has been given to each patient (VaxigripTetra/placebo) and when (date and time) will be prepared, signed and kept by the unblinded study nurse who is not otherwise involved or participating in this study. To ascertain blinding, the physician can lay a piece of foil around the syringe to ensure that the patient cannot see what is administered during the vaccination. The influenza vaccination, or placebo, is given as a deep subcutaneous injection up to 72 hours of hospital admission following NSTEMI and STEMI patients, Figure 2. Patients will be observed for 20 minutes after vaccination/placebo to monitor, and potentially treat, side effects. This strategy is chosen to optimize compliance with randomization and ensure simplicity. According to ICH GCP 4.3.1 A senior local cardiac consultant and a mid level cardiac consultant will be assigned at the local hospital and together with the local study investigator both will be responsible for all medical decisions regarding the study. Thus, if deemed necessary for serious and unexpected adverse experiences that are associated with the use of the drug the investigator will be able to unblind the study drug immediately, without restrictions and without prior contact to the sponsor or the monitor.

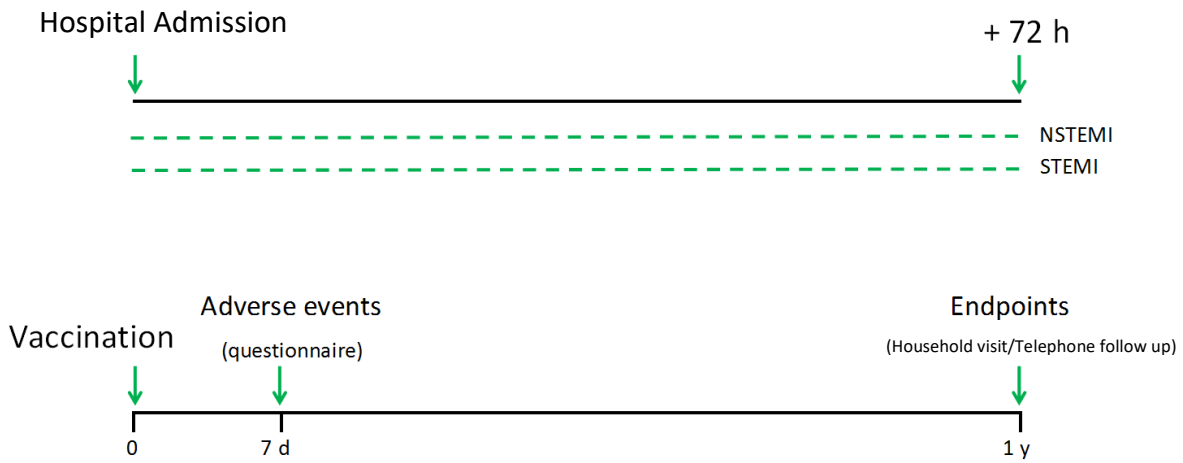


Figure 2. *Timing of vaccination (upper panel, dotted line) and follow-up (fu) till 12 months (lower panel).*

The chosen type of influenza vaccination (VaxigripTetra, Sanofi Pasteur Europe - suspension for injection in pre-filled syringe) may, in contrast to other vaccines given via the intramuscular route, be administered as a deep subcutaneous injection and is chosen to minimize the risk of bleeding. For patients in the placebo group, 0.9% sodium chloride (normal saline) will be used.

VaxigripTetra (appendix VIII) will be labeled for the study by Sanofi Pasteur. Each center will order VaxigripTetra from local Sanofi Bangladesh office and the vaccine will be delivered to the centers as a refrigerated temperature-controlled transport (+2 to +8°C). Placebo will be ordered according to each participating unit ordinary requisition routines and will not be marked with any study specific information.

Endpoints
Primary endpoint

- The primary endpoint is time to all-cause death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year. These data will be obtained by following up patiently actively quarterly over one-year time and review medical records (prescription notes/discharge certificate notes) received as scanned images/pictures by patients' attendants or if needed by household visits. All primary endpoints up to 350 days will be adjudicated by a central adjudication committee.

Secondary endpoints
- Time to all-cause death till 1 year.
- Time to cardiovascular death till 1 year.
- Time to stent thrombosis till 1 year.
- Time to revascularization till 1 year.
- Time to myocardial infarction till 1 year.
- Time to cardiovascular death, a new myocardial infarction or stent thrombosis (first occurring) till 1 year.
- Time to stroke, including transient ischemic attack (TIA) till 1 year.
- Time to hospitalization for heart failure.
- Length of hospital stay.

Because influenza may precipitate plaque rupture [11] it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques [33].

Endpoint definition
Death: All reasons for death, i.e. cardiac, non-cardiac or unknown. Myocardial infarction: ICD codes I21, I21.4 and I22, heart failure as I50 and stroke as I63.9. New PCIs and stent thrombosis as reported during following up. All endpoints will be adjudicated according to a separate Adjudication Charter.

Statistics and data management

The data will be passed on from the participating centers to Örebro University Hospital where data management work and statistical analyses will be performed in collaboration with the accredited Swedish clinical research organization, Lytics, which will be in charge of external web-randomization (http://lytics.ai/company). A copy of the data will also be saved in icddr,b server.

Data analysis
The results will be analyzed according to the intention-to-treat principle. Differences between groups in time-to-event endpoints will be assessed with the log-rank test. For the primary endpoint, patients will be censored at 1 year; analyses at other time points will be handled in a similar way. Survival probabilities will be displayed and calculated using Kaplan-Meier methodology.

Hazard ratios (HR) with 95% confidence intervals between study groups will be calculated using Cox proportional hazard model, if violation to proportional hazard assumption time-dependent HR will be calculated and adjustment will be made for stratification variables, center and STEMI/NSTEMI.

Differences between study groups will be assessed with unpaired t-tests on original scale or log scale as appropriate. Ordinal variables will be assessed with chi-2 test for trend or Mann-Whitney U test and Pearson's chi-square test or Fisher's exact test will be used to test differences between proportions. Two-sided statistical significance levels of 5% will be used and estimates will be presented with 95% confidence intervals.

Subgroup analyses will first and foremost be carried out for the primary endpoint and its components. All subgroup analyses of event data will be performed using a proportional hazards model with factors treatment, subgroup, and treatment-subgroup interaction, and will be presented with within-group hazard ratios with 95% confidence intervals and the interaction p-value. The primary subgroup analyses will focus on the STEMI and NSTEMI populations and the effect of intervention in each of the three influenza seasons, with the purpose of evaluating effect in each subgroup.

Interim Safety Analysis
A maximum of 3 months following inclusion of the first 100 patients an independent endpoint committee (IEC) will monitor study endpoints. Variables to be assessed are all-cause death, a new myocardial infarction and stent thrombosis.
Premature termination of the study will be mandated in the event that one of the treatment strategies shows statistically significance at the 0.001 alpha level for the composite of time to all-cause death, a new myocardial infarction or stent thrombosis.

Analysis population
The results will be analyzed according to the intention-to-treat principle, i.e. patients randomized to a certain group will be followed and assessed irrespectively of the actual treatment. Protocol violations will be monitored continuously and the responsible centers notified. Data collected during the study will be coded so that no subjects can be identified.

Sample size calculations
Sample size is calculated on the basis of three smaller randomized studies [8-10], demographic data from annual SCAAR reports (accessible at http://www.ucr.uu.se/swedeheart/) and from the TASTE tria`l in which the number of high risk patients included was lower than expected [25].
The combined 1-year primary endpoint of all-cause death, a new AMI or stent thrombosis is estimated at 10.0% (expected survival probability of 0.9) for individuals randomized to placebo.
For patients with stable coronary artery disease an analysis of data from SCAAR on 11761 individuals identified a subgroup of patients with approximately the same 1-year risk of cardiovascular events as for patients with NSTEMI or STEMI. After applying enrichment criteria for risk individuals ≥75 years of age with at least one additional risk criterion - previous myocardial infarction, previous PCI, previous CABG, diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40 ml/min the risk for the primary composite endpoint of this study was calculated to be on par with patients having a myocardial infarction.

With a 5% two-sided significance level we calculated that 386 events would be needed to have a 80% statistical power to detect a 25% reduction of the primary endpoint in the influenza vaccination group, corresponding to a hazard ratio of 0.75 [34]. With this estimation 2186 patients are needed per study group, power calculation utilized with STATA release 11 (College Station, TX, USA). In order to control for dropouts and crossing from one group to the other (both were negligible in TASTE), 4400 patients will be included.
For Bangladesh, We assume that 20% MI cases have future cardiovascular events, therefore, having 95% confidence interval, 10% non response rate with a design effect of 2 to detect a 10% reduction with 80% power would be needed 500 patients for each group. Hence we expect to enroll 1000 patients in two infleunza season of years 2019 and 2020.

Randomization procedure
An external web-page for randomization coupled to relevant national registries in the participating countries will be constructed (Figure 3). Following written informed consent randomization is stratified by center with a 1:1 allocation within each stratum using predefined block sizes. Block randomization is by a computer-generated random number list prepared by Lytics, the clinical research organization in charge of external web-randomization (http://lytics.ai/company). The patient, investigators and all other medical staff are kept blinded to the allocation.

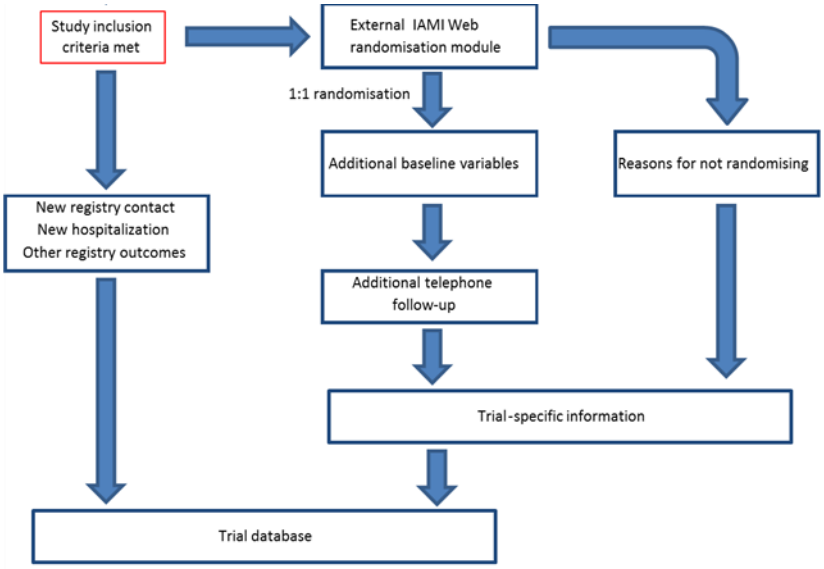


Figure 3. External Web-based randomization and relation to a national clinical registry.

Study sites in Bangladesh:
Bangladesh will be one of the participating sites in this multi-site study. Currently, the study is ongoing in 7 countries (Appendix VI). If engaged, then Bangladesh will be the 8th country joining in this collaborative international network study. In Bangladesh the study will be implemented at Naitonal Institute of Cardiovascular Diseases (NICVD) in Dhaka, National Heart Foundation hospital (NHFH) and Ibrahim Cradiac hospital (ICH) Dhaka. NICVD, NHFH and ICH are three of the largest tartiary care hospitals in Dhaka managaing patients with cardiovascular disorders from Dhaka and also from surrouding sub-urban and rural areas. A senior cardiac consultant and a mid level cardiac consultant will be involved and a medical officer will be deployed at the public cardiac hospital, and one medical officer will work on the remaining two private cardiac hospitals. There will be researchers deployed in the hospital setting for following up of the patients and perform household visit for documenting medical records (prescription notes/discharge certificate notes), if available.

What are the planned trial interventions?

Experimental (inactivated influenza vaccine or IIV):

Participants at high risk for adverse vascular events will be immunized with inactivated trivalent influenza vaccine (VAXIGRIP® vaccine) recommended for the influenza season. A 0.5 ml dose of the vaccine will be administered intramuscularly annually.

Control (Saline):

Participants at high risk for adverse vascular events will be immunized with sterile normal saline (0.9% sodium chloride). A 0.5 ml dose will be administered intramuscularly annually.

Safety assessments
Safety parameters

The following listed safety parameters will be monitored during the study treatment administration: Vital signs, allergic reactions, bleeding, arrhythmia and consciousness. If indicated, basic blood chemistry analyses and blood gases will be examined.

Adverse Events – AE

Registration of adverse events will start after informed consent and when treatment with study medication has been given and continue until the patient leaves the hospital up to a minimum of 7 days following influenza vaccination. The same time limit will be used in both treatment groups. The patients will be informed to contact the investigator or study nurse if any adverse event should occur during this timeframe. At 7 days after the vaccination patients will be requested to return a postage paid standard questionnaire to asses if any adverse event has occurred following vaccination.

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal investigational product.

Medical occurrences that are symptoms of existing disease, and that do represent an exacerbation of that disease, or the PCI procedure are not defined as AE's in this clinical trial. Also, elective hospitalisations for pre-treatment conditions are not AE's nor expected reactions to vaccination: redness, swelling, pain, fever and chills. AEs not to be reported are also those defined as study endpoints. IEC will evaluate for safety after 100 patients.

Serious Adverse Event – SAE

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing inpatients' hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect,
- other important medical event

Hospitalisation or prolongation for existing inpatient hospitalisation disease and that do represent an exacerbation of that disease and the coronary angiography/PCI procedure as well as other events non-related to the study medication will not be reported as an SAE.

Suspected Unexpected Serious Adverse Reaction – SUSAR

All serious adverse events (SAE) must be evaluated unexpected and drug related or not. The definition of an unexpected adverse reaction is an adverse event, which has not been documented or reported earlier.

If the responsible investigator judges the SAE as being drug related and unexpected it must be promptly reported to the sponsor, who is responsible for reporting SUSARs to the Regulatory Authorities and the Ethics Committee. Whether the reaction is expected or not will be assessed against the SPC.

Definitions of severity and relationship

Assessment of severity

For all adverse events, serious as well as non-serious, the investigator must assess severity. Relationship should be classified according to the following definitions.

- **Mild**: Awareness of sign or symptom, but easily tolerated and cause no interference with daily activities.

- **Moderate**: Discomfort enough to cause interference with daily activities.
- **Severe:** Inability to perform normal daily activities.

Relationship to study drug

The investigator will judge whether or not, in his/her opinion, the adverse event is associated with the study treatment. Relationship should be classified according to the following definitions:

Probable: An adverse event, which might be due to the use of the drug. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely, e.g. concomitant drug(s), concomitant disease(s).

Possible: An adverse event, which might be due to the use of the drug. An alternative explanation, e.g. concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Unlikely: An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Reporting procedures for Adverse Events and Serious Adverse Events

Only adverse events and serious adverse events that are not considered as signs and symptoms expected and related to STEMI or NSTEMI or known side effects from the study drug will be reported in this study. Events defined as endpoints in the study (e.g. all-cause death, a new myocardial infarction or stent thrombosis) will not be reported as adverse events. This means that other clinical signs and symptoms, which are reported by the patient and observed by the investigator, and in the opinion of the investigator are unexpected in relation to actual diagnosis, will be reported to a minimum 7 days post vaccination**.**

SUSAR reporting procedure

If the responsible investigator judges the SAE as being drug-related and unexpected the event must be reported to the sponsor within one working day. The documentation will be on a CIOMS form (http://www.cioms.ch/index.php/cioms-form-i). The sponsor is then responsible for reporting SUSAR to the regulatory authorities and ethics committee. The sponsor is also responsible for information to all involved investigators in the study.

- A SUSAR resulting in death or judged as life threatening must be reported to regulatory authorities and the ethics committee within 7 days after the sponsor has been notified about the event. A full report has to be sent to the authorities within 15 days.
- A SUSAR which is not resulting in death or is life threatening has to be reported to regulatory authorities and ethics committee within 15 days after the sponsor has been notified about the event. A full report has to be sent to the authorities as soon as possible.

Annual report

A safety report, including assessment of overall safety and all reported SUSARs will be submitted yearly to the Regulatory Authorities and if requested to the Ethics Committee.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

This committee will be responsible for safety oversight of the study (including monitoring of adverse reactions). The DSMB (Data Safety Monitoring Board), composed of cardiologists, an infectious disease physician, vaccine trial expert and an epidemiologist will be responsible for making recommendations on safety issues, premature trial termination, and unblinding of study groups. In case of any adverse event, this team will respond within 24 hours to address the adverse event. The DSMB, which will be blinded to study group, will be asked to review safety data on an annual basis for each arm of the study. If safety concerns arise, more frequent meetings will be initiated. The DSMB will receive immediate notification and reports of serious adverse reactions.
The following members are nominated for the DSMB:

1. **Prof. Dr. M. Atahar Ali** MD, FCPS, Professor Department of Cardiology, NICVD
2. **Dr. Mushtuk Husain**, Former Principal Scientific Officer, IEDCR
3. **Dr. Khaleda Islam**, Former Director Primary Health Care & Program Manager
4. **Dr. Ashraful Islam Khan**, Scientist, IDD, icddr,b

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The current protocol will attempt to determine if influenza vaccine may reduce frequency of adverse vascular events among patients with heart failure. The will be implemented National Institute of Cardiovascular Institute (NICVD), National Heart Foundation hospital (NHFH) and Ibrahim Cradiac hospital (ICH) Dhakawhich are tertiary care cardiac hospitals in Dhaka. The study activities will include collection of sociodemographic as well as clinical data and adminitering the enrolled patients with either influenza vaccine or normal saline placebo. This work will not need to collection any biological samples from the study participants.

This project will be entirely based within hospital settings.

Sources of Material

After enrollment and taking consent, the study physician along with a cardiologist will randomize the patient into either the influenza vaccine group or the normal saline placebo group. Then the study physician along with the cardiologist will abstract clinical information by interviewing and clinically examining the patient as well as reviewing the patient's current and past medical records (prescription notes/discharge certificate notes), if available. The physicians may need to interview the patient's attendents to obtain the required information. All of the information will be recorded into a structured questionnaire electronically. The informaiton of the participant will be recorded agianst a unique identification number. Only the study staff will have access to these records. The data will be entered into a password protected handheld computers.

Potential Risks

The major risk of influenza vaccine, although rare (1 per 200,000 doses), is an anaphylactic reaction, characterized by hives, swelling of mouth and throat, difficulty breathing, and low blood pressure. Such a rare event occurs immediately after injection. After administration of study medication patients will be observed by trained health care professionals (both from study site hospital and also icddr,b) for a minimum of 15 minutes. Patients will be observed for the following symptoms and signs: skin reactions such as hives, flushed skin, or paleness, suddenly feeling warm, difficulty of swallowing, nausea, vomiting, or diarrhoea, abdominal pain, low blood pressure and tachycardia, runny nose and sneezing, swollen tongue or lips and wheezing or difficulty breathing. Relevant precautions for treating anaphylactic reactions (epinephrine, CPR equipment) will be at hand since the study sites are tertiary level hospitals and have adequate facilities to address such immediate adverse event.
Less than one third of individuals receiving study vaccine may experience some soreness or redness at the site for 1-2 days. Fever, malaise, nausea, loss of appetite, muscle aches occur infrequently and may last 1-2 days. It is unclear whether Guillain-Barré syndrome is associated with influenza vaccination, however it has been estimated that if there was such an association, it would be at a rate of 1 or 2 incremental episodes of Guillain-Barré syndrome per million doses of influenza vaccine given. There is no evidence that receiving two influenza vaccines (i.e. for participants who are randomized to influenza vaccine and also receive a vaccine outside of the trial) within a given season leads to any additional adverse events other than those described above. In fact, children under the age of 9 years are routinely immunized with two doses of influenza vaccine four weeks apart to increase protection against influenza(33).

Recruitment and informed consent

The study physician/cardiologist will approach the potential case-patient to informhim/her about the objectives of the study, study procedures, risks involved and invite him/her to participate. The patient will be enrolled into this study only after s/he has provided written informed consent.

Protection against risk

We will explain to participants and their families the objectives, study procedures and the risks involved in this study. The patient will be enrolled only after provision of written informed consent. Risks to violation of confidentiality will be minimized restricting the access to the electronic database toeverone except the study staff. De-identified data will be prepared before sharing then with any researchers outside the circle of the study co-investigators. All patients involved in the study will be receiving routine care through the normal mechanism during their hospital stay.

Potential benefits

The benefit to the participant through enrolment is that, the study physicians will take additional clinical history and do additional clinical examination. Moreover, past and present medical records (prescription notes/discharge certificate notes) of the participants, if available will be reviewed. All these clinical activities for the participants will be repeated during the subsequent follow-up visits. This increased medical attention may result in improved clinical care.

The benefit to society is a better understanding of the efficacy of infuenza vaccine for reduction of adverse vascular events among high risk patients. The information gained from the study findings would help the policy makers of the country to assess if the governement should increase its effort such as increased finance to campaign for influenza vaccination among high risk population to reduce the burden of adverse vascular events.

The risks are minimal and the benefits are substantial, thus the activity does not put too onerous a burden on the study subjects.

Importance of the knowledge to be gained

This proposed randomized trial has important implications for the management of patients at high risk for major adverse vascular events. We anticipate that such a trial would influence management decisions by physicians for patients at high risk for major vascular events. If influenza vaccine is shown to reduce adverse vascular events, it will represent an important change in how prevention of adverse vascular events is thought about.

Inclusion of women and minorities

Patients will be enrolled based upon their meeting the eligibility criteria. Sex/gender and racial/ethnic group will not be a criterion for enrolment. However, since males are more likely to access the formal health care system than females in Bangladesh, we expect to enrol more males in the hospital-based study than females.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable.

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

Funding will be made by Sanofi Pasteur through Örebro University Hospital, Sweden. This study will be jointly implemented by icddr,b and Örebro University Hospital, Sweden in the participating hospitals. A MOU will be signed between the two institution and also between icddr,b and participating hospitals.
In the participating hospitals, there will be a senior cardiologist, a mid-level cardiologist and we will assign a medical officer and research assisstants for receuirment and following up. An honorarium for the senior and mid-level cardiologists will be provided during the study period.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

The participating hospitals:

The present protocol will be implemented in National institute of Cardiovascular diseases (NICVD), National Heart Foundation hospital (NHFH) and Ibrahim Cradiac hospital (ICH) Dhaka which are the largest tertiary care cardiac hospital of Bangladesh located in urban Dhaka. The range of cardiovascular diseases managed include Acute myocardial infarction, Hypertensive heart disease, Multiple valve disease, Congenital malformations of cardiac chambers and connections, Heart failure, Atherosclerosis, Acute and subacute infective endocarditis, Left ventricular failure etc. The hospitals have both outpatient and inpatient units.

The hospital selected is as follows:

Sl.	Hospitals	District	Administration
1	National Institue of Cardiovascular Diseases (NICVD)	Dhaka	Governement
2	National Heart Foundation Hospital and Research Institute	Dhaka	Private
3	Ibrahim Cardiac Hospital and Research Institute	Dhaka	Private

Data management: A study data base with all patients included in the study will be generated based on the ordinary national registry process and a study specific randomization module. An electronic case report form (CRF) will be generated automatically based on the ordinary registration form and stored at Lytics for each patient included. The patient's identity will always be confidential. A copy of the data will be stored with the local PI in icddr,b server
The investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the CRFs. All data from the 7 day follow up questionnaire and telephone calls/household visits after 350±10 days and SUSARs will be imported into the study data base.

Quality Control and assurance: The quality control process has been integrated into the overall data management process. Quality assurance or audit process will be performed by staff at the coordinating centre. A sample of participant records (10%) will be audited quarterly using our participant record audit tool. This retrospective review will focus on the following indicators: consent forms; eligibility; vaccine administration and reactogenicity; adverse event/serious adverse event reporting; study endpoints; missed vaccinations and blood draws; signatures, as required; and study discontinuation. Regulatory records will be audited annually using a regulatory file audit tool. The following indicators will be included during this process: ethics approvals, safety reports, protocol and consent, sample CRFs, and monitoring reports.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

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Budget

Name of the Project/Protocol: Effect of influenza vaccination on recurrent cardiovascular events among Myocardial Infarction (MI) patients in Bangladesh: A multicounty, randomized clinical trial

Budget Period: April 01, 2019 to September 30, 2021

Particulars	Pay level	Month Rate (S)	# of Staff	% Time	No. of Month			Inflation %		2019	2020	2021	Total Cost US$
			2019	2019	2019	2020	2021	2020	2021				
Personnel:													
Sr.RI, Dr. Aleem	NOB/2	2,551	1	20%	9	12	9	1.10	1.21	4,592	6,735	5,556	16,883
Sr.RI, Dr. Zubair	NOB/2	2,551	1	50%	9	12	9	1.10	1.21	11,480	16,837	13,890	42,206
Medical Officer, CSA (TBD)	NOA/1	1,346	2	100%	9	12	9	1.10	1.21	24,228	35,534	29,316	89,078
FRA, CSA (TBD)	GS3/2	434	2	100%	9	12	9	1.10	1.21	7,812	11,458	9,453	28,722
PMC Mr. Mahbub	NOB/8	3,188	1	20%	9	12	9	1.10	1.21	5,738	6,312	5,208	17,258
Sr. Admin Officer, Mr. Mustak	GS6/8	1,545	1	20%	9	12	9	1.10	1.21	2,781	4,079	3,365	10,225
										-	-	-	-
Subtotal										**56,631**	**80,954**	**66,787**	**204,372**
Consultant:		**$ Rate**	**No.**										
Local Consultant		$ 3,500	$ 1							3,500	3,500	3,500	10,500
Subtotal										**3,500**	**3,500**	**3,500**	**10,500**
Travel and Perdiem		**$ Rate**	**No.**										
Local Transport including hiring		500	1							500	1,200	400	2,100
ICDDR,B Transport		500	1							500	2,000	200	2,700
Perdiem and Lodging		1,000	1							1,000	800	800	2,600
International Travel with perdiem		5,000	1							5,000	5,000	5,000	15,000
Subtotal										**7,000**	**9,000**	**6,400**	**22,400**
Supplies & Materials (Stock/Non Stock)		**$ Rate**	**No.**										
Office Supplies Stock/Non Stock		60	4							240	300	360	900
Vaccination, cold chain logistics & technical s		200	1							200	200	200	600
Subtotal										**440**	**500**	**560**	**1,500**
Others		**$ Rate**	**No.**										
Staff Development Training (Int. and local)		$ 300	$ 1							300	330	370	1,000
Workshop / Seminar (Int. and local)		$ 1,000	$ 1							500	500	560	1,560
Other services, Stipend / Labor charge		$ 100	$ 1							100	110	123	333
Communication(Fax, Phone bill, Courier, pos		$ 410	$ 1							410	451	584	1,445
Printing and photocopy		$ 200	$ 1							200	220	247	667
General service and utility maintenance		$ 400	$ 1							400	400	400	1,200
Publication charge (Intl)		$ 457	$ 1							457	503	564	1,523
Subtotal										**2,367**	**2,514**	**2,847**	**7,728**
Equipment		**$ Rate**	**No.**										
Tabs		$ 500	$ 6							3,000	-	-	3,000
Refriegrator		$ 1,200	$ 1							1,200	-	-	1,200
Subtotal										**4,200**	**-**	**-**	**4,200**
Total direct cost										**74,138**	**96,468**	**80,095**	**250,700**
Indirect Cost 20%										13,988	19,294	16,019	49,300
Total Budget										**88,125**	**115,762**	**96,114**	**300,000**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item, including the use of human resources, major equipment, and laboratory services.

In total $300,000 will be incurred to run the study acitvities. The justification for direct cost is described as below:

Personnel

The PI will oversee the study dedicating 50% of his time. One full time medical officer will be placed at NICVD and another medical officer will be work in both the private hospitals in recruiting and administering vaccines/placebpo to pateints. The field research assisstants will help the medical officer providing logistic support and also help in following up of the patients. For admin activities, A project manager and an admis officer will contribute 20% of their time.

Six **cardiologists study coordinator** (One senior and one mid-level cardiologists from each site: NICVD, NHF&RI and ICH&RI) will perform to oversee the implementation of the study activities according to the study protocol and coordination of the acitivities. The **Director** of each hospitals will assign duties to the implementing

study personnel at their respective hospitals and he will play a supervisory role. He will be the point of contact to resolve administrative issues.

Travel
The investigators may need to travel to the patients' home for follow-up and data collection which may be outide Dhaka and also there will be at least one international travel for the PI/Co-PI of the study during the study peroid.

Supplies
All necessary supplies will be provided in each hospital settings and the vaccines and placenbo will be provided by the local office of Sanofi Pastuer, Bangladesh. Other costs will include that for staff development, training, workshop, services, communication, printing, photocopying etc. In addition cost will be incurred to pay the cardiologists (Director, a mid-level cardiologist and a senior cardiologist) of sites involved in this study.

Equipment:
The study will need 6 tabs for data collection for date collection, storage and transfer. A refrigerator will be needed to store vaccines.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration.

None

Biography of the Principal Investigator (Internal):

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name: Abu Muhammad Zubair Akhtar**

2. **Present Position:** Senior Research Investigator

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
MPH	James P Grant School of Public Health, BracUnversity	2013
BDS	Bangladesh Dental College, Dhaka University	2005

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**1338175**	**Taken on 12/03/2013**

Note: If the response is “no”, please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-18002	Co-PI	May 2018	April 2019	20
PR -2007-002	CO-PI	March 2007	Sep 2019	40

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	0
b. Peer reviewed articles and book chapters	0
c. Papers in conference proceedings	8
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	0
e. Working papers	2
f. Monographs	0

7. Five recent publications including publications relevant to the present research protocol

7.1.

7.2.

7.3.

7.4.

7.5.

Biography of the Co-principal investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Professor Allen G P Ross

2. **Present Position:** Senior Director, Infectious Diseases Division (IDD), icddr,b.

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
Doctorate of Science (D.Sc., Medical Sciences)	University of Queensland, Faculty of Science, Queensland, Australia	2017
Doctorate of Medicine (Ch.B., M.D., Honours)	University of Queensland, School of Medicine, Queensland, Australia	2010
Doctorate of Philosophy (Ph.D., Tropical Health, Distinction)	University of Queensland, School of Medicine, Queensland, Australia	1998
Masters of Science (M.Sc., Human Biology)	University of Guelph, Guelph, Ontario, Canada	1994
Bachelor of Science (B.Sc., Biology)	Acadia University, Wolfville, Nova Scotia, Canada	1990

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☒	Yes ☐			

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

6. Publications

Types of publications	Numbers
g. Original scientific papers in peer-review journals	104
h. Peer reviewed articles and book chapters	7
i. Papers in conference proceedings	
j. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
k. Working papers	
l. Monographs	

7. Five recent publications including publications relevant to the present research protocol

7.1 He P, Gordon CA, Williams GM, Li Y, Wang Y, Hu J, Gray DJ, **Ross AG**, Harn D, McManus DP. Real-time PCR diagnosis of Schistosoma japonicum in low transmission areas of China. Infect Dis Poverty. 2018 Jan 31;7(1):8. doi: 10.1186/s40249-018-0390-y.

7.2 Inobaya MT, Chau TN, Ng SK, MacDougall C, Olveda RM, Tallo V, Landicho JM, Malacad CM, Aligato MF, Guevarra JR, **Ross AG**. Mass drug administration and the sustainable control of schistosomiasis: community health workers are vital for global elimination efforts. Int J Int J Infect Dis. 2017 Nov 8. pii: S1201-9712(17)30280-1. doi: 10.1016/j.ijid.2017.10.023.

7.3 Weerakoon KG, Gordon CA, Williams GM, Cai P, Gobert GN,4, Olveda RM, **Ross AG**, Olveda DU, McManus DP. Droplet digital PCR diagnosis of human schistosomiasis japonica: parasite cell-free DNA detection in diverse clinical samples. J Infect Dis. 2017 Sep 27. doi: 10.1093/infdis/jix521.

7.4 Cai P, Weerakoon KG, Mu Y, Olveda DU, Piao X, Liu S, Olveda RM, Chen Q, **Ross AG**, McManus DP. A Parallel Comparison of Antigen Candidates for Development of an Optimized Serological Diagnosis of Schistosomiasis Japonica in the Philippines. EBioMedicine. 2017 Sep 18. pii: S2352-3964(17)30364-X. doi: 10.1016/j.ebiom.2017.09.011.

7.5 **Ross AG**, Papier K, Luceres-Catubig R, Chau TN, Inobaya MT, Ng S. Poverty, dietary intake intestinal parasites and nutritional status among school-age children in the rural Philippines. Trop. Med. Infect. Dis. 2017, 2, 49; doi:10.3390/tropicalmed2040049

Biography of the investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name: Professor Dr. Mahmudur Rahman**

2. **Present Position: Consultant,**
   Program for Emerging Infections (PEI), Infectious Diseases Division (IDD), icddr,b.

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
MBBS	Chittagong Medical College, Bangladesh	1983
Masters of Public Health (MPH)	ASEAN Institute of Health Development, Mahidol University, Bangkok, Thailand	1988
Ph.D	University of Cambridge, UK	1996

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**2036218**	

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
2007-002	Co-PI	18/02/2007	29/09/2019	
PR-18060	Co-PI	01/10/2018	29/09/2019	

6. **Publications**

Types of publications	Numbers
m. Original scientific papers in peer-review journals	130
n. Peer reviewed articles and book chapters	
o. Papers in conference proceedings	
p. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
q. Working papers	
r. Monographs	

7. **Five recent publications including publications relevant to the present research protocol**

7.1. **Rahman Mahmudur** (Joint first author): NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. www.thelancet.comhttp://dx.doi.org/10.1016/S0140-6736(17)32129-3.

7.2. **Rahman Mahmudur** (Equal Contribution):NCD Risk Factor Collaboration (NCD-RisC). A century of trends in adult human height. eLife 2016;5:e13410. DOI: 10.7554/eLife.13410

7.3 . Syed M. Satter, Negar Aliabadi, Catherine Yen, Paul A. Gastañaduy, Makhdum Ahmed, Abdullah Mamun, Khaleda Islam, Meerjady S. Flora, **Mahmudur Rahman**, Mustafizur Rahman, James D. Heffelfinger, Stephen P. Luby, Emily S. Gurley, K. Zaman, Umesh D. Parashar. Epidemiology of childhood intussusception in Bangladesh: Findings from an active national hospital based surveillance system, 2012–2016. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.08.092..

7.4 . Fahmida Chowdhury, Katharine Sturm-Ramirez, Abdullah Al Mamun, A Danielle Iuliano, Mejbah Uddin Bhuiyan, Mohammod Jobayer Chisti, Makhdum Ahmed, Sabbir Haider, **Mahmudur Rahman**, Eduardo Azziz-Baumgartner. Factors driving customers to seek health care from pharmacies for acute respiratory illness and treatment recommendations from drug sellers in Dhaka city, Bangladesh. Patient Preference and Adherence 2017:11.

7.5. Repon C. Paul, **Mahmudur Rahman**, Eric Wiesen, Minal Patel, Kajal C. Banik, Ahmad R. Sharif, Sharmin Sultana, Mizanur Rahman, Jayantha Liyanage, Nihal Abeysinghe, Saleem Kamili,4 Trudy Murphy, Stephen P. Luby, and Eric E. Mast. Hepatitis B Surface Antigen Seroprevalence among Prevaccine and Vaccine Era Children in Bangladesh. Am. J. Trop. Med. Hyg., 99(3), 2018, pp. 764–771 doi:10.4269/ajtmh.17-0721.

Biography of the investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** K. Zaman
2. **Present Position:** Senior Scientist and Epidemiologist, icddr,b

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
PhD	Johns Hopkins Bloomberg School of Public Health, USA	1999
MPH	Johns Hopkins Bloomberg School of Public Health, USA	1992
MBBS	Rajshahi Medical College, Bangladesh	1978

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1712891	Taken on 02/28/2015

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR 15004	PI	Feb 2015	Feb 2018	15
PR 15085	PI	October 2015	October 2018	25
PR 15050	PI	July 2015	July 2018	10
PR 16014	PI	March 2016	Dec 2019	10
PR 17034	PI	Sep 2016	Sep 2018	25

PR 15036	PI	June 2015	June 2018	15

6. Publications

Types of publications	Numbers
1. Original scientific papers in peer-review journals	170
2. Peer reviewed articles and book chapters	3
3. Papers in conference proceedings	120
4. Letters, editorials, annotations, and abstracts in peer-reviewed journals	4
5. Working papers	
6. Monographs	

Five recent publications including publications relevant to the present research protocol

1. **Zaman K**, Estívariz CF, Morales M, Yunus M, Snider CJ, Gary HE Jr, Weldon WC, Oberste MS, Wassilak SG, Pallansch MA, Anand A. Immunogenicity of type 2 monovalent oral and inactivated Poliovirus vaccines for type 2 poliovirus outbreak response: An open -level randomized controlled trial. Lancet ID 2018 Jun;18(6):657-665.
2. **Zaman K**, Zaman SF, Zaman F, Aziz A, Faisal SB, Traskine M, Habib MA, Ruiz Guiñazú J, Borys D. Immunologic non-inferiority and safety of the investigational pneumococcal non-typeable Haemophilus influenzae protein D-conjugate vaccine (PHiD-CV) 4-dose vial presentation compared to the licensed PHiD-CV 1-dose vial presentation in infants: A phase III randomized study. Vaccine. 2018 Jan 29;36(5):698-706. doi: 10.1016/j.vaccine.2017.12.034. Epub 2017 Dec 23.
3. **Zaman K**, Sack DA, Neuzil KM, Yunus M, Moulton LH, Sugimoto JD, Fleming JA, Hossain I, Arifeen SE, Azim T, Rahman M, Lewis KDC, Feller AJ, Qadri F, Halloran ME, Cravioto A, Victor JC. Effectiveness of a live oral human rotavirus vaccine after programmatic introduction in Bangladesh: A cluster-randomized trial. Effectiveness of a live oral human rotavirus vaccine after programmatic introduction in Bangladesh: A cluster-randomized trial. PLoS Med. 2017 Apr 18;14(4):e1002282. doi: 10.1371/journal.pmed.1002282. eCollection 2017 Apr.
4. **Zaman K**, Fleming JA, Victor JC, Yunus M, Azim T, Rahman M, Mowla SMN, Bellini WJ, McNeal M, Icenogle JP, Lopman B, Parashar U, Cortese MM, Steele D, Neuzil KM. Non-interference of rotavirus vaccine with measles-rubella vaccine at 9 months and improvements in anti-rotavirus immunity: a randomized trial. J Infect Dis 2016;213(11):1686-93
5. **Zaman K**, Naser AM. Power M, Yaich M, Zhang L, Ginsburg AS, Luby SP, Rahman M, Hills S, Bhardwaj M, Jorge Flores J. Lot -to-lot consistency of live attenuated SA 14-14-2 Japanese encephalitis vaccine manufactured in a Good Manufacturing Practice facility and non-inferiority with respect to an earlier product. Vaccine 2014; 32: 6061-66

Biography of the investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Dr. Firdausi Qadri, PhD

Present Position: Emeritus Scientist and Acting Senior Director (IDD), Enteric and Respiratory Infections, Infectious Disease Division (IDD), icddr,b

2. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
Postdoctoral fellowship	icddr,b, Bangladesh	1986-1988
PhD	Liverpool University, United Kingdom	1980
Masters	University of Dhaka, Bangladesh	1977
B.Sc.	University of Dhaka, Bangladesh	1975

3. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**		

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

4. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

5. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	**247**
b. Peer reviewed articles and book chapters	
c. Papers in conference proceedings	
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
e. Working papers	
f. Monographs	

6. Five recent publications including publications relevant to the present research protocol

1. Sugimoto JD, Koepke AA, Kenah EE, Halloran ME, Chowdhury F, Khan AI, LaRocque RC, Yang Y, Ryan ET, **Qadri F**, Calderwood SB, Harris JB, Longini IM Jr. Household Transmission of Vibrio cholerae in Bangladesh.PLoSNegl Trop Dis. 2014 Nov 20;8(11):e3314

2. Rychert J, Creely D, Mayo-Smith LM, Calderwood SB, Ivers LC, Ryan ET, Boncy J, **Qadri F**, Ahmed D, Ferraro MJ, Harris JB. Evaluation of MALDI-TOF Mass Spectrometry for Identification of Vibrio cholerae.J Clin Microbiol. 2014 Nov 12. pii: JCM.02666-14.
3. Ahmad SM, Raqib R, **Qadri F**, Stephensen CB. The effect of newborn vitamin A supplementation on infant immune functions: Trial design, interventions, and baseline data.Contemp Clin Trials. 2014 Sep 28. pii: S1551-7144(14)00145-1.
4. Uddin MJ, Wahed T, Saha NC, Kaukab SS, Khan IA, Khan AI, Saha A, Chowdhury F, Clemens JD, **Qadri F**. Coverage and acceptability of cholera vaccine among high-risk population of urban Dhaka, Bangladesh.Vaccine. 2014 Aug 20. pii: S0264-410X(14)01134-7.
5. Leung DT, Bhuiyan TR, Nishat NS, Hoq MR, Aktar A, Rahman MA, Uddin T, Khan AI, Chowdhury F, Charles RC, Harris JB, Calderwood SB, **Qadri F**, Ryan ET. Circulating Mucosal Associated Invariant T Cells Are Activated in Vibrio cholerae O1 Infection and Associated with Lipopolysaccharide Antibody Responses.PLoSNegl Trop Dis. 2014 Aug 21;8(8):e3076.

Biography of the investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Sayera Banu

2. **Present Position:**SeniorScientist

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
PhD	University of Dhaka, Bangladesh	2003
Post graduate training	Institute Pasteur, Paris	2000
MS	University of Tsukuba, Japan	1997
MBBS	University of Dhaka, Bangladesh	1989

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	CITI Program	1158017	06/12/2019

5. List of ongoing research protocols/ activities

5.1. As Principal Investigator

Protocol number	Starting date	End date	Percentage of time
PR13003	01-03-13	26-06-19	85
PR17098	29-03-18	28-03-19	05
PR17072	28-08-17	30-06-19	05
PR15121	05-05-16	04-05-19	05

5.2. As Co-Investigator

Protocol Number	Starting date	End date	Percentage of time

6. Publications

Types of publications	Numbers
s. Original scientific papers in peer-review journals	50
t. Peer reviewed articles and book chapters	1
u. Papers in conference proceedings	20
v. Letters, editorials, annotations, and abstracts in peer-reviewed journals	10
w. Working papers	6
x. Monographs	

7. Recent publications including publications relevant to the present research protocol

7.1. Rahman SM, Maliha UT, Ahmed S, Kabir S, Khatun R, Shah JA, Banu S, 2018. Evaluation of Xpert MTB/RIF assay for detection of Mycobacterium tuberculosis in stool samples of adults with pulmonary tuberculosis. PloS one 13: e0203063.

7.2. Sahrin M, Rahman A, Uddin M, Kabir S, Kabir S, Houpt E, Banu S, 2018. Discordance in Xpert® MTB/RIF assay results among low bacterial load clinical specimens in Bangladesh. The International Journal of Tuberculosis and Lung Disease 22: 1056-1062.

7.3. Kabir S, Uddin MKM, Chisti MJ, Fannana T, Haque ME, Uddin MR, Banu S, Ahmed T, 2018. Role of PCR method using IS6110 primer in detecting Mycobacterium tuberculosis among the clinically diagnosed childhood tuberculosis patients at an urban hospital in Dhaka, Bangladesh. Int J Infect Dis 68: 108-114.

7.4. Uddin MKM, Ahmed M, Islam MR, Rahman A, Khatun R, Hossain MA, Maug AKJ, Banu S, 2018. Molecular characterization and drug susceptibility profile of Mycobacterium tuberculosis isolates from Northeast Bangladesh. Infect Genet Evol 65: 136-143.

7.5. Heysell SK, Ahmed S, Rahman MT, Akhanda MW, Gleason AT, Ebers A, Houpt ER, Banu S, 2018. Hearing loss with Kanamycin treatment for multidrug-resistant tuberculosis in Bangladesh. European Respiratory Journal: 1701778.

Biography of the investigator- Internal

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Fahmida Chowdhury

2. **Present Position:** Deputy Project Coordinator, Respiratory Viruses Working Group, IDD, PEI, icddr,b

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
Masters in Public Health	Uppsala University, Sweden	2009
MBBS	Bangladesh Medical College, Dhaka	1999

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1297541	09/08/2015

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-15011	PI	23.04.2015	31.12.2017	20%
PR-13088	PI	01.02.2014	31.12.2017	20%
PR-14122	PI	20.05.2015	30.09.2019	25%
PR-13016	PI	01.06.2013	20.05.2018	20%
PR-15024	PI	24.05.2015	19.05.2018	5%
PR-2006-054	PI	01.02.2007	31.10.2018	10%

6. Publications

Types of publications	Numbers
y. Original scientific papers in peer-review journals	8
z. Peer reviewed articles and book chapters	1
aa. Papers in conference proceedings	8
bb. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
cc. Working papers	
dd. Monographs	

7. Five recent publications including publications relevant to the present research protocol

1. Md Mahbubur Rashid,[1,2] Mohammod Jobayer Chisti,[3]Dilruba Akter,[4]Malabika Sarkar,[2] **Fahmida Chowdhury[1]**.Antibiotic use for pneumonia among children under-five at a pediatric hospital in Dhaka city, Bangladesh. Patient Preference and Adherence. 2017 August 3; Volume 2017:11 Pages 1335—1342.

2. **Fahmida Chowdhury[1]**, Katharine Sturm-Ramirez[1,2], Abdullah Al Mamun[1], A Danielle Iuliano[2], Mejbah Uddin Bhuiyan[1], Mohammod Jobayer Chisti[1], Makhdum Ahmed[1], Sabbir Haider[3], Mahmudur Rahman[3], Eduardo Azziz-Baumgartner[2]. Factors driving customers to seek health care from pharmacies for acute respiratory illness and treatment recommendations from drug sellers in Dhaka city, Bangladesh. Patient Preference and Adherence. 2017 March 6; Volume 2017:11 Pages 479—486.

3. Shahid AS, Ahmed T, Shahunja KM, Kabir S, **Chowdhury F**, Faruque AS, Das SK, Sarker MH, Bardhan PK, Chisti MJ. Factors Associated with Streptococcal Bacteremia in Diarrheal Children under Five Years of Age and Their Outcome in an Urban Hospital in Bangladesh. PLoS One. 2016 May 2;11(5):e0154777

4. **Chowdhury F**, Chisti MJ, Hossain MI, Malek MA, Salam MA, Faruque AS. Association between paternal smoking and nutritional status of under-five children attending Diarrhoeal Hospital, Dhaka, Bangladesh. Acta Paediatr. 2011 Mar; 100(3):390-5.

5. **Chowdhury F**, Chisti MJ, Khan AH, Chowdhury MA, PietroniMA.Salmonella Typhi and Plasmodium falciparum co-infection in a 12-year old girl with haemoglobin E trait from a non-malarious area in Bangladesh. J Health PopulNutr. 2010 Oct; 28(5):529-31.

Biography of the Principal Investigators (External):

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

7. Name: Ole Fröbert

8. **Present Position:**Senior Consultant and Professor of CardiologyDepartment of Cardiology Örebro University Hospital, Sweden

9. **Educational background**:(last degree and diploma& training relevant to the present research proposal)

	Institution	Year
MD	Aarhus University	1992
PhD	Aarhus University	1996

10. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☒	Yes ☐	**This is not required in Sweden**		-

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

11. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

12. Publications

Types of publications	Numbers
ee. Original scientific papers in peer-review journals	138
ff. Peer reviewed articles and book chapters	6
gg. Papers in conference proceedings	90
hh. Letters, editorials, annotations, and abstracts in peer-reviewed journals	7
ii. Working papers	
jj. Monographs	

13. Five recent publications including publications relevant to the present research protocol

1. Xaplanteris P, Fournier S, Pijls NHJ, Fearon WF, Barbato E, Tonino PAL, Engstrøm T, Kääb S, Dambrink JH, Rioufol G, Toth GG, Piroth Z, Witt N, **Fröbert O**, Kala P, Linke A, Jagic N, Mates M, Mavromatis K, Samady H, Irimpen A, Oldroyd K, Campo G, Rothenbühler M, Jüni P, De Bruyne B: Five-Year Outcomes with PCI Guided by Fractional Flow Reserve. **N Engl J Med. 2018;379: 250-259.**

2. Andell P, Berntorp K, Christiansen EH, Gudmundsdottir IJ, Sandhall L, Venetsanos D, Erlinge D, **Fröbert O**, Koul S, Reitan C, Götberg M.: Reclassification of Treatment Strategy With Instantaneous Wave-Free Ratio and Fractional Flow Reserve: A Substudy From the iFR-SWEDEHEART Trial. **JACC Cardiovasc Interv. 2018 Oct 22;11(20):2084-2094**.

3. Kwakkenbos L, Juszczak E, Hemkens LG, Sampson M, **Fröbert O**, Relton C, Gale C, Zwarenstein M, Langan SM, Moher D, Boutron I, Ravaud P, Campbell MK, Mc Cord KA, van Staa TP, Thabane L, Uher R, Verkooijen HM, Benchimol EI, Erlinge D, Sauvé M, Torgerson D, Thombs BD. Protocol for the development of a CONSORT extension for RCTs using cohort and routinely collected health data. **Res Integr Peer Rev. 2018 Oct 29;3:9.**

4. Mohammad MA, Koul S, Rylance R, **Fröbert O**, Alfredsson J, Sahlén A, Witt N, Jernberg T, Muller J, Erlinge D.: Association of Weather With Day-to-Day Incidence of Myocardial Infarction: A SWEDEHEART Nationwide Observational Study. **JAMA Cardiol. 2018 Nov 1;3(11):1081-1089.**

5. Mohammad MA, Karlsson S, Haddad J, Cederberg B, Jernberg T, Lindahl B, **Fröbert O**, Koul S, Erlinge D.: Christmas, national holidays, sport events, and time factors as triggers of acute myocardial infarction: SWEDEHEART observational study 1998-2013. **BMJ. 2018 Dec 12;363:k4811.**

Biography of the Investigators- External

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Mohammad Abdul Aleem

2. **Present Position:** Research investigator, Program for Emerging Infections (PEI), Infectious Diseases Division (IDD), icddr,b.

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
Masters of Public Health (MPH Epidemiology)	National Institute of Preventive and Social Medicine (NIPSOM)	2012
Maters of Philosophy (M.Phil Immunology)	Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), University of Dhaka, Bangladesh	2011

MBBS	Bangladesh Medical College, University of Dhaka, Bangladesh	2004
Training	Medicine	2007
Training	Cardiology	2005

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**1120805**	

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
2007-002	PI	30/09/2016	29/09/2017	100%
PR-16074	PI	03/01/2017	02/28/2018	20%

6. Publications

Types of publications	Numbers
kk. Original scientific papers in peer-review journals	1
ll. Peer reviewed articles and book chapters	0
mm. Papers in conference proceedings	6
nn. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
oo. Working papers	
pp. Monographs	

7. Five recent publications including publications relevant to the present research protocol

7.1. **Aleem MA**, Chowdhury Ashesh, Taher MA et al. Study of serum levels of hsCRP, IFN-γ and TNF-α in patients with Acute Coronary Syndrome. *Bangladesh Heart Journal*, January 2010; 25(1); 11-17

7.2.Haque N., **Aleem MA**., Haque M., Use of alternative medicine among women in an urban and rural area of Bangladesh. *Journal of medical science and research*, 2015 Jan, 24 (1); p.13-21.

7.3.Md. Ariful Islam, Anisur Rahman, **Mohammad Abdul Aleem**, Sheikh Mohammed Shariful Islam. Prevalence and associated factors of depression among post-stroke patients in Bangladesh. *Int J Ment Health Addiction*. DOI 10.1007/s11469-015-9582-x.

7.4 Ashesh Kumar Chowdhury, Humaira Tabassum, Monisha Chowdhury, **Mohammad Abdul Aleem**, Md. Abu Taher Sarkar and Mansura Khan. Comparative evaluation of

antibody test and quantitative RNA assay for accurate diagnosis of Hepatitis C Virus infected patients of Bangladesh. *Journal of medical science and research*, 2016, July, 25(1); p.20-28.

Biography of the Investigators- External

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name: Raina MacIntyre, PhD**
2. **Present Position:**

3. **Educational background**: (last degree and diploma& training relevant to the present research proposal)

	Institution	Year
PhD	Australian National University	1998
FAFPHM	Australasian Faculty of Public Health Medicine	1995
FRACP	Royal Australasian College of Physicians	1994
MAE	Australian National University	1994
MBBS	University of Sydney	1988

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☒	Yes ☐	**This is not required in Australia**		

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

6. **Publications**

Types of publications	Numbers
g. Original scientific papers in peer-review journals	~310
h. Peer reviewed articles and book chapters	7
i. Papers in conference proceedings	7
j. Letters, editorials, annotations, and abstracts in peer-reviewed journals	~20
k. Working papers	
l. Monographs	NA

7. **Five recent publications including publications relevant to the present research protocol**

1. **MacIntyre CR**, Mahimbo A, Moa A, Barnes M. Influenza vaccine as a coronary intervention for prevention of myocardial infarction. Heart, 2016;102(24):1953-56.
2. **MacIntyre CR,** Seale H, Dung TC, Hien NT, et al. A cluster randomised trial of cloth masks compared

with medical masks in healthcare workers. *BMJ Open* 2015; 5:e006577.

3. **MacIntyre CR**, Heywood AE, Kovoor P. Influenza virus vaccine reduces risk of ischemic events: time for a large-scale randomized trial? (Commentary) Future Cardiol**.** 2014; 10(1): 35–37.
4. **MacIntyre CR**, Heywood AE, Kovoor P, Ridda I, Seale H, Tan T, Gao Z, Katelaris AL, Siu HWD, Lo V, Lindley R, Dwyer DE. Ischaemic heart disease, influenza and influenza vaccination: a prospective case control study. *Heart* Published Online First: 2013,99(24):1843-8.
5. **MacIntyre CR**, Wang Q, Seale H, Peng Y, et al. A randomised clinical trial of three options for N95 respirators and medical masks in health workers. American Journal of Respiratory and Critical Care Medicine 2013; 187(9):960-966.

Check-List

Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]

1. Has the proposal been reviewed, discussed and cleared by all listed investigators?

   ☒ Yes ☐ No

   If the response is No, please clarify the reasons:

2. Has the proposal been peer-reviewed externally?

   ☐ Yes ☒ No ☒ External Review Exempted

   If the response is 'No' or "External Review Exempted", please explain the reasons:

   Protocol has already achieved ethics clearance at Läkemedelsverket / Swedish Medical Products Agency; We request exemption.

   If the response is "Yes", please indicate if all of their comments have been addressed?

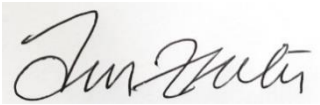
   ☐Yes (please attach)

   ☐No (please indicate reason(s)):

3. Has the budget been reviewed and approved by icddr,b's Finance?
   ☒Yes☐ No (reason): _____

4. Has the Ethics Certificate(s) been attached with the Protocol?

   ☒ Yes☐ No
   If the answer is 'No', please explain the reasons:



_____ _____

Signature of the Principal Investigator Date

Appendix I
Voluntary Consent Form

Protocol No.PR-19005	Version No.3.0	Date:25-03-2019

Protocol Title: Effect of influenza vaccination on recurrent cardiovascular events among Myocardial Infarction (MI) patients in Bangladesh: A multicounty, randomized clinical trial

Investigator's name: Dr. Zubair Akhtar

Purpose of the research

Hello/Assalamualaikum/Adab. My name is……., working with icddr,b (Cholera Hospital). With prior approval from Directorate General of Health Services of Bangladesh, we are doing a study in collaboration with Örebro University Hospital, Sweden to investigate whether influenza vaccination of patients with heart attacks or stable coronary artery disease is better than no vaccination in terms of reducing cardiovascular events such as heart attack, stroke and new hospitalizations.

Background

Influenza vaccination is recommended in Sweden, the rest of Europe and the United States and also in Bangladesh among pilgrims (haji) before the Holy Hajj in Mecca, but it is not known whether vaccination prevents cardiovascular disease and further studies are needed to evaluate the effect in patients with myocardial infarction. In this study we will invite patients to participate and half of them will be included in a group receiving regular influenza vaccine and the other half (the control group) will receive an injection of placebo (inactive substance, normal saline) it is important that you do not know whether you have been given the real influenza vaccine or the placebo. This is one of the best ways we have for knowing what influenza vaccination really does. In the study influenza vaccine Vaxigrip ® is used. This is an approved vaccine for influenza prevention both in adults and children from 3 years and up.

Why invited to participate in the study?

We have invited to you to participate in this study as you are a patient with recent myocardial infarction (MI) or patients with a diagnosis of stable coronary artery disease, and >18 years of age or ≥75 years of age and you have one of them- previous myocardial infarction, previous Percutaneous coronary intervention (PCI), previous coronary artery bypass grafting (CABG), diabetes mellitus, current smoking or an estimated glomerular filtration rate (eGFR) <40 and you are not allergic to chicken egg.

What is expected from the participants of the research study?

If you choose to participate we will ask you to sign an informed consent form for the collection of data related to the study. Vaccination (Vaxigrip® / saline) will be conducted in relation to the medical treatment procedure that you may undergo for myocardial infarction or stable coronary artery disease.

Risk and benefits

There are no major risks involved in participating in this study but reaction**s** to vaccination may include soreness, redness or swelling at the vaccination site and fever. More serious reactions are rare, but signs of serious allergic reactions may be breathing problems, hoarseness, hives, paleness, weakness, a fast heartbeat or dizziness. The reason why you cannot participate in the study if you are allergic to eggs is that the virus strains used in influenza vaccination is cultivated in chicken eggs. Should any serious reactions occur after vaccination you will be treated immediately according to hospital routine. Patients receiving influenza vaccination will have a protective effect against influenza during this year's influenza

season. Otherwise there are no direct benefits in participating to the individual participants. But your participation will help us to know how vaccination can be helpful to prevent cardiovascular disease and further studies are needed to evaluate the effect in patients with myocardial infarction. Moreover, your inputs will help us to guide government to introduce measures such as an influenza vaccination that may benefit you and other in near future.

Privacy, anonymity and confidentiality
We affirm you that your privacy, anonymity, and confidentiality will be strictly maintained. We will keep your information private and will not share any of them with people who aren't involved in our study. To protect your privacy, we will keep the records under a code number rather than by your name. The code that links a number to your name will be kept by study staff in locked files. Your name and other information about you will not appear when we talk about this study or publish its results.

Future use of information
If the information we collect needs to be used for future use by other researchers, we will not supply any personal information and will maintain strict privacy.

Right not to participate and withdraw
Your participation in this study is totally voluntary. You may refuse to take part in the study now or at any time during the trial. You may also withdraw from the study at any time. Whether you choose to participate or not or withdraw, all the services you receive at this hospital will continue and nothing will change.

Principle of compensation
There is neither any cost nor any payment for participating in this study.

Answering your questions/ Contact persons
Dr..... (to be recruited), Medical officer, Infectious Diseases Division, icddr,b, Mohakhali, Dhaka. Contact Mobile: 01…….. (to be subscribed). If you have questions about your rights as a participant of a research study, or if you think some harm has been done to you because of the study, you may contact or meet personally with the below mentioned person at following address M. A. Salam Khan, IRB Secretariat, Phone No: 9827084

If you agree to our proposal of enrolling you/your patient in our study, please indicate that by putting your signature or your left thumb impression at the specified space below

Thank you for your cooperation

_____	_____
Signature or left thumb impression of participant	Date
_____	_____
Signature or left thumb impression of Parent/ Guardian/ Attendant	Date
_____	_____
Signature or left thumb impression of the witness	Date
_____	_____
Signature of the PI or his/her representative	Date

Appendix II

Effect of influenza vaccination on recurrent cardiovascular events among Myocardial Infarction (MI) patients in Bangladesh: A multicounty, randomized clinical trial.

❖ **Reporting by letter at 7 (+3) days.**

1. Please describe if you, during the first week after vaccination, have experienced:
    i. Shivering (date and for how many days)
    ii. Fever (date and for how many days)
    iii. Headache (date and for how many days)
    iv. Muscle ache (date and for how many days)
    v. A feeling of general discomfort (date and for how many days)

2. Please rate any discomfort after your vaccination:
    i. Pain (1. None at all; 2. A little; 3. Moderate; 4. Very; 5. Extreme, and for how many days).
    ii. Redness (1-5, days).
    iii. Swelling (1-5, days).
    iv. Itching (1-5, days).
    v. Hardening (a bump) (1-5, days).
    vi. Bruising (1-5, days).
    vii. Sleep affected (1-5, days).
    viii. Other (1-5, days). Describe:_____

Appendix III

❖ **Telephone/ household quarterly follow-up at 3 months interval.**

1. Have you been hospitalized since your myocardial infarction 3 months ago?
    a. No.
    b. Yes:
        a. For myocardial infarction (y (please state name of hospital and date (if unsure, state approximate date)/n).
        b. For stroke (y (hospital and date (if unsure, state approximate date)/n).
        c. For heart failure (y (hospital and date (if unsure, state approximate date)/n).
        d. Other (hospital and date (if unsure, state approximate date). Describe:- _____

2. Do you think that you have had influenza since you left hospital 3 months ago?
    a. No.
    b. Yes:
        i. Did you visit a doctor for influenza symptoms?
            1. fever
            2. coughing
            3. runny or stuffy nose
            4. sore throat
            5. headache
            6. fatigue
            7. chills
            8. body aches
        ii. Were you hospitalized with influenza?
            1. For how many days?
            2. Which hospital?
        iii. Did you receive antibiotics or antiviral medicine? (yes/no/unknown)
        iv. Approximately when did you have influenza and for how long?
            1. First episode _____
            2. Second episode _____

3. Have you received influenza vaccination since you left hospital 3 months ago?
    a. No.
    b. Yes:
        i. Approximately when did you receive influenza vaccination?
            1. First vaccination _____
            2. Second vaccination _____

4. Have you received pneumococcal vaccination since you left hospital 3 months ago?
    a. No.
    b. Yes:
        i. Approximately when did you receive vaccination?
            1. First vaccination _____
            2. Second vaccination _____

5. How anxious do you feel about receiving influenza vaccination in the future?

    1. Not at all; 2. A little; 3. Moderately; 4. Very; 5. Extremely.

6. ONLY if patient or relative cannot be contacted OR a patient is deceased:
    a. Please tick this box if patient or relative cannot be contacted: □
    b. If available from hospital records or other sources, please tick all boxes below that apply and state FIRST hospitalization if more than one for the same condition:
        i. Patient is alive □
        ii. Patient is deceased: □
            - Date (if known):
            - Cause of death (if known):
        iii. Patient was hospitalized for myocardial infarction □
            - Date (if known):
        iv. Patient was hospitalized for stroke □
            - Date (if known):
        v. Patient was hospitalized for heart failure □
            - Date (if known):
        vi. Patient was hospitalized for other serious illness □
            - Date (if known):
            - Type of illness:

Appendix IV

Variables to enter in eCRF form

Age - yr. (mean (± SD))

Male sex - no. (%)

Body-mass index (mean (± SD)) [1)]

Diabetes mellitus - no. (%)

Smoking status - no. (%)

- Never smoked
- Former smoker
- Current smoker
- Unknown

Hyperlipidemia - no. (%)

Hypertension - no. (%)

Previous myocardial infarction - no. (%)

Previous PCI - no. (%)

Previous coronary artery by-pass grafting - no. (%)

STEMI - no. (%)

NSTEMI - no. (%)

Killip class ≥ 2 – no. (%)

Number of diseased vessels - no. (%)

- 1-vessel disease (not left main)
- 2-vessel disease (not left main)
- 3-vessel disease (not left main)
- Left main disease
- Not available

Revascularization at baseline

Treated with PCI

Stent no. per procedure. Mean (± SD)

Drug-eluting stent implantation - no. (%)

Drug-eluting balloon - no. (%)

N0. of treated vessels - no. (%)

0

1

2

3

Treated vessel - no. (%) [2)]

RCA

LM

LAD

LCx

By-pass graft

Procedural success - no. (%)

Complete revascularisation - no. (%)

Treated with coronary artery by-pass grafting

Medical treatment only

Left ventricular ejection fraction at discharge [3)]

Normal

Slightly reduced

Moderately reduced

Severely reduced

Unknown

Discharge medication

ASA - no. (%)

P2Y12 inhibitor - no. (%)

β-blocker - no. (%)

ACE-I/ARB - no. (%)

Statin - no. (%)

Appendix V

Gender Analysis Tool

In Relation to severe acute respiratory infection among elderly patients:	Are there sex differences in	How do biological differences between women and men influence their :	How do the different roles and activities of men and women affect their	How do gender norms / values affect men and women's	How do access to, and control over resources affect men and women's
Vulnerability: Incidence ** Prevalence ** (male/female)	Yes.	Men are more likely to have lower respiratory tract infection. Woman are more likely to have upper respiratory infection. Incidence of death or ICU admission are higher among men.	Men are more likely to exposed to smoking, alcoholism and have co-morbidities like COPD, cardiac illness.	Social structural and psycho social determinants for women and behavioural determinants for men.	
Health seeking behaviour	Yes. Married men are more likely to seek health care. Women are less likely to report morbidity.	Not applicable.	Male patients are more likely to seek emergency health care.	Social structural and psycho social determinants for women and behavioural determinants for men.	

Ability to access health services	Yes.	Not applicable.		Woman may be more disadvantaged in terms of functional disability.	Possible gender bias in ICU admission. Men are more likely to receive ICU care.
Experience with health services and health providers	Yes. Women are more likely to utilize health services and health providers.	Not applicable.			
Preventive and Treatment options, responses to treatment or rehabilitation	Yes.	Yes. Men are more likely to suffer from cardiovascular illness hence needing different preventive and treatment options. Due to difference in causative respiratory pathogens antibiotic responses differ.	Not applicable.		
Outcome of health problem	Yes. Outcome of health problem is more	Outcome of health problem is more adverse	Women are more likely to cope		Possible gender bias in receivi

	adverse among men.	among men. Men are more likely to have COPD and underlying chronic cardiac illness that leads to poorer prognosis.	well to any distressing condition. Due to adverse lifestyle related risk factors men more frequently have co-morbidities.		ng aggressive treatment. Men are more likely to receive aggressive treatment which influences the outcome.
Consequences (economic & social, including attitudinal)	Yes. More frequency among male patients.	Not applicable.	Economic consequences are more frequent among male patients. This is derived from the perspective of men playing the role of family head in most cases in low income countries like Bangladesh.		

Appendix VI

Countries participating in this trial

1. United Kingdom
2. Australia
3. Sweden
4. Denmark
5. Norway
6. Latvia
7. Czech Republic

Appendix VII

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
স্বাস্থ্য অধিদপ্তর
হাসপাতাল এবং ক্লিনিক সমূহ শাখা
মহাখালী, ঢাকা-১২১২

স্মারক নং: স্বাঃঅধিঃ/হা:সা:ম্যা:/NGO/INGO/২০১৮-১৯/ ৪৭৭ তারিখ: ০৯-০১-২০১৯ খ্রিস্টাব্দ

বিষয়ঃ **"Influenza vaccination After Myocardial Infarction (IAMI trial); A multicenter, prospective, randomized controlled clinical trial in selected hospitals in Dhaka, Bangladesh"** শীর্ষক একটি গবেষনা পরিচালনার জন্য সহযোগিতাকরণ প্রসঙ্গে।

সূত্রঃ Icddr'b, তারিখঃ ২৬.১২.২০১৮ খ্রি.

এ মর্মে জানানো যাচ্ছে যে, সংশ্লিষ্ট পরিচালক/তত্ত্বধায়ক জাতীয় হৃদরোগ ইন্সটিউট ও হাসপাতাল / ন্যাশনাল হার্ট ফাউন্ডেশন এন্ড রিসার্চ ইন্সটিউট / ইব্রাহীম কার্ডিয়াক হসপিটাল এন্ড রিসার্চ ইন্সটিউটকে জানানো যাচ্ছে যে, Örebro University Hospital, Sweden, icddr'b এবং স্বাস্থ্য অধিদপ্তর (হাসপাতাল এবং ক্লিনিক সমূহ শাখা), বাংলাদেশ যৌথভাবে বাংলাদেশের রাজধানী ঢাকায় **"Influenza vaccination After Myocardial Infarction (IAMI trial); A multicenter, prospective, randomized controlled clinical trial in selected hospitals in Dhaka, Bangladesh"** শীর্ষক একটি গবেষনা পরিচালনা করবেন। উক্ত গবেষণার ফলাফল মায়োকার্ডিয়াল ইনফার্কশন প্রতিরোধে ইনফ্লুয়েঞ্জা টীকার কার্যকারিতা জানতে সহায়তা করবে। এ বিষয়ে সার্বিক সহযোগিতার জন্য সংশ্লিষ্ট সবাইকে বিশেষভাবে অনুরোধ করা হলো।

এতে মহাপরিচালক মহোদয়ের অনুমোদন আছে।

কার্যার্থে:

১. পরিচালক, জাতীয় হৃদরোগ ইন্সটিউট ও হাসপাতাল , ঢাকা।
২. পরিচালক, ন্যাশনাল হার্ট ফাউন্ডেশন এন্ড রিসার্চ ইন্সটিউট, ঢাকা।
৩. পরিচালক, ইব্রাহীম কার্ডিয়াক হসপিটাল এন্ড রিসার্চ ইন্সটিউট, ঢাকা।

(অধ্যাপক ডাঃ মোঃ আলী খান)
পরিচালক (হাসপাতাল ও ক্লিনিক সমুহ) এবং
লাইন ডাইরেক্টর (হসপিটাল সার্ভিসেস ম্যানেজমেন্ট)
স্বাস্থ্য অধিদপ্তর, মহাখালী, ঢাকা।
Email: ihsm@ld.dghs.gov.bd
ফোন: ০২-৫৫০৬৭১৫০ফ্যাক্স নং- ৫৫০৬৭১৫১

স্মারক নং: স্বাঃঅধিঃ/হা:সা:ম্যা:/NGO/INGO/২০১৮-১৯/ ৪৭৬ তারিখ: ০৯-০১-২০১৯ খ্রিস্টাব্দ

অনুলিপি সদয় অবগতির জন্য প্রেরণ করা হলো :

১. মহাপরিচালক, স্বাস্থ্য অধিদপ্তর, মহাখালী, ঢাকা। দৃ: আ: সহকারী পরিচালক (সমন্বয়)।
২. পরিচারক (গবেষণা ও উন্নয়ন), স্বাস্থ্য অধিদপ্তর, মহাখালী, ঢাকা।
৩. সিনিয়র ডাইরেক্টর, ইনফেকশাস ডিজিজ ডিভিশন, আইসিডিডিআর,বি ।
৪. অ্যাক্টিং হেড, প্রোগ্রাম ফর ইমারজিং ইনফেকশনস, ইনফেকশাস ডিজিজ ডিভিশন, আইসিডিডিআর,বি ।
৫. অফিস নথি।

(অধ্যাপক ডাঃ মোঃ আলী খান)
পরিচালক (হাসপাতাল ও ক্লিনিক সমুহ) এবং
লাইন ডাইরেক্টর (হসপিটাল সার্ভিসেস ম্যানেজমেন্ট)
স্বাস্থ্য অধিদপ্তর, মহাখালী, ঢাকা।
Email: ihsm@ld.dghs.gov.bd
ফোন: ০২-৫৫০৬৭১৫০ফ্যাক্স নং- ৫৫০৬৭১৫১

Appendix VIII

Vaxigrip Tetra product details

1. NAME OF THE MEDICINAL PRODUCT

Vaxigrip Tetra, suspension for injection in pre-filled syringe
Quadrivalent influenza vaccine (split virion, inactivated)

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Influenza virus (inactivated, split) of the following strains*:

A/California/7/2009 (H1N1)pdm09 - like strain (A/California/7/2009, NYMC X-179A)
..... 15 micrograms HA**

A/Texas/50/2012 (H3N2) - like strain (A/Texas/50/2012, NYMC X-223A)..... 15 micrograms HA**

B/Massachusetts/2/2012 (Yamagata lineage)..... 15 micrograms HA**

B/Brisbane/60/2008 (Victoria lineage)..... 15 micrograms HA**

Per 0.5 ml dose

* propagated in fertilised hens' eggs from healthy chicken flocks
** haemagglutinin

This vaccine complies with the WHO recommendations (Northern Hemisphere) and EU decision for the 2014/2015 season.

For the full list of excipients, see Section 6.1.

Vaxigrip Tetra may contain traces of eggs, such as ovalbumin, and of neomycin, formaldehyde and octoxinol-9, which are used during the manufacturing process (see Section 4.3).

3. PHARMACEUTICAL FORM

Suspension for injection in pre-filled syringe.
The vaccine, after shaking gently, is a colourless opalescent liquid.

Protocol Number: IAMI-2014 protocol version 8.0 September 2018

Influenza vaccination After Myocardial Infarction (IAMI) trial. A registry-based randomized clinical trial

Statistical Analysis Plan

Prepared for: Department of Cardiology, Örebro University Hospital

Clinical Trial Registration Number: NCT02831608

Version Number: v.1.4

Date: 16/February/2021

Prepared by: Tim Collier, Stuart Pocock

Protocol No. IAMI-2014 protocol version 8.0 September 2018

SIGNATURE PAGE

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17-02-2021

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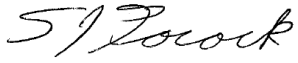
Reviewed by:



17-02-2021

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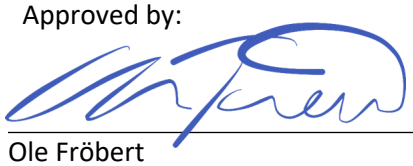
Department of Medical Statistics, London School of Hygiene & Tropical Medicine

Approved by:



17 February 2021

Ole Fröbert <Date>

MD, PhD, Cardiologist, adjunct Professor

Department of Cardiology, Faculty of Health, Örebro University

Table of Contents

1 INTRODUCTION

1.1 STUDY BACKGROUND AND RATIONALE

In a multicenter, prospective, randomized registry-based controlled clinical trial based on the SWEDEHEART platform and other national registries (in countries with available registries) to compare influenza vaccination and placebo in reducing future major adverse cardiac and cerebrovascular events in patients with myocardial infarction. In countries with no national registries the study is carried out as a conventional “pragmatic” clinical trial with collection of only key baseline and outcome variables.

1.2 HYPOTHESES AND OBJECTIVES

We test the hypothesis that influenza vaccination is superior to placebo in reducing time to the composite endpoint of all-cause death, a new myocardial infarction (AMI) or stent thrombosis (first occurring) at 12 months in patients with ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) or high risk patients with stable coronary artery disease (primary end point). Key secondary endpoints are each component of the composite primary endpoint and cardiovascular death. Other secondary endpoints are listed below.

2 STUDY METHODS

2.1 OVERALL DESIGN

Parallel group, 1:1 allocation to influenza vaccine (Vaxigrip, Vaxigrip Tetra or FluQuadri, Sanofi Pasteur) or to placebo (saline).

2.2 RANDOMIZATION

An external web-page for randomization was constructed. Following written informed consent randomization is stratified by center with a 1:1 allocation within each stratum using predefined block sizes. Block randomization is by a computer generated random number list prepared by Lytics, the clinical research organization in charge of external web-randomization (*http://lytics.ai/company*). The patient, investigators and all other medical staff are kept blinded to the allocation. Unblinded study nurses, not otherwise involved in study conduction or follow-up, prepare the study medication (Vaxigrip, Vaxigrip Tetra or FluQuadri /placebo).

2.3 SAMPLE SIZE

We estimated that 2186 patients per arm would provide 80% power at the 5% significance level to detect a 25% relative reduction in the risk of the primary endpoint. To allow for loss to follow-up a total of 4400 patients will be enrolled. Please refer to point 4.4 (p 16) in the study protocol.

2.4 FRAMEWORK

This study tests whether influenza vaccination is superior to placebo regarding the listed primary endpoint and key secondary endpoints. The results will be analyzed according to the intention-to-treat principle with one modification (see 3.3 below).

2.5 DATA SAFETY MONITORING BOARD ANALYSES AND STOPPING GUIDANCE

A maximum of 3 months following inclusion of the first 1000 patients an independent endpoint committee (IEC/ data safety monitoring board) will monitor study endpoints. Variables to be assessed

are all-cause death, a new myocardial infarction and stent thrombosis. Premature termination of the study for efficacy will be mandated if p<0.001 in favour of the influenza vaccine group for the composite of time to all-cause death, a new myocardial infarction or stent thrombosis.

2.6 TIMING OF FINAL ANALYSIS

Final analysis for the primary and secondary endpoints will be conducted when 12 months' data is available for all patients enrolled. From a hypothesis generating perspective we aim to follow up patients through registries (from countries where such information is available) beyond 1 year and up to 5 years. Because influenza may precipitate plaque rupture it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques. Endpoints beyond 1 year will be regarded as exploratory.

3 STATISTICAL PRINCIPLES

3.1 CONFIDENCE INTERVALS AND P VALUES

1) *Level of statistical significance*
   All applicable statistical tests will be 2-sided and will be performed using a 5% significance level.
2) *Adjustment for multiplicity and, if so, detailing how the type 1 error is to be controlled*
   No adjustment for multiplicity is planned for the primary endpoint. If statistical significance is demonstrated for the primary endpoint then the three components of the composite and cardiovascular (CV) death will be tested sequentially in the following predefined hierarchy: death, CV death, AMI, stent thrombosis. All other secondary endpoints will be considered as exploratory.
3) *Confidence intervals to be reported*
   All confidence intervals (CI) presented will be 95% and two-sided.

3.2 ADHERENCE AND PROTOCOL DEVIATIONS

Adherence to the intervention is defined by whether a randomized participant has received study medication according to randomization. Please refer to 3.3 below.

Protocol deviations (number and type of protocol deviations by intervention group and listing of all deviations) to be reported and summarized:

1) wrong intervention being administered (major);
2) incorrect data being collected and documented (major);
3) errors in applying inclusion/exclusion criteria (as it is unlikely that patients not undergoing a percutaneous intervention are randomized. Errors in applying additional inclusion/exclusion criteria will be considered minor deviations);
4) or missed follow-up visits (major if no follow-up data are collected, minor if the components of the primary endpoint. i.e. death, myocardial infarction, stent thrombosis, are collected).

3.3 ANALYSIS POPULATIONS

- Intention-to-Treat (ITT) population: the ITT population consists of all randomized patients according to their randomized treatment assignment.

- Modified Intention-to-Treat (m-ITT) population: because the study intervention (influenza vaccine/placebo) is administered only once during the index hospitalization and because most protocol deviations regarding the intervention are purely logistical (patients have left the hospital before study medication was administered) we will define the m-ITT population as all randomized patients according to their randomized treatment assignment and who received the study medication.
- Safety population: the safety population consists of all randomized patients who received treatment according to the actual treatment received.
- Per-Protocol population: no per-protocol analysis is planned.

4 STUDY POPULATION

4.1 SCREENING DATA

The following summaries will be presented for all screened patients: Enrolment: the number of days recruiting, the number of patients screened, and the number of patients recruited, and the reason for non-recruitment. This summary will be provided overall. Patients of both sexes >18 years are eligible for inclusion.

4.2 ELIGIBILITY

Eligibility criteria are summarized in the protocol (points 3.1.2 and 3.1.3). The number of ineligible patients randomized, if any, will be reported, with reasons for ineligibility.

4.3 RECRUITMENT

Recruitment Information to be included in the CONSORT flow diagram (Figure 1) is:

1) number of patients screened;
2) number of patients randomized, number of patients enrolled in error*, number of patients that did not receive study medication according to randomization*;
3) number of patients randomized receiving study medication according to randomization, number of patients allocated to influenza vaccine, number of patients allocated to placebo;
4) number of patients followed-up at 12 months, number of patients lost to follow-up at 12 months*;
5) number of patients allocated to and receiving influenza vaccine and followed-up for the primary endpoint at 12 months, number of patients allocated to and receiving placebo and followed-up for the primary endpoint at 12 months, number of patients allocated to and receiving influenza vaccine and lost to follow-up at 12 months*, number of patients allocated to and receiving placebo and lost to follow-up at 12 months*.

*Reasons will be provided.

4.4 WITHDRAWAL / FOLLOW-UP

1) We will report withdrawal from follow-up but data collected to date of withdrawal will be used. Also lost to contact/follow-up at 12 months will be reported.
2) Timing of withdrawals will be reported. Timing of lost to follow up can only occur at 12 months since this is the only stipulated patient contact following the index hospitalization.

3) The numbers will be presented in CONSORT diagram with reasons for withdrawal and/or exclusion from analysis given at each stage (baseline, 12 months follow-up). The numbers over the course of the trial will be summarized by treatment arms.

5 STUDY ASSESSMENTS

5.1 BASELINE PATIENT CHARACTERISTICS

1) Patients will be described in Table 1 with respects to age, gender, smoking status and comorbidities, separately for the two randomized arms.

Table 1. Baseline characteristics

	Placebo	Vaccine
N		
Age - yr. (mean (± SD))		
Male sex - no. (%)		
Body-mass index (mean (± SD)) [1)]		
Diabetes mellitus - no. (%)		
Smoking status - no. (%)		
Never smoked		
Former smoker		
Current smoker		
Unknown		
Hyperlipidemia - no. (%)		
Hypertension - no. (%)		
Previous myocardial infarction - no. (%)		
Previous PCI - no. (%)		
Previous coronary artery by-pass grafting - no. (%)		
STEMI - no. (%)		
NSTEMI - no. (%)		
Stable angina – no. (%)		
Killip class ≥ 2 – no. (%)		
Number of diseased vessels - no. (%)		
1-vessel disease (not left main)		
2-vessel disease (not left main)		
3-vessel disease (not left main)		
Left main disease		
Not available		

1) Body-mass index = kg/m^2.

Revascularization at baseline will be described in Supplementary Table S1, separately for the two randomized arms.

Supplementary Table S1. Revascularization at baseline

	Placebo (N=#)	Vaccine (N=#)
Treated with PCI		
Stent no. per procedure -no. (%)		
0		
1		
2		
3+		
Drug-eluting stent implantation - no. (%)		
Drug-eluting balloon - no. (%)		
NO. of treated vessels - no. (%)		
0		
1		
2		
3		
RCA		
LAD		
LCx		
LM		
By-pass graft		
Procedural success - no. (%)		
Complete revascularisation - no. (%)		
Treated with coronary artery by-pass grafting		
Medical treatment only		
Left ventricular ejection fraction at discharge *		
Normal		
Slightly reduced		
Moderately reduced		
Severely reduced		
Unknown		
Discharge medication		
ASA - no. (%)		
P2Y12 inhibitor - no. (%)		
β-blocker - no. (%)		
ACE-I/ARB - no. (%)		
Statin - no. (%)		

ACE-I: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; ASA: acetylsalicylic acid; LAD: left anterior descending artery; LCx: left circumflex artery; LM: left main coronary artery; RCA: right coronary artery.
** Left ventricular function is considered to be normal if the left ventricular ejection fraction (LVEF) is 50% or more, slightly reduced if the LVEF is 40 to 49%, moderately reduced if the LVEF is 30 to 39%, and severely reduced if the LVEF is less than 30%.*

5.2 EFFICACY ASSESSMENTS

5.2.1 PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the composite of all-cause death, AMI or stent thrombosis at 12 months post randomisation. The results for the primary efficacy endpoint will be reported using Kaplan-Meier plots and in Table 2.

The effect of treatment on the primary endpoint will be analysed in eight pre-specified subgroups:

- Sex: male, female
- Age: <65, ≥65
- Diabetes: yes, no
- Current smoker: yes, no
- Previous myocardial infarction: yes, no
- Inclusion criteria: STEMI, NSTEMI, stable angina
- Influenza season: 2016-17, 2017-18, 2018-19, 2019-20
- Hemisphere: North, South

The results of the subgroup analyses will be reported as a forest plot in Figure 3 including p-values from interaction tests.

5.2.2 SECONDARY EFFICACY ENDPOINTS

The three components of the primary composite efficacy endpoint and CV death, all at 12 months, will be considered as key secondary efficacy endpoints. Other secondary efficacy endpoints are: unplanned revascularization, stroke/TIA, the composite of CV death, AMI and stent thrombosis, hospitalization for heart failure, and hospitalization for arrhythmia, all at 12 months post randomisation. Kaplan-Meier plots will be presented separately for each of the 3 components of the composite endpoint in Figure 2. Results for all secondary endpoints will be reported in Table 2 and Table S2.

Table 2. 12 months' efficacy

	Placebo	Vaccine	Hazard Ratio (95% CI)	P-value
Primary Endpoint, no. (%)				
All-cause death, AMI or stent thrombosis				
Key Secondary Endpoints, no (%)[$]				
All-cause death				
Cardiovascular death				
AMI				
Stent thrombosis [*]				
Other Secondary Endpoints, no. (%)				
Unplanned revascularization				
Cardiovascular death, a new AMI or stent thrombosis				
Stroke, including TIA				
Hospitalization for heart failure				
Hospitalization for arrhythmia				

** Stent thrombosis is defined as angiographically verified stent occlusion with acute clinical presentation.*

5.3 SAFETY ASSESSMENTS

5.3.1 ADVERSE EVENTS

The number and percentage of patients with serious adverse events (SAE) will be reported by treatment group. Comparisons between the two treatment groups will be carried out using a Fisher's exact test or a Chi-squared test as appropriate.

Table 3 Serious Adverse Events

Serious Adverse Event	Placebo	Vaccine	P-value
SAE term #1			
SAE term #2			
…			

5.3.2 CLINICAL LABORATORY ASSEMENT

No laboratory assessments will be reported.

5.3.3 OTHER SAFETY ASSEMENT

No other safety assessments are planned.

6 STATISTICAL ANALYSES

6.1 ANALYSIS METHODS

6.1.1 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the composite of all-cause death, AMI or stent thrombosis from baseline to 12-months. The number of patients with an event and Kaplan-Meier cumulative percentage at 12 months will be reported by treatment arm in the m-ITT population. The event rates in the two groups will be compared using a log-rank test. A Hazard Ratio (HR) and two-sided 95% Confidence Interval (95% CI) will be estimated using a Cox Proportional Hazards (PH) model including treatment group as a covariate with adjustment for the stratification variables, center and STEMI/NSTEMI/stable angina. The absolute difference in the 12 month cumulative percentage and corresponding 95% CI will also be reported. Kaplan-Meier survival plots will be presented by treatment group. A two-sided p-value less than 0.05 will be considered statistically significant. No imputation of missing values due to loss to follow-up will be carried out for the primary analysis. Randomized patients who are lost to follow-up after receiving the study treatment will be censored with 0.5 days of follow-up or at the date of last contact.

6.1.2 ANALYSIS OF THE SECONDARY ENDPOINT(S)

If superiority is demonstrated for the primary efficacy composite endpoint a log rank test will be used to compare event rates in the two randomised arms for the four key secondary efficacy endpoints. A fixed sequence hierarchical testing approach will be used to control the overall type-1 error rate; the sequence is all-cause death, CV death, AMI, and stent thrombosis. For example, a test for CV death will be carried out only if superiority is demonstrated for all-cause death, and a test for AMI will be carried out only if superiority is demonstrated for both all-cause death and CV death. A two-sided p-value less than 0.05 will be considered statistically significant for each test. All other secondary endpoints will be considered exploratory and hypothesis generating.

Kaplan-Meier survival plots will be presented by treatment group for each of the four key secondary endpoints. All time-to-event secondary endpoints will be analysed using the same methods as for the primary endpoint.

6.1.3 SAFETY ANALYSES

The number of treatment related serious adverse events (SAE), including treatment related deaths, are reported divided by their relationship as ‘definitely’, ‘probably’ and ‘possibly’ related to treatment. The proportion of patients with SAE will be compared descriptively across treatments and differences assessed for clinical significance. The number (and percentage) of patients experiencing each AE/SAE will be presented for each treatment arm categorized by severity. For each patient, only the maximum severity experienced of each type of AE will be displayed. The number (and percentage) of occurrences of each AE/SAE will also be presented for each treatment arm.

6.1.4 BASELINE DESCRIPTIVE STATISTICS

Categorical data will be summarized by numbers and percentages. Continuous data will be summarized by mean, standard deviation (SD) and range if data are normal and median, interquartile range (IQR) and range if data are skewed. No tests of statistical significance will be undertaken for baseline characteristics.

6.1.5 SUB-GROUP ANALYSES

Subgroup analyses will be performed for the primary efficacy endpoint using a Cox PH model including treatment group, subgroup and a treatment group-subgroup interaction. The pre-specified subgroups are listed in 5.2.1. Subgroup level HRs and 95% CIs for the primary efficacy endpoint will be presented using forest plots along with the interaction p-value.

6.1.6 TABULATION OF INDIVIDUAL PARTICIPANT DATA

No tabulation of individual participant data will be presented.

6.1.7 EXPLORATORY ANALYSES

From a hypothesis generating perspective we aim to follow up patients through registries beyond 1 year and up to 5 years. Because influenza may precipitate plaque rupture it is possible that a single influenza vaccination in the early phase after a myocardial infarction may stabilize non-culprit coronary plaques. Endpoints beyond 1 year will be regarded as exploratory.

6.1.8 SENSITIVITY ANALYSES

The primary efficacy analysis will be repeated in the m-ITT population. A sensitivity analysis for the primary efficacy endpoint will be repeated with missing outcomes imputed as described in 6.2.

6.2 MISSING DATA

Missing data will be reported for all baseline and outcome variables. Imputation of missing values for baseline variables or outcomes will be carried out only as a sensitivity analysis for the primary endpoint. Imputation of missing values will be carried out with multiple imputation using chained equations.

6.3 STATISTICAL SOFTWARE

The analysis will be carried out using Stata version 16.1. Other packages such as SAS 9.4 M4, R 3.5.0, or Python 3.6 may be used if necessary.

7 ABBREVIATIONS

ACE-I	Angiotensin-converting-enzyme inhibitor
AE	Adverse Event
AFT	Accelerated failure time
AMI	Acute myocardial infarction
ARB	Angiotensin II receptor blocker
ASA	Acetylsalicylic acid
CI	Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
HR	Hazard ratio
IAMI	Influenza vaccination After Myocardial Infarction trial
IEC	Independent endpoint committee
IQR	Interquartile range
IRR	Incidence rate ratio
ITT	Intention-to-Treat
LAD	Left anterior descendent coronary artery
LCx	Left circumflex coronary artery
LM	Left main coronary artery
MI	Myocardial infarction
MIm	Multiple imputation
NSTEMI	Non-ST-segment elevation myocardial infarction
P2Y12	(Specific type of receptor found on platelets)
PCI	Percutaneous coronary intervention
PH	Proportional hazards
RCA	Right coronary artery
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SHR	Subhazard ratio
STEMI	ST-segment elevation myocardial infarction

8 SAP AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	Apr 25, 2018	First draft	
1.1	May 30, 2018	Sections 3 and 6 were completed. Minor revision for other sections.	
1.2	May 31, 2018	Abbreviations in Section 7 were completed.	
1.3	June 16, 2020	Clarification of key secondary endpoints and the procedure for controlling the overall type-1 error. Clarification of the method of analysis for the primary efficacy endpoint and key secondary endpoints. Clarification of the analysis populations.	An independent review of the SAP version 1.2 was carried out Tim Collier and Stuart Pocock, LSHTM. The wording of the SAP was vague in places and methods of analysis required clarification.
1.4	February 16, 2021	Length of stay in hospital removed from the list of other secondary endpoints.	Before database lock and unblinding it was discovered that length of stay in hospital was very poorly recorded mostly due to patients being discharged to other healthcare settings.