PROJECT TITLE: Randomized Controlled Trial Comparing Intracoronary Administration of Adenosine or Sodium Nitroprusside to Control for Attenuation of Microvascular Obstruction During Primary Percutaneous Coronary Intervention

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1. PROJECT TITLE: Randomized Controlled Trial Comparing Intracoronary Administration of Adenosine or Sodium Nitroprusside to Control for Attenuation of Microvascular Obstruction During Primary Percutaneous Coronary Intervention (EME project number: 09/150/28)

2. SUMMARY

2.1. DESIGN: Randomized, controlled, open label trial with blinded endpoint analysis.

2.2. STUDY POPULATION: Patients presenting with STEMI and undergoing proposed P-PCI at 2 major tertiary cardiac centres (Leicester and Leeds). **Inclusion criteria**: (1) ≥ 18 years age. (2) Informed ASSENT (verbal consent) prior to angiography and then written informed consent within 24 hours from the procedure. (3) STEMI ≤ 6 hrs of symptom onset, requiring primary reperfusion by PCI. (4) Single-vessel coronary artery disease (non culprit disease ≤70% stenosis at angiography). (5) TIMI flow 0/I at angiography. **Exclusion criteria:** (1) Contraindications to P-PCI/ MRI scanning/ contrast agents /study medications. (2) SBP <90mmHg. (3) Cardiogenic shock. (4) Previous Q wave MI. (5) Culprit lesion not identified or located in a by-pass graft. (6) Stent thrombosis. (7) Left main disease. (8) Severe asthma. (9) GFR<30ml/min. (10) Pregnancy.

2.3. INTERVENTIONS: All patients will be treated with Bivalirudin and thrombus aspiration and will be randomly assigned, with stratification for symptoms to balloon <3 hrs or >3hrs and anterior MI or not, to one of the following 3 groups: 1) Standard PCI (control). 2) Distal intracoronary (IC) adenosine (1mg) via the flushed aspiration catheter used as a microcatheter (AMC) followed by 2mg to LCA or 1mg to RCA via guide catheter. 3) Distal IC SNP (250 mcg) via AMC followed by 250mcg via guide catheter..

2.4. OUTCOME MEASURES: Primary: CMR measured infarct size (IS) at 48-72 hrs post procedure. CMR analysis will be performed blinded to treatment allocation Secondary: CMR incidence and extent of MVO, myocardial salvage, incidence of haemorrhage, LV volumes and function in the acute stage, Major Adverse Clinical Events (MACE) and components at 6 months, angiographic markers of MVO, LV volumes and functions, ECG ST resolution and cardiac enzymes.

2.5. SUB-STUDY: Blood samples will be taken from patients to assess the effect of these treatment agents on platelet activation within the coronary circulation.

2.6. PROPOSED SAMPLE SIZE: to detect a 5% difference in infarct size with 90% power, alpha 0.05 and two-tailed, assuming expected infarct size of 20% with a SD 10%, will require 86 pts/group. To allow for dropouts before CMR a total of 297 patients will be recruited.

2.7. STATISTICAL ANALYSIS: Data will be examined for normal distribution. Groups will be compared by ANOVA, and multivariate analysis will take into consideration possible confounders. Primary analysis will be by intention-to-treat with a secondary analysis by treatment received. For secondary endpoints, time-to-event regression methods will be used to investigate potentially important predictors of MACE. Initial analyses will adopt a 'complete case' approach, but sensitivity analyses using multiple imputation methods will also be undertaken to assess the plausibility of the 'missing at random' assumption. The study will be monitored by an independent data safety monitoring board. There will be an interim analysis when 35 patients have been included in each arm. Keith Abrams, Prof. of Medical Statistics and NIHR Senior Investigator, will oversee the statistical analysis.

3. Background:

3.1. Existing research

During primary PCI (P-PCI) micro-vascular obstruction (MVO) may occur to varying degrees ranging from true "no-reflow" phenomenon (was flow and then there isn't) to less degrees of impairment of distal vessel micro-angiographic flow. MVO in its various degrees has been reported to occur in 40 to 60% of patients with ST-segment elevation myocardial infarction (STEMI) and treated with P-PCI, and often

despite optimal reperfusion (Thrombolysis in myocardial infarction (TIMI) flow grade 3) in the main epicardial coronary artery(1)(2). MVO is associated with Major Adverse Cardiac Events (MACE) rate in up to 30% at 1 month and 60% at 12 months (2). In a large clinical series of >10,000 PCI patients, MVO was associated with an overall incidence of in-hospital death of 15% and MI of 31% (3). The presence of MVO, as detected by cardiac magnetic resonance imaging (CMR), is a strong predictor of left ventricular (LV) remodelling (4) and MACE at one year (5). Treating MVO has been shown to be associated with improved left ventricular remodelling even in the absence of significant improvement in regional contractile function (3). Several review articles (6-8) addressing MVO management in the setting of P-PCI suggests that currently there is no consensus on the optimal strategy to limit MVO during P-PCI. Furthermore our own discussions with UK physician colleagues support the notion that there is little consensus on the best method for detecting MVO and almost none on how best to treat it once detected. Degrees of MVO less than "total no-reflow" are under-recognised and under-investigated despite contributing to subsequent MACE.
Multiple factors may be responsible for the development of MVO, but predominantly it is the result of distal embolization, vaso-active substance release and development of microvascular ischemia-reperfusion injury (9-12). Thus, it may be hypothesized that combining a mechanic and pharmacologic approach may achieve optimal microvascular reperfusion. Indeed the TAPAS (13) trial showed benefit (improving angiographic microvascular flow) was seen following aspiration occurred irrespective of presence of visible thrombus. Moreover, while manual thrombectomy has been shown, in two recent trials, to reduce infarct size and preserve microvascular integrity assessed by CMR (EXPIRA trial) (14), and to improve LV function and tissue perfusion assessed by myocardial contrast echocardiography (MCE) (15), it is believed this is not the sole pathology. Given these results, it has become generally accepted that most patients undergoing P-PCI should undergo thrombus aspiration. However residual mortality and subsequent MACE rates suggest there is room for improvement even in those patients who do not suffer classical “no-reflow” phenomenon.

Adenosine and sodium nitroprusside in P-PCI
Attempts to manage MVO have led to the evolution of several treatment regimens including the use of vasodilators (adenosine, verapamil, sodium nitroprusside (SNP) and nicorandil) (16-24). Among these, adenosine and SNP are the two most commonly used agents to induce hyperaemia amongst most operators.

Adenosine is a potent vasodilator of arterioles and has been shown to reduce adverse outcome and death in the setting of no-reflow albeit in small studies (25). It has cardio-protective effects, although the mechanisms are not fully understood. In experimental animal models, adenosine reduces ischemia-reperfusion injury, limits infarct size, and improves ventricular function (26). The mode of action appears to be receptor-mediated inhibition of neutrophil-related processes (27). In isolated, perfused rat hearts, adenosine given at time of reperfusion increases glucose oxidation and inhibits glycolysis, reduces tissue lactate levels, and increases ATP levels. These effects decrease cellular acidosis and Ca^{2+} overload and are associated with beneficial effects on mechanical function (28). Adenosine also decreases post-ischaemic cardiac TNF (tumour necrosis factor), which contributes to post-ischaemic myocardial dysfunction by direct depression of contractility and induction of myocyte apoptosis (29). Furthermore, prophylactic adenosine infusion is effective in reducing myocardial reperfusion injury (30). Almost all the clinical studies with adenosine to date have examined routine administration on classical “no-reflow” following P-PCI. For example, Assali (21) reported that in patients receiving IC adenosine boluses, (24–48 µg) before and after each balloon inflation during P-PCI there was significantly less no-reflow compared to those who did not (5.9 v 28.6%; P=0.014). Some data also suggests adenosine may improve microvascular function and reduce infarct size. Micari (31) reported that infarct size expressed as a percentage of the MCE area at risk (AAR), was less in patients treated with IV adenosine (50 to 70 µg/kg/min) for 3-h at 3-5 days and at 4 weeks after P-PCI. However in the AMISTAD II study (32), of 2118 patients with anterior STEMI, randomized to a 3-h infusion (started within 15 mins of reperfusion, fibrinolysis or PCI) of either IV adenosine (50 or 70 µg/kg/min) or to placebo, there was no reduction in the composite primary endpoint of death from any cause during 6 months follow up, or in-hospital CHF and first re-hospitalization for CHF. Interestingly, a recent post hoc analysis of this study reported that a

3-h adenosine infusion administered within the first 3-h of onset of AMI decreased 1 month (5.2 v. 9.2%, respectively, P = 0.014) and 6-month mortality (7.3 v. 11.2%, P = 0.033) compared with placebo, and reduced the composite clinical endpoint of death or CCF at six months (12.0 v 17.2%, P = 0.022) (33). In a trial of anterior STEMI, a very high dose (4 mg) IC bolus of adenosine, given before P-PCI significantly reduced no-reflow rate, (TIMI flow 30 mins post-procedure), without reported complications. However, temporary pacemaker wires were needed prophylactically prior to drug injection to counter AV block (25). Contrary to this in a recent trial of 448 patients presenting with STEMI randomized to 2 bolus injections of IC adenosine (2x120 μg in 20 ml NaCl) or placebo there were no significant differences in the primary (incidence of residual ST-segment deviation <0.2 mV, 30-60 mins post PCI) or secondary endpoints (ST-segment elevation resolution, myocardial blush grade (MBG), TIMI flow on angiogram post PCI, enzyme infarct size, nor clinical outcome at 30 days) (34).
There has been a recent publication that has clarified the dosing conundrum somewhat (ref Grygier et al. Am J Cardiol 2011;107:1131–1135) The authors studied 70 patients presenting with STEMI for P-PCI andthey were randomised to either AD: 2x 2 mg bolus for the (LAD) 2x1mg bolus for the (RCA) in 35 patient or Placebo (35 pts) This high dose AD regime was associated with significant improvements in Myocardial Blush grade 3,, 23 patients (65.7%) in the adenosine group and 13 (37.1%) in the placebo group (p<0.05). Additionally, resolution of ST-segment elevation (>50%) was more frequently observed in the adenosine than placebo group: 27 (77%) versus 15 (43%) respectively (p <0.01). There was a borderline significant reduction in TIMI grade 3 flow after PCI. 32 patients (91.4%) in the adenosine group and 27 patients (77.1%) in the placebo group (p= 0.059).
This is a larger dose than originally planned in this study but was given via the guide catheter rather than via micro catheter as had been proposed. This may have further advantage as described below . There is always the concern that with increased doses, complete heart block may occur (see ref 25 above). However even though they had the temporary pacing wire available on the table it was not used in any of the cases.

There appears thus conflicting data as to whether adenosine is effective at attenuating MVO and reducing MACE associated with STEMI. Basic research suggests that there are beneficial effects but the available clinical data are contradictory. The failure of the recent randomised clinical trials to show a reduction in MVO may be in part related to the lack of a sensitive imaging modality such as CMR to detect its MVO presence and/or the failure to deliver adenosine closer to the site through a distally placed micro-catheter.
Changing the dose from the data we had available on first submission to what we have recently available through publication will improve the chances of demonstrating the efficacy of AD in this trial.

Sodium Nitro-Prusside is a direct nitric oxide (NO) donor that requires no intracellular metabolism. Experimental studies have demonstrated that NO has multiple vascular functions, including vasodilatation of arterioles, inhibition of platelet adhesion and anti-inflammatory activity (35). Local delivery of SNP is effective in reducing no-reflow in animal reperfusion-injury models (36, 37). In isolated reperfused guinea pig hearts, exogenous NO has been shown to have cardioprotective effects during post-ischemic reperfusion, including prevention of coronary interstitial oedema (38). Based on previous reports, in which Doppler flow wires were used, the hyperaemia induced by both SNP and adenosine greatly exceeded the hyperaemia induced by calcium channel blockers. Furthermore, IC SNP appears to produce an equivalent but more prolonged coronary hyperaemia than adenosine (39-42). Shinozaki (39) reported that selective IC administration of SNP (120 μg), in repeated doses, through a *drug delivery catheter* before each balloon dilatation, significantly reduced no-reflow and improved reperfusion of infarcted myocardium assessed by post-PCI MBG and corrected TIMI flow count (CTFC). Hillegass et al (43) retrospectively analysed 19 consecutive patients who underwent standard PCI procedures on either SVGs or native coronary vessels complicated by either no-reflow or impaired flow. They used high doses of IC SNP (50–1000μg; mean cumulative dose=435μg) and noted a significant improvement in coronary flow as assessed by TIMI flow grade and TIMI frame counts, without any significant hypotension. In the setting of P-PCI, Pasceri (44) studied 23 consecutive patients developing no-reflow, who were given a mean dose 90+/-50 μg of SNP via the guide catheter and reported successful reversal with no significant

associated adverse effects other than transient hypotension. However, this study had no control group and was indeed a study of treatment rather than prevention as was Wang's (45) who reported beneficial effects of IC administration of (100 μg) SNP in multiple boluses (the total drug dose varied from 100 to 700 μg) through the guiding catheter using a 3 ml syringe, in 11 patients undergoing P-PCI. Hendler (42) assigned 40 patients with STEMI referred for P-PCI to one of 4 groups: IC injection of SNP (100-500 μg), adenosine (60-120 μg) or verapamil (100-500 μg), which were compared with the control group-IC injection of nitroglycerin (200-400 μg). Despite the small number of the studied patients, they showed that SNP was the most beneficial of the agents tested in terms of improving myocardial reperfusion assessed by MBG compared to the control group (p=0.023), which correlated with an improvement in LVEF compared to baseline (p =0.048). In the adenosine group, there was a clear trend toward a better MBG (p = 0.28), but this did not reach statistical significance. In comparison with the control group, verapamil did not show any beneficial effect on the MBG. Only one study has examined the effect of SNP for the prevention of no-reflow in a prospective, controlled clinical trial. Amit (46) randomized 98 patients presenting with STEMI to receive either a single dose of SNP (60 μg) selectively into the infarct related artery or placebo. The study concluded that selective IC administration of a fixed dose of SNP in patients with STEMI did not improve coronary flow and myocardial tissue reperfusion but improved MACE, including death, recurrent MI, and target lesion revascularization (TLR), (6.3% of the SNP group v. 20% of the placebo group, P=0.05) at 6 months (results may be due to a type I statistical error). These numerous various studies are difficult to interpret since they were heterogeneous in the presentation (acute STEMI v elective PCI), methodology (drug, dose, route and frequency of administration) with decisions on adjunctive management largely at operator discretion, resulting in treatment heterogeneity, and the outcome measures used. All included angiographic but various endpoints (epicardial arterial TIMI flow grade or CTFC).

Bivaluridin, a direct thrombin inhibitor, has been shown recently in the ACUITY (47) and HORIZONS-AMI (48) trials to provide similar efficacy with less bleeding compared with UF heparin plus GPIIb/IIIa receptor inhibitors in P-PCI patients. Bivalirudin inactivates thrombin and blocks thrombin-mediated platelet activation and aggregation (49). It has demonstrated encouraging results when administered via IC route to treat MVO due to distal microembolization in a complex patient with AMI (50). IV Bivalirudin is becoming standard of care in P-PCI due to lower bleeding rates but paradoxically MVO rates may increase since it may reduce NO availability in endothelial cells by trapping myeloperoxidase in the vascular wall (51). The proposed study will inform on the impact of bivalirudin use on MVO - the negative effects shown by Rudolph (51) was in a trial of a small number of elective PCI patients, the end point being brachial artery flow mediated dilatory response in association with plasma MPO levels. Accepting there may be an attenuation of vasodilatory agent response with the use of Bivalirudin, this makes our proposal important, to ensure we understand the interaction between these various agents - We plan to and look to secure funding through medicinal companies to measure plasma myeloperoxidase levels in trial patients.

3.2. Rationale for use of CMR as primary outcome measure in trials of MVO

Prognosis following Acute Myocardial Infarction

LV systolic dysfunction has long been recognised as an important event in survivors of acute myocardial infarction (AMI) (52). In 605 male survivors of AMI, LV ejection fraction (EF), LV end diastolic volume (EDV) and end systolic volume (ESV) were all predictive of mortality at mean 78 months follow-up but only ESV was an independent predictor (52). The importance of reductions in EF, infarct size and increases in LV volumes were confirmed in 2300 survivors of STEMI receiving reperfusion therapy (53). Limiting infarct size leads to improved LV function and attenuation of subsequent cardiac remodelling (LV dilatation > 20%). CMR is the gold standard technique for quantification of LV volumes and function, and delayed contrast imaging can detect and quantify myocardial infarction with unique precision (54,55). In an dog model of reperfused MI, MVO and infarct size were strongly related (r=0.89 and r=0.81 resp) to early LV remodelling. MVO was the best independent predictor(r^2=0.71, p<0.001) of remodelling(56). In humans, CMR measured MVO correlates strongly with ST-segment resolution in patients undergoing P-PCI but relatively weakly with MBG and not with TIMI flow(4). CMR measured

MVO is not simply an oversensitive measure of small vessel obstruction, however. Larger infarcts detected on CMR are consistently associated with larger ventricular volumes, reduced EF and increased MVO which occurs in 40-60% of P-PCI patients (4,57-59). Infarct size and MVO are consistently related to adverse ventricular remodelling. In P-PCI studies which included MVO in a multivariate model, it predicts remodelling independently of infarct size, EF and cardiac volumes (4, 57, 59-61).

Prognostic value of CMR following P-PCI

Infarct size and MVO correlate with medium-term prognosis. For example, in 122 patients with STEMI undergoing P-PCI, LVEF, LVEDV and ESV all correlated with infarct size ($r=-0.75$, $r=0.42$, $r= 0.69$ respect, all $p<0.001$) and outcome. Infarct size on CMR was the only independent predictor of MACE (1 death, 1 MI and 16 heart failure admissions) at 2 years (61). MVO was also associated with MACE but not included in the multivariate model in this study (61). In another study of 184 patients undergoing successful P-PCI, the presence of MVO on CMR was independently predictive of MACE at 1 year (5). MACE in those with no MVO v MVO was 6.1% v 43.7% respectively. In a multivariate model including MVO, Global Registry of Acute Coronary Events score, infarct size and EF the presence of MVO was associated with an odds ratio of 8.7 (3.6-21.1, $p<0.001$) for the occurrence of MACE at 1 year(5).

Importance of myocardial salvage following STEMI

The extent of myocardial necrosis after an acute coronary occlusion is variable and dependent on a number of factors. These include - time to reperfusion, collateral blood flow, metabolic tissue demand etc, but with total area at risk (AAR), as determined by amount of tissue acutely hypoperfused at time of coronary occlusion, probably being most important (62). Although infarct size is undoubtedly of major prognostic significance following AMI, extent of salvaged myocardium may be an equally important predictor of outcome. In a retrospective study of 765 patients receiving various reperfusion strategies, including P-PCI in 634 patients, myocardial salvage was assessed by single photon emission tomography (SPECT) 7-14 days post STEMI (63). In those patients with myocardial salvage index (AAR-infarct size/AAR) below the median, mortality was 5.0% compared to 1.0% in those with above median salvage index (63). Salvage index remained an independent predictor of mortality with AAR and age although measures of LVEF and MVO were not included in the multivariate model. When revascularisation strategies are to be compared myocardial salvage may be an even more important measure of outcome (64). When the efficacy of reperfusion strategies in STEMI is assessed (attenuation of MVO), then quantified AAR may influence final infarct size as much as the different therapies being studied and therefore should be routinely taken in to consideration.

CMR and myocardial salvage index

CMR can accurately quantify myocardial salvage index. During ischaemia or infarction, myocardial tissue develops oedema which can be detected as high signal intensity on T2 weighted (T2w) images, and the oedema area is greater than the area of irreversibly damaged, necrotic myocardium (65-67). Myocardium with high T2 signal closely correlates with AAR, as confirmed in experimental models of both reperfused (68) and non-reperfused myocardial infarction (69). As expected the extent of salvaged myocardium decreases with increasing infarct size, as measured by CMR (66). Two small clinical studies have validated the myocardial salvage index with CMR v SPECT (70, 71) and the calculated AAR appears to be stable for at least 1 week post STEMI (70). A major advantage of CMR is that salvage index can be measured during a single examination, in addition to, quantification of volumes, function, infarct size and MVO. Although T2w imaging has been prone to artefact, recent advances including increased slice thicknesses, use of coil signal intensity correction algorithms and motion correction have made the assessment of oedema much more robust (65). In 137 post P-PCI patients it was demonstrated that myocardial salvage index was strongly related to ECG ST segment resolution and was a stronger predictor of LV remodelling at 4 months than MVO (72).The prognostic importance of myocardial salvage index in predicting outcome has been confirmed in a single centre study with 208 patients following PPCI (73). Myocardial salvage index was the strongest predictor of MACE (death, re-infarction or new heart failure) at six months and this effect was independent of infarct size and MVO (73).The findings need to be confirmed in further studies but suggest that myocardial salvage index is a better marker of prognosis than infarct size and MVO.

3.3. Risks and benefits of managing MVO

Potential **benefits** of prevention/attenuation of MVO at the time of P-PCI are:

1. reduce the risk of recurrent MI/death, since MVO itself is associated with five- ten-fold increase in mortality and a high incidence of re-infarction (in up to 32%) (11,65,74,75)
2. reduce infarct size leading to less ventricular remodelling resulting in better clinical outcomes in the medium-term (death, ventricular arrhythmias and most importantly heart failure admissions).
3. provide economic benefits, associated with improved clinical outcomes (shorter LOS/readmissions).

Potential **risks** of adjuvant pharmaco-therapy for MVO at the time of P-PCI are:

1. haemodymanic instability, including hypotension due to vasodilatory action of adenosine and SNP, or tachy/brady-arrhythmias and adenosine administration may induce bronchospasm in asthmatic subjects. However, it has been shown in previous studies that IC administration of either drug even in very high doses (25,43) does not cause significant adverse effects. Minor effects which occur resolve spontaneously, probably as a consequence of the very short half life of both drugs.
2. infarct size may be increased as injection of the study drugs prior to PCI could cause embolisation of thrombus to the microcirculation. However, selective IC administration of the drugs in the distal coronary bed *after* thrombus aspiration as in this study, will minimise the chances of this occurrence and will maximize drug delivery locally and reduce systemic effects.
3. the combined mechanic and pharmacological strategy may not lead to either prognostic benefit to the patient or reduced costs to the NHS.

3.4. Rationale for current study: >100,000 patients suffering STEMI present in the UK each year (76). P-PCI in the UK is increasing exponentially. In 2004 there were <1500 P-PCI and in 2007 and 2008 these figures had increased to 5902 and 9224 respectively (BCIS database). Although P-PCI delivered quickly is more effective than thrombolysis, the efficacy of this, essentially mechanical, technique is limited by the unpredictable phenomenon of no-reflow and the under-stated lesser degrees of MVO. As more UK centres adopt P-PCI the dilemma of how to attenuate MVO will remain. Currently there is no consensus on the optimal management to prevent or attenuate MVO particularly when thrombus laden lesions are treated with P-PCI. There is divergent clinical practice, even within institutions, in the UK and worldwide. This is because there is no solid evidence base to inform clinicians. The current options for interventional cardiologists are: 1. Routinely aspirate thrombus and give IC vasodilator during the intervention but only in high burden thrombus formation lesions. 2. Perform a standard P-PCI only and then give IV vasodilator if angiographic no-reflow develops. 3. Routinely consider that angiographically silent MVO (i.e a grade below true “no-reflow”) may have important impact on infarct size and clinical outcome and treat prophylactically. Few if any clinicians follow this thinking. Indeed, it appears impossible to predict the incidence of (no-reflow/MVO) from the presenting angiogram (pre or post wire or balloon) and it can be argued that irrespective of thrombus burden it would be better to undertake prophylactic treatment in all patients, following the use of aspiration catheter, with delivery of agents able, in theory at least, to reduce (angiographically undetectable) MVO. Several studies of IC adenosine or SNP have shown favourable effects in attenuating MVO. However, the size of effect with either drug and whether indeed there is a difference between them in reducing MVO and infarct size is undetermined.

In summary, the proposed study will test the magnitude of effect of adenosine and SNP in reducing or preventing MVO and infarct size, administered optimally by distal intra-coronary injection and with MVO detected optimally (using CMR).

This study is unique in using the following potentially optimum measures to prevent MVO:

1- The delivery method; we will use the thrombus aspiration catheter as a microcatheter to deliver the assigned drug distally in the IRA instead of non-selective guide-catheter delivery as used in other studies (34). Only the REOPEN-AMI study (below) has a similar technique but with a standard microcatheter.

(Nb We plan to use a thrombus aspiration device which is standard of care for pre stenting thrombus aspiration in the 2 centres to deliver the study drugs. While the thrombus aspiration catheter has no indication for delivery of drugs it is has a short over the wire segment and then it is monorail allowing the wire to be maintained in the coronary artery. It has a lumen (used to aspirate the thrombus) which

can be used easily to deliver study drugs. The other option is to use microcatheters that are merely a lumen. Such catheters would require extending the intracoronary guidewire, so prolonging the procedure and with the other risk of losing distal wire position. We anticipate that the aspiration catheter would be suitable in ~80% cases. Unsuitability is defined by not being able to easily pass the catheter beyond the lesion pre stenting or the stent post stenting. If this happens in normal clinical situations aspiration is normally abandoned. In this study a microcatheter will be taken to deliver the agent distally whether aspiration has been successful or not.

Three scenarios

a, Aspiration catheter - aspirate thrombus - remove catheter from guide catheter- flush externally - re-introduce maintaining distal wire position- inject first dose study drug- remove aspiration catheter- stent- re-introoduce aspiration catheter- give second dose study drug Likely success ~80%

b. Aspiration catheter - will not pass- exchange for microcatheter (Finecross™ by extending wire- inject first dose study drug- remove microcatheter- stent- re-introduce microcatheter- give second dose study drug Likely needed in ~ 15%

c. Aspiration catheter - aspirate thrombus - remove catheter from guide catheter- flush externally - difficult to reintroduce past proximal or mid vessel stent- remove aspiration catheter- introduce microcatheter (Finecross ™) by extending guidewire- give second dose study drug Likely needed in~5%

2- The contemporary treatment with Bivalirudin which has been shown to have net clinical benefit compared to GPIIb/IIIa receptor inhibitors in STEMI patients treated with P-PCI.

3- Uniquely chosen drug dosing regimens

4- The use of CMR for the primary endpoint. As outlined above, CMR offers a unique and robust assessment of the success of optimal reperfusion for STEMI. CMR infarct characteristics are the best proven *surrogate* markers of medium-term outcome in patients with STEMI treated by PPCI (5,61). CMR detected MVO will provide a more robust assessment of the differences in the *efficacy* and *safety* of the pharmaco-therapeutic strategies being tested that may only be seen with a much larger population if there were reliance on angiographic, electrical (ECG), or clinical outcomes alone. We will also obtain a greater understanding of the *mechanisms by* which differences in outcome between the three groups may result. The effect on myocardial salvage and subsequent ventricular remodelling and medium term outcome will be well established through the use of CMR in this trial population.

The proposed study design is similar to the **REOPEN-AMI study** (77) which is currently recruiting, in terms of distal IC administration of the same agents as in our proposed study in P-PCI after performing thrombus aspiration. However, there are notable differences, namely:

1. The primary end point of the REOPEN-AMI is ECG ST resolution which is not as sensitive as the CM R marker of MVO as we are planning.
2. Different doses and control arms are being used. Drug doses in REOPEN-AMI, are: adenosine 80µg bolus + 2mg infusion and SNP 60µg bolus followed by 100µg in 33ml over 2 min and placebo 33ml saline infused over 2 min). In the current proposal and after careful review of all the available data, two IC boluses of the assigned treatment agents; adenosine (1 x 1mg followed by 1mg RCA or 2mg LCA) or SNP (2x250 µg) will be delivered via thrombus aspiration catheter, the first bolus being given after thrombus aspiration, the second after stenting (see justification of dosing below). The control arm will have standard P-PCI following thrombus aspiration *without* microcatheter instrumentation and saline infusion, which theoretically could increase infarct size by causing further micro-embolisation.
3. In REOPEN-AMI the imaging modality is myocardial contrast echocardiography which gives only a semi-quantitative score for MVO and cannot absolutely quantify MVO or measure infarct size as is the case with CMR.
4. In REOPEN-AMI GPIIb/IIIA is used which is being contemporarily replaced by the use of Bivalirudin.
5. No mechanistic investigations are included in REOPEN-AMI.

We believe that our trial design using CMR to detect endpoints will give a more robust assessment of the efficacy of adenosine and SNP in preventing/attenuating MVO than the REOPEN-AMI study. Given some similarities in trial design however, the two studies could be compared for clinical outcomes and we have thus extended our clinical outcomes time-point measure to 6 months. Heterogeneity measure would be used to determine if a meta-analysis was useful

4. Research objectives: The objectives of our **proposed study** are to determine:-
1) Whether adjunctive pharmaco-therapy at time of P-PCI and following thrombus aspiration, reduces CMR-determined MVO and infarct size.
2) Whether there is a difference between adenosine and SNP in reducing CMR-detected MVO and infarct size, both given selectively and distally via a thrombus aspiration catheter or a coronary microcatheter.
3) The correlation of angiographic, including the recently designed computer-assisted myocardial blush quantification 'Quantitative Blush Evaluator'(QuBE), and other myocardial perfusion markers, with CMR detected MVO and infarct size, as well as with clinical outcome at six months.

Study questions:
1) How might we attenuate MVO in patients undergoing P-PCI?
2) Should all or no patients receive MVO pharmaco-therapy routinely in addition to catheter aspiration and if so with which drug?
3) Can we determine which sub-group, if any, of studied patients would most benefit from prophylactic MVO management in the setting of PCI for STEMI?

Study hypothesis: Intracoronary adenosine or sodium nitroprusside delivered selectively via thrombus aspiration catheter (or if unsuccessful via a coronary microcatheter) following thrombus aspiration in P-PCI reduces MVO parameters and infarct size as measured with CMR, compared with standard treatment following thrombus aspiration.

5. Research design:

5.1. Study design: Two-centre, randomized, controlled, open label, clinical trial with blinded end-point analysis of primary and secondary outcome measures.

5.2. Consent: Patients will be asked to provide 'ASSENT' to the study after reading a shortened information sheet. This will be presented to the patient before angiography to limit potential researcher bias. Patients will then be asked to confirm their continued participation in the study (including CMR scans) the day following their heart attack and after reading all the patient information sheets. Patients will also be asked to consent to have their health records flagged with the NHS Information Centre Medical Research Information Service. Use of ASSENT followed by CONSENT within 24 hours is used in several studies.

5.3. Randomisation: If angiographic inclusion criteria are satisfied and there are no exclusions, the patient randomisation will be on the cath lab table, prior to PCI, using a dedicated 24/7 computerised telephone service, with stratification for "symptoms to balloon <3hrs or ≥3hrs", "anterior infarction" or not and by hospital. Randomisation will be via the telephone using the Sealed Envelope company.

5.4. Monitoring: Coordinating Centre
A central coordinating centre will be established and liaise with the principal investigators. The coordinating centre will be responsible for overall trial management, including production of final protocol, Case Record Forms, Manual of Operations, arranging meetings, data handling, quality assurance and statistical reporting. Regular progress reports and newsletters will be provided to all relevant parties. The coordinating centre will be based at Glenfield Hospital, Leicester with support from the University of Leicester Clinical Trial Unit (LCTU). The study will be overseen by an **Independent Data and Safety Monitoring Board** (DSMB) (Chair Prof Jennifer Adgey Belfast). There will be an interim analysis when 35 patients have been included in each arm and thereafter as determined by the DSMB.

5.5. Premature Termination: The trial may be terminated prematurely if the recruitment target cannot be met within the projected recruitment phase. The trial can be terminated by the co-ordinating centre

at an individual centre after agreement with the Steering Committee if the following occurs: The centre cannot comply with the requirements of the protocol. The centre is unable to comply with the required data standards. On the advice of the DSMB.

6. Study population:

6.1. Inclusion Criteria

1. ≥ 18 years age.
2. Informed ASSENT (verbal consent) prior to angiography.
3. STEMI ≤ 6 hrs of symptom onset, requiring primary reperfusion by PCI.
4. Single-vessel coronary artery disease (non culprit disease ≤70% stenosis at angiography)
5. TIMI flow 0/I at angiography.

6.2. Exclusion Criteria

1. Contraindications to: P-PCI *, CMR**, contrast agents, or study medications: Adenosine***, SNP****, Aspirin, Thienopyridine and Bivalirudin.
2. SBP ≤ 90mmHg
3. Cardiogenic Shock
4. Previous Q wave myocardial infarction
5. Culprit lesion not identified or located in a by-pass graft
6. Stent thrombosis. 7. Left main disease. 8. Known severe asthma.
9. Known stage 4 or 5 chronic kidney disease (eGFR<30ml/min). 10. Pregnancy.

*Exclusion criteria for P-PCI (presentation timing, inadequate arterial access etc); patient unable to tolerate "prolonged" PCI procedure (in operators' opinion).

**Absolute contra-indication to CMR (Pacemaker, ICD, intra-cranial metal clips).

***Contraindications to Adenosine (known hypersensitivity to Adenosine, sick sinus syndrome, second or third degree atrio-ventricular block – except in patients with functioning artificial pacemaker, long QT syndrome has been defined as QTc > 450 ms at baseline).

ECG will be undertaken just after the first dose of the study drug and QT/QTc will be recorded and compared to the baseline. If the QTc recorded after the first dose of the study drug exceeds 450ms or there is an increase in the QT/QTc of > 60 ms from baseline, the second dose will be abandoned and this will be recorded.

**** Contraindications to SNP (known hypersensitivity to SNP, compensatory hypertension - as may be seen in arteriovenous shunts or coarctation of the aorta, high output failure, congenital optic atrophy or tobacco amblyopia)

6.3. Withdrawal criteria: • Withdrawal of consent.

7. Planned interventions:

Those patients agreeing to be included will be pre-treated with Prasugrel and Bivalirudin (N.I.C.E and HORIZONS trial (48) and thrombus aspiration irrespective of visible evidence of thrombus according to TAPAS [14] and EXPIRA (13, 14), provided TIMI Grade 0/I flow. The first dose of one of the two trial agents will then be given via the thrombus aspiration (TA) catheter* according to randomisation group

Group 1: The control (comparator) group; A standard PCI including thrombus aspiration will be performed.

Group 2: Two separated** adenosine boluses will be delivered to the IRA. Following aspiration but pre PCI, 1 (one) mg Adenosine will be delivered to the infarct related artery via the aspiration catheter (ie to the distal Right CA, distal Left Anterior Descending or distal Circumflex arteries). If delivery of the aspiration catheter has not been possible as can sometimes happen due to vessel tortuosity then 1 mg will be given via the guide catheter to the right coronary artery or 2 mg via the guide catheter to the left coronary artery (see Grygier et al. Am J Cardiol 2011;107:1131–1135). The absolute new change is that in order to avoid crossing the newly deployed stent with the aspiration catheter post PCI, the second doses of Adenosine will be given via the guide catheter (1 mg if the IRA was the Right coronary artery and 2 mg if the IRA was in the Left CA (LAD or CX)

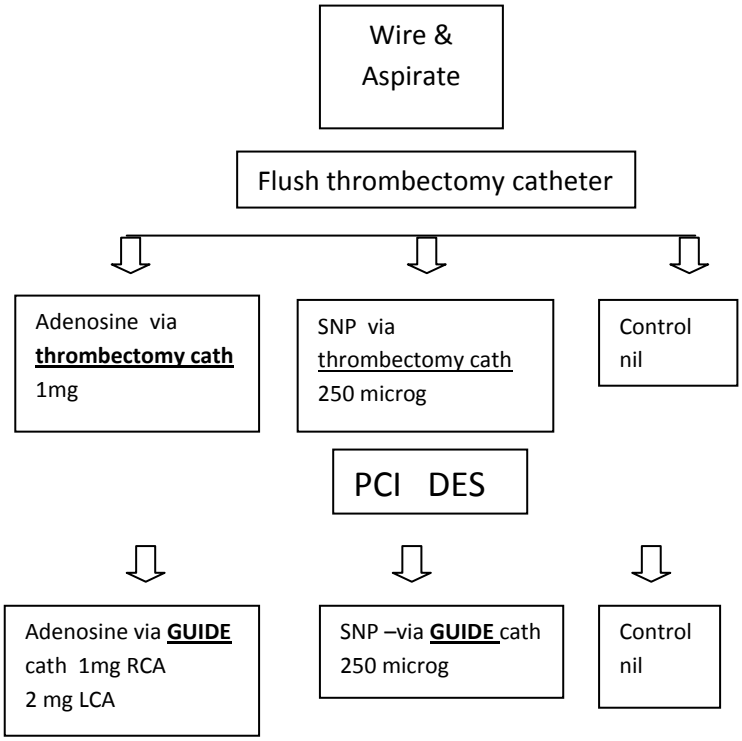
Previously all doses including the second dose were given via the aspiration catheter both pre and post procedure It has become clear that as the study was explained to our interventional colleagues some felt concern crossing a newly placed stent with the aspiration catheter to deliver the post PCI dose. Now that Grygier has shown some efficacy with the drug delivered via the guide catheter this overcomes the problem. However the reason we continue to give the doses pre stenting via the aspiration catheter is because Grygier's study was a small trial of only 70 patients in total, there were no clinical end points and some of the differences only just reached significance.

Group 3: Similarly the first of the two SNP boluses (2x250 µg) will be delivered IC via TA catheter placed distally in the IRA. The second post stent deployment dose will be delivered via the guide catheter. We have no new data to suggest a dose change from that previously indicated unlike with the Adenosine
***** Both drugs will be administered through a **Thrombus aspiration (Export XT 6F) catheter** placed distal to the angioplasty site following wire crossing the occlusion/lesion. Aspiration is routine at this point. This aspiration catheter will be used to deliver the first dose of drug . It is an atraumatic monorail aspiration thrombectomy catheter which offers several advantages. It is a 6Fr-compatible system, effectively reduces the thrombus burden and could be used for selective distal delivery of pharmacological agents into the IRA. In up to 20% of cases we may not be able to deliver the aspiration catheter and then will give the first bolus down the guide catheter .
***** In all cases** the first bolus will be given after thrombus aspiration, the second after stenting but via the guide catheter. The dose of the Adenosine will be 1 mg via the aspiration catheter to the IRA and post 1 mg for the RCA or 2 mg for the LCA. MVO occurs at the microcirculatory level, and based on previous data (29, 33, 78), it is postulated that distal delivery of a drug will provide sufficient concentration to the affected vessel without significant systemic adverse effects. All patients will be pre-treated with dual antiplatelet therapy with aspirin (300mg), followed by daily dose of 75 mg, and Prasugrel or Clopidogrel as follows:
Prasugrel dosage based on TRITON-TIMI 38 (79), NICE guidance (80) and summary of product characteristics (SmPC):
Loading dose of 60 mg, followed by maintenance dose 10 mg/day for up to 12 months
It has been agreed amongst our clinicians and in conjunction with our PCT, that considering the potential excess bleeding risk in patients > 75 years or < 60 kg or those with previous TIA or stroke will not be given Prasugrel. These patients will instead be given Clopidogrel doses (600mg loading and 75 mg per day for 12 months)
Bivalirudin will be given to all patients (0.75 mg/Kg bolus plus infusion of 1.75 mg/Kg/hr) in the absence of specific contraindication or modified dose if there is renal insufficiency, and will be discontinued at the completion of PCI, but could be continued for 4 hours if clinically indicated (operator unhappy with final stent result).The procedures will be performed with percutaneous (preferable) radial or femoral approaches using a 6-7 Fr sheath. Careful angiographic images with long acquisition will be acquired before and after wiring to enable determination of angiographic markers of MVO. Identical further angiography will be taken after thrombus aspiration, injection of the first bolus of drug, stenting and after the second bolus (at the time of final best result). ECG will be recorded and retained at each angiography time point. Blood samples will be drawn at baseline and at 4, 12 hrs after angioplasty, and pre-discharge for cardiac enzymes estimation and NT-proBNP (discharge only). ECG will be undertaken at 90 minutes. At any time the operator can choose to undertake any further treatment they see fit for example if no/slow reflow despite study treatment. All further treatments, delivery routes and timing will be carefully recorded. For all groups optimal post-MI medical therapy will consist of ACE-Inhibitor, beta-blocker at post MI target dose or highest tolerated dose, High dose statin (target total cholesterol <4mmol/L and LDL-Cholesterol <2mmol/L), eplerenone if indicated for heart failure/LV dysfunction). Further anti-anginals will be added as per the COURAGE trial(81).

8. Justification of doses and time of administration for Adenosine and Sodium nitroprusside:

Adenosine: Previous studies have shown that IC adenosine boluses up to *120 µg* (42) or even with double boluses of 120 µg (*i.e. 240 µg)* (34) failed to improve myocardial reperfusion in the setting of

STEMI. In a study comparing different IC doses of adenosine to achieve maximal hyperaemia equivalent to the standard IV route, incremental doses of IC adenosine (60, 90, 120, and *150 µg* as boluses) and a standard IV infusion of 140 µg/kg/min were administered in a randomised fashion. It has been shown that there is a dose-response relationship on hyperaemia measured by fractional flow reserve (FFR) for IC adenosine >60 µg and FFR decreased with increasing adenosine doses, with lowest values observed with *150 µg* IC bolus. The injection of high IC adenosine boluses was safe and associated with fewer systemic adverse effects than standard IV adenosine (82). Based on these data and the local clinical protocol at UHL where *140 µg* IC adenosine is administered ad *hoc* in no-reflow, we chose a total of *300 µg* IC adenosine divided in two boli (i.e. 2 x150= 300 µg) as it had been shown to produce a maximum hyperaemia without significant adverse effects. Furthermore we are delivering the agent via thrombus aspiration catheter as a microcatheter. This dosing was acceptable and based on the evidence at the time. New data has led us to the dose change and concerns about re crossing a new stent to the post PIC delivery change (from distally placed catheter to guide catheter)



SNP: Hillegass (43) used high doses of IC SNP (50-1000 µg; mean total given dose=*435 µg*) to treat 19 patients with no-reflow and noted a significant improvement in coronary flow as assessed by TIMI flow grade and frame counts, without any significant hypotension. In the setting of P-PCI, Hendler (42) reported a significant improvement in tissue myocardial perfusion (TMP) grades in the SNP group (*100-500 µg*) compared to the IC injection of nitro-glycerine (control group) (3 v 1.8; p = 0.023). Wang (45) also found that the effective total dosage of IC SNP, given in multiples of 100 µg, in the setting of P-PCI generally did not exceed *500 µg*. Thus, we have chosen to give a total of *500 µg* IC SNP divided in two boli (i.e.2 x 250= 500 µg), based on the previous data that demonstrated safety and efficacy of SNP given at approximately this dose. Importantly, the fixed doses we will use are equivalent to the average dose given by some previous investigators. The choice of using a fixed rather than a weight-adjusted dose was based upon simplicity and to avoid bias which may result by giving different doses to the study's patients. The reasons why we have chosen to give two boluses are: 1. Both drugs have short half lives and thus, it would be better to give a bolus just after thrombus aspiration and the second one just after stenting. 2. We assume that any risk of adverse effects might be decreased by dividing the total dose. We acknowledge the significant variability in doses used previously. We have chosen doses shown to be safe and we believe to be optimal at inducing physiological hyperaemia which may reduce the burden of microvascular obstruction. If these fail then operators may not waste time and money in the future.

9. Proposed outcome measures:

9.1. Primary: CMR measured infarct size (% LV mass) at 48-72 hours post procedure. Infarct size has been chosen rather than the MVO as the primary end point since there is a wealth of published data on IS following P-PCI. We are also uncertain as to the expected size of effect on MVO (incidence and absolute reduction in MVO as % of LV mass) of both SNP and adenosine. MVO and infarct size measured on CMR are closely inter-related and are both important predictors of prognosis.

9.2. Secondary:
1. CMR incidence and extent of MVO (% LV mass) at 48-72 hours post procedure.
2. CMR measured myocardial salvage index, haemorrhage, LV EF and volumes in the acute stage.
3. Angiographic markers of MVO including the recently designed computer-assisted myocardial blush quantification 'Quantitative Blush Evaluator' (QuBE)(83). (Assessed by two interventional cardiologists blinded to treatment arm).
4. Incidence pre and post procedure angiographic true "no-reflow".
5. Incidence of angiographic slow/no-reflow after PCI with the three different management strategies.
6. Any in-patient clinical events (re-occlusion), need for repeat PCI, recurrent chest pain with new ECG changes, incidence of clinical heart failure (symptoms plus basal crackles plus X-ray evidence pulmonary congestion) proven cerebrovascular accident (CVA).
7. Overall MACCE and its components at 6 months: namely death, need for TLR, recurrent MI, severe heart failure, and CVA.
8. Comparing CMR markers with other myocardial perfusion markers: angiographic (TIMI grade CTFC, MBG, and computer-assisted myocardial blush quantification), ECG (ST segment resolution) and cardiac enzymes.
9. Degree of ST resolution.
10. Echocardiography assessment of LV (EDV, ESV and EF) at 8-12 weeks during a routine follow up visit.

10. Assessment and follow up:

10.1. Assessment of efficacy/effectiveness:

10.1.1. Angiographic Analysis, Assessment of epicardial coronary blood flow and microvascular circulation: Careful and identical angiograms (i.e. views, table height) for assessment of myocardial reperfusion and to compare the effect of SNP v Adenosine on the epicardial coronary blood flow and integrity of the IRA microvasculature, with the aim of allowing TIMI flow (84), CTFC (85), MBG (86), and QuBE (83) assessment. Thrombus score will be graded as described by the TIMI study group (87).

ST segment resolution: The sum of ST elevation will be assessed in three contiguous leads in the infarct zone, 60 ms from J point. The extent of ST segment resolution will be expressed as a percentage of the ST elevation shown on the initial presenting ECG. Incomplete reperfusion is defined as <70% ST-segment resolution on electrocardiography (4).

10.1.2. Myocardial reperfusion assessment using CMR:

CMR protocol: All scans will be performed on dedicated 3T research scanners located at each centre. A comprehensive assessment incorporating, functional, rest perfusion, T2w spin echo (STIR), prior to the administration of contrast and scar imaging will be performed (69). Functional assessment of LV ejection fraction, volumes and mass will be according to current standards with the use of steady state, free precession sequence of the whole LV with 8-12 contiguous short axis slices. Rest perfusion will be performed after functional imaging and T2w imaging. Early (fixed TI 440ms) and late enhancement images acquired using an inversion recovery prepared T1 weighted gradient-echo sequence in identical short axis slices commencing will be obtained after contrast injection in all patients. Quantification of LV volumes, mass, oedema and scar characteristics will be performed on short axis data sets in a random,

blinded fashion. LV mass and volumes will be corrected for body surface area, and scar size assessed manually by delineation of the hyper-enhanced area on each short axis slice, adding all slices to generate infarcted mass. Hypo-enhanced areas within the infarcted zone will be quantified separately to indicate the extent of MVO and will be included in total scar volume. The area at risk will be quantified by adding all areas of signal intensity on T2w greater than 2 standard deviations higher than remote myocardium. Salvaged myocardium will be calculated as the difference between hyper-enhanced T2w mass and necrotic myocardium on delayed contrast imaging. Dark areas in the infarct core will be taken to represent intra-myocardial haemorrhage. Salvaged myocardium will be expressed as percentage of area at risk and total LV size and MVO will be quantified on late enhanced images (4), expressed as a percentage of infarct size, area at risk and LV mass. Patients will ideally be imaged 48-72 hours after AMI to limit variation in MVO and infarct size (88) which is a dynamic process (74). However with very short length of stay following P-PCI, patients admitted on a Thursday may be scanned on day 1 as they may be discharged over the weekend when CMR scanning is unavailable. Similarly, patients presenting on Fridays may be scanned on day 4 if there is no scanning time on day 3 (for example Bank Holidays).

10.1.3. Assessment of platelet activation and inflammation during the procedures:
This mechanistic sub-study will be carried out on patients entered into the study at Glenfield hospital, and will be performed in **Professor Goodall's laboratory** by the Clinical Fellow, using established methods, and with support from experienced technical staff.
Sample collection: Small samples of blood (≤2ml) will be taken from the patients to assess the effect of these short lived treatment agents on platelet activation within the coronary circulation.
Samples will be collected from within the coronary vessels at 3 time points;(i) after thrombus aspiration – before treatment with the study drug; (ii) immediately after the first drug infusion, and (iii) immediately after the second drug infusion following stent insertion. Samples will be collected into tri-sodium citrate anticoagulant and processed immediately for flow cytometry to detect platelet-monocyte aggregates, which are a sensitive marker of platelet activation. In order to relate the degree of MVO to the underlying platelet and haemostatic activity in the patients (i.e. to determine whether the patients were receiving adequate antiplatelet (aspirin and Prasugrel) and antithrombotic (Bivalirudin) therapy), samples of blood from the Arterial Sheath (10ml) will be collected prior to procedure for measurement of platelet response to ADP and Arachidonic acid in a Multiplate whole blood impedance aggregometer. Plasma samples will also be collected from this time point and at the 6-12 hour time point (i.e. at the same time as samples are taken for measurement on cardiac enzymes) and analysed subsequently for endogenous thrombin generation (by the calibrated automated thrombogram method) and for inflammatory cytokines (TNF, IL6) as markers of thrombotic potential and inflammatory response respectively. These studies should help to provide evidence of the *mechanisms of action* of the two trial drugs and of the *efficacy* of the underling antiplatelet and antithrombotic therapy. All necessary methodology and equipment for these studies exist at Glenfield and have been established as possible in this study by Prof Goodall.

10.2. Assessment of Cost-Effectiveness:
Using a EQ5D Health questionnaire which will be completed at 6 months (appendix 1)

10.3. Data recording: Procedural data will be recorded as per trial guideline with the operator primarily responsible for these data. Patients will have follow-up as per routine clinical practice. In addition patients will be contacted by telephone 6 months post discharge and asked about further hospitalisations: to be checked against patient records. Patients will be flagged with the Office of National Statistics to ensure subsequent mortality capture. The study will employ a dedicated on-line password protected electronic case-report form (e-CRF). Designated centre investigators will have administrator rights to the system. It will allow rapid update of patient details/events. A part-time (0.5WTE) research nurse will be employed at each of the two participating centres and will be primarily responsible for data collection.

10.4. Data analysis: data will be entered in to an electronic database to allow statistical analysis. This will be performed primarily by a LCTU statistician under the supervision of Prof. K Abrams.

10.5. Assessment of safety: Given that additional pharmaco-therapeutic interventions will be performed in this study, safety is a major priority. Monitoring of adverse and clinical events starts at randomisation and continues until study end.

10.5.1. Safety Measures: All P-PCI patients are managed during their immediate post procedural period in a high intensity monitored bed (CCU or CHDU). Routine monitoring includes regular nursing observations (hourly BP and heart rate for 4 hours and if stable 4 hourly thereafter) and continuous ECG monitoring for the first 24 hours. Following sheath removal observations are every 30 mins for 1 hour. This is standard. Additionally trial patients will be observed carefully for any changes in heart rate or rhythm. All alterations in heart rhythm will be recorded on automated monitoring system and will be reviewed. All patients will be reviewed by the clinical team at least daily and any complications noted (rate, rhythm, chest pain, ECG or BP changes, bleeding from access site) will be treated in the routine acute manner. Members of the clinical team will monitor the clinical notes and patients for adverse and clinical events and report them accordingly. In addition, the research team will also review the patients daily for AEs, to be able to enter data into the CRF and allow for timely reporting of SAEs. After 24 hours, patients may be transferred to a specialised cardiology bed until discharge. Discharge is when clinically appropriate. Patients will have follow-up at the outpatient clinic as per routine clinical practice. In addition study patients will be contacted by telephone 6 months post discharge and asked about further hospitalisation: to be checked against patient records. Patients will be flagged with the Office of National Statistics to ensure subsequent mortality capture. A clinical events committee will be established to review the details of key trial adverse events and will adjudicate events using original source documents. Their reports will be used in the assessment of endpoints and for presentation of data to the DSMB (Chaired and constituted by Professor Jennifer Adgey).

10.5.2. Adverse Events: It is recognized that most adverse events will be expected as complications of the STEMI, or revascularization procedure. If serious, these events will be recorded for the evaluation of outcome measures and for safety monitoring. See below for definitions. The DSMB will assess whether AEs are complications of study treatments or possible complications of having a STEMI. Case Record Forms have been structured to capture adverse events which are most likely to occur during hospitalisation.

10.5.2.1. Adverse events definitions for this study

An Adverse Event (AE) is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient in a clinical investigation as a result of being part of a study that involved procedures pharmaceutical products beyond standard care. The event does not necessarily have to have a causal relationship with this treatment.

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that

- results in death,
- is immediately life-threatening,
- results in persistent or significant disability / incapacity,
- requires or prolongs patient hospitalisation,
- is a congenital anomaly / birth defect,
- or is to be deemed serious for any other reason representing a significant hazard, which is comparable to the aforementioned criteria.

A Suspected Unexpected Serious Adverse Reaction (SUSAR): is defined as an adverse reaction, the nature and severity of which is not consistent with the applicable product information (eg as in Investigator's Brochure if product is unlicensed; SmPC if it has a license)

10.5.2.2 Expected Events that will be captured

Some serious adverse events occurring in this trial will be expected as a consequence of the underlying disease, routine treatments or diagnostic tests as well as study-related investigational procedures. The CRF will be designed to capture expected clinical outcomes, their complications and all events related to the trial itself.

Hypotension requiring intervention (eg; IABP insertion, inotropic drugs or intravenous fluids).

Any sustained arrhythmia requiring intervention.

QT/QTc prolongation: ECG will be undertaken directly after the first and second dose of the study drugs and QT/QTc will be recorded and compared to the baseline. If the QTc recorded after the first dose of the study drug exceeds 450ms or there is an increase in the QT/QTc of > 60 ms from baseline, the second dose will be abandoned and this will be recorded. On the other hand, if the ECG recorded, after the second dose, shows an increase in QT/QTc of > 500 ms or of > 60 ms from baseline, this will be reported.

Major bleeding: according to Horizons-AMI trial, a contemporary P-PCI trial using the bleeding definition in other studies, major bleeding is defined as intracranial or intraocular haemorrhage, bleeding at the access site with a haematoma that was 5 cm or larger in diameter or that required intervention, a decrease in haemoglobin concentration of 40 g/L or more without an overt source of bleeding or 30 g/L or more with an overt source of bleeding, reoperation for bleeding, or blood product transfusion.

Death

Recurrent Myocardial Infarction (MI)

MI will be defined differently in specific clinical situations in this trial. The ESC/ACC criteria for acute, evolving or recent MI will apply.

- ***Re-infarction:*** During index admission: further chest pain lasting > 20 minutes accompanied by new Electrocardiographic changes (new Q waves >0.04 second or ST-segment elevation > 0.1 mV in two leads for > 30 minutes), further enzyme rise*, or both. *Following the index MI, any new symptoms of ischaemia suggestive of recurrent infarction on clinical or electrocardiographic grounds should prompt immediate estimation of a biomarker (e.g. troponin) followed by a second sample at 6-12 hours. Recurrent MI is diagnosed if there is a ≥20% rise in the value of the biomarker in the second sample, provided the absolute value is > the 99% percentile upper reference limit. For patients who die and for whom no cardiac markers were obtained, the presence of new ST segment elevation and new chest pain would meet criteria for MI.
- **Contrast-induced nephropathy:** The most commonly quoted definition of Contrast-induced nephropathy is 25% increase in serum Creatinine concentration from the baseline value, or absolute increase of at least 0.5 mg/dL (44.2 µmol/L), appearing within 48 hours of administration of contrast media, and maintained for 2–5 days(49, 50, 89).

Cerebrovascular Events:

- **Stroke** is defined as a new focal neurological deficit of presumed vascular aetiology persisting > 24 hours with a neurological imaging study that does not indicate a different aetiology. All strokes will undergo imaging confirmation. Review by a neurologist should be obtained where possible and documentation of the consultation included with the CRF.
- ***Transient Ischaemic Attack*** A transient ischaemic attack is any focal ischaemic neurological deficit of abrupt onset which resolves completely within 24 hours. Review by a neurologist should be obtained where possible and documentation of the consultation included in the CRF.

Severe Heart Failure: Early heart failure: any new onset cardiogenic shock or heart failure with radiographic evidence of pulmonary oedema requiring intravenous diuretic therapy occurring during the

index admission and after randomization. Late heart failure: admission to hospital for treatment for documented NYHA class III or IV heart failure.
Patients will be flagged with the NHS IC MRIS which will notify the coordinating centre of deaths (and the cause) and hospital episode statistics to ensure that all SAE's occurring after hospital discharge are captured. This will also allow long-term (5 year) clinical outcome data to be collected.

10.5.2.3 Classifying SAEs.
All SAEs will be assessed for causality and expectedness.

- Related events are those considered to have resulted from the administration of any study procedures.
- Unexpected events are those not listed in the protocol or in the Summary of Product Characteristics of any drugs administered.

The basis for judging the intensity of the AE as well as the causal relationship between the investigational product and the AE is described below.
Intensity of event

- Mild: Awareness of sign(s) or symptom(s) which is/are easily tolerated
- Moderate: Enough discomfort to cause interference with usual activity
- Severe: Incapacitating or causing inability to work or to perform usual activities

10.5.2.4. Reporting SAEs
Investigators will be required to report all SAEs to the Chief Investigator (CI) within 24 hours of identification of the event. Upon receipt, by the coordinating centre, these will be reviewed by the CI to assess expectedness & causality.
All SAEs must be entered by the investigator on the AE page of the CRF.
The following events will trigger the completion of an SAE form for expedited reporting:

- Serious and treatment-related SAEs
- Serious, not treatment-related and not mentioned on the 'list of STEMI-related events'

Any SAE, whether or not considered related to the investigational products, and whether or not the investigational products have been administered, must be reported immediately in the CRF, if it meets the criteria for expedited reporting described above. Expedited reporting of SAEs, e.g. SUSARs, will be done according to local regulatory requirements.

All serious adverse events not related to any study treatment, but related to STEMI will only be recorded on the AE page of the CRF. Only serious adverse events (SAEs) will be recorded in the CRF.
An immediate report (within 24 hours) must be made orally or in writing. The immediate report must be followed by a detailed written report on the event. Requests from the sponsor for further information of the serious adverse event must be promptly responded to.
A summary of safety report will be included in the annual progress report to the Ethics Committee.
All SAEs will be appropriately logged & forwarded on to the DSMB in a timely manner.

10.5.2.5. Investigator's responsibilities:
Investigators must ensure that all relevant local approvals have been obtained prior to the start of the study. Investigators are responsible for performing the study in accordance with the principles of Good Practice Guidelines & the Declaration of Helsinki, NHS Governance & local laws.
Investigators will be required to report any SAEs as well as the time of onset, end and intensity of these events. A carefully written record of all SAEs shall be kept by the Chief Investigator. Records shall include data on the time of onset, end time and intensity of the event as well as any treatment or action required for the event and its outcome. Investigators are required to notify the sponsor, in an expedited manner (within 24 hours) if any adverse events meet the definition of serious as in section 10.5.2.1
Patients experiencing serious adverse events will be managed as per best practice dictated by the responsible clinician.

10.5.2.6. Sponsor's responsibilities:
The Sponsor's role is clearly set out in the NHS Research Governance documents. The Sponsor is the University Hospitals of Leicester NHS Trust. Research agreements will be held with the participating sites. The Sponsor is responsible for ensuring that the study is conducted to the standards set out in the NHS Research Governance Framework. The sponsor shall ensure that all relevant information about a suspected unexpected serious adverse reaction (SUSAR) which occurs during the course of the trial in the UK is recorded and reported:
-To the MHRA
- To the appropriate Research Ethics Committee
- Not later than **7 days** after the sponsor was first aware of the reaction (fatal or life threatening)
- Not later than **15 days** for those not classified as fatal or life threatening.
The sponsor must ensure that all investigators are notified of any SUSAR that occurs in relation to the trial, although these duties may be delegated to the Chief Investigator & coordinating centre.

11. Sample size:
Is based on previous observations of significant correlation between the extent of MVO and infarct size (which was estimated to be up to 20% of LV mass as detected by CMR after P-PCI)(4). Since there are no available data regarding the incidence of MVO with the study drugs assessed by CMR, and a wealth of published data on infarct size following P-PCI, we have estimated the sample size based on the ability to detect a reduction in infarct size from 20% to 15% LV mass. Assuming 90% power with alpha=0.05, two-tailed and a standard deviation of 10%(1,14,58,59,61,72,75,90,91), see also (appendix 2), 86 subjects will be required per group to detect a mean difference of 5% in infarct size between the treatment groups and control (258 total). Allowing for a dropout rate of 15% between P-PCI and CMR, a further 13 subjects will be required per group (i.e. 99 patients / group, total = 297 patients). Given the exclusion criteria and need for ASSENT pre P-PCI recruitment at 4 patients per week (average 2/centre) is felt to be a conservative estimate of the anticipated recruitment rate (and achieved by both centres in other studies). It will take 18 months to recruit this number of patients and this rate.

12. Statistical analysis:
Keith Abrams, Professor of medical statistics and NIHR Senior Investigator, will oversee the statistical analysis supported by Leicester CTU. Demographics will be presented and infarct size and MVO will be summarized, both overall and by treatment group. The distribution of infarct size will be investigated and the data will be transformed if found to be non-normally distributed. Primary analysis will be by intention to treat with a secondary analysis by treatment received. Patients entering in to the study but not completing the CMR will be continued to be followed-up for MACE. An Analysis of Variance will compare mean infarct size between groups. Each drug will be compared to the control (i.e. Adenosine v Control and SNP v Control). Multivariable analysis using linear regression will take into consideration possible confounders such as sex, age, and other co-morbidity although the major confounders of location of infarct (anterior/non anterior) and time from symptom onset to reperfusion will be addressed by the stratified randomisation process. For secondary endpoints, time-to-event regression methods will be used to investigate potentially important predictors of MACE. Initial analyses will adopt a 'complete case' approach, but sensitivity analyses using multiple imputation methods will also be undertaken to assess the plausibility of the 'missing at random' assumption. A planned subgroup analysis will look at those with and without angiographically obvious thrombus at the time of P-PCI to determine whether those without thrombus additionally benefit from prophylactic pharmacotherapy for the prevention of no reflow. However, this subgroup analysis will be limited due to small numbers and will therefore be interpreted in an exploratory manner as hypothesis generating. The study will be monitored by an Independent Data Safety Monitoring Board and there will be an interim analysis after 35 have been assessed in each group, with subsequent interim analyses at the direction of the IDSMB

13. Ethical arrangements:

Given the need for rapid reperfusion treatment in STEMI, patients will be asked to provide 'ASSENT' to the study after reading a shortened form before angiography. If the patient meets all inclusion criteria after angiography they will be asked for further verbal assent prior to randomisation. Those included will be given the full patient information sheets within 24 hours, assuming medically fit, and asked for full written informed consent to continued participation in the study. The assent process has been utilized in another trial of STEMI (STREAM) and has ethical committee approval. At all times patients will be told they are under no obligation to participate in the study and consent can be withdrawn at any time without prejudice to their treatment.

14. Research Governance:

14.1. Sponsor: This study will be sponsored by the University Hospitals of Leicester NHS Trust where a number of clinical trials are conducted.

14.2. Steering Committee: The trial Steering Committee (TSC) will be responsible for maintaining the scientific integrity of the trial and will monitor the progress of the trial. The TSC will approve the trial protocol and any subsequent amendments and the case record forms. It will meet prior to the start of the trial and as required during the trial. A lay member will be invited to join the TSC .The Steering Committee will be chaired by **Professor Bob Wilcox** with **2 other independent members** Dr. **Peter Ludman & Dr. Jim Nolan**

14.3. Data and Safety Monitoring Board: An independent DSMB will be established and consist of a cardiologist, a statistician, and clinicians experienced in clinical trials. The chair (**Prof Jennifer Adgey**) has chosen 3 other DSMB members (2 physicians **Mazhar Khan, Ian Menown** and 1 statistician **Cathal Walsh**). The main role of the DSMB is to consider the data from any interim analyses and specifically to assess any safety issues such as (unexpected adverse events) and report back to the TSC. The DSMB will **develop a charter outlining their responsibilities and operational detail and timing of data review**.

14.4. Coordinating Centre: A central coordinating centre will be established to liaise with the principal investigators. The coordinating centre will be responsible for overall trial management, including production of final protocol, Case Record Forms, Manual of Operations, arranging meetings, data handling, quality assurance and statistical reporting. Regular progress reports and e-newsletters will be provided to all relevant parties. The coordinating centre will be based at Glenfield Hospital, Leicester with support from the University of Leicester CTU (LCTU).

14.5. Study Operators: The trial operators will be nominated before enrolment at the centre and recorded as Co-investigators. All trial operators will be experienced in P-PCI procedures.

14.6. Data retention: All data pertaining to the study will be retained for 10 years and will be available for independent inspection.

15. Project timetable and milestones:

Timetable: January 2011 MREC submission and clinical trial registration.
February 2011 DSMB & trial staff appointed, ethics approval anticipated
January – February 2011 Preparation of eCRF, study database
February 2011 TSC meeting and pre-trial site visits, R and D approvals
March 2011- recruitment commences
Oct 2011- Interim analysis completed
December 2011-halfway stage- expecting ~150 pts recruited
August 2012- recruitment finishes
September 2012- February 2013: 6 month follow up: MACE
March 2013- June 2013- data cleaning, analysis and writing up for publication

16. Gantt chart: see appendix 3

17. Expertise: Professor Gershlick is the overall PI and an interventional cardiologist of International standing. **Dr Blackman** from Leeds (an Interventionist with a strong academic record in P-PCI) is the PI at Leeds. The PI's will ensure that eligible patients are approached to participate in the study. Interventional colleagues at each centre will be co-investigators. **Dr's McCann** and **Greenwood** (CMR sub-specialists with strong academic records) are jointly responsible for the CMR protocol, performance and analysis. Dr McCann will be responsible for the supervision of the fellow primarily undertaking the blinded CMR analysis at the core lab in Leicester. Dr Greenwood and McCann will agree all areas of MVO and will check the quantitative analysis. Twenty scans will be randomly selected for double analysis by Drs Greenwood and McCann. Interobserver variability <5% will be accepted for all quantitative measures. Prof Gershlick will supervise the fellow in analysing ECG and coronary angiography. **Professor Goodall** is an internationally recognised expert on platelet biology and haemostasis and will be responsible for design and supervision of the laboratory-based sub-studies on haemostatic and inflammatory markers in blood samples obtained during the procedures. The trial will be run (i.e. the randomisation and analysis) via the LCTU with direct input from **Professor Keith Abrams,** an experienced clinical trialist and statistician. Dr Islam Zakarya will co-ordinate the trial and has been responsible for all first drafts he will be directly under the supervision of the PI's.

18. Service Users: Service users have not been specifically involved in the design of this study. There will be a lay representative on the TSC who has experienced a previous MI or PCI.

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20. Flow diagram: See appendix 4

Appendix 1: EQ5D Health questionnaire

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ☐

I have some problems in walking about ☐

I am confined to bed ☐

Self-care

I have no problems with self-care ☐

I have some problems washing or dressing myself ☐

I am unable to wash or dress myself ☐

Usual Activities (e.g. work, study, housework, family or leisure activities)

I have no problems with performing my usual activities ☐

I have some problems with performing my usual activities ☐

I am unable to perform my usual activities ☐

Pain/Discomfort

I have no pain or discomfort ☐

I have moderate pain or discomfort ☐

I have extreme pain or discomfort ☐

Anxiety/Depression

I am not anxious or depressed ☐

I am moderately anxious or depressed ☐

I am extremely anxious or depressed ☐

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which a best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever a point on the scale indicates how good or bad your health state is today.

Your own

health state

today

Best imaginable health state

Worst imaginable health state

Ge

Appendix 2:

Table 1: STEMI studies with infarct size measured on CMR with data presented as mean and SD

Author	Year	No Pts	P-PCI	IS ± SD%	CMR days Post STEMI
Lund(2)	2007	55	47	19 ±10	5±3
Nijveldt(3)	2007	60	All TIMI 2/3	17 ±10	5±2
Hahn(4)	2008	39	ALL	26 ±8	3 (2-6)
Wu E(5)	2008	122	ALL	25± 17	IP?
Beek(6)	2009	45	All TIMI 2/3	18±10	5±2
Ganame(7)	2009	98	All	19 ±11	<7
Sardella(8)	2009	75	All	14± 9.5	3-5
Nijveldt(9)	2009	63	All TIMI 2/3	16 ± 7	4-7
Masci(10)	2010	137	All	18 ± 13	< 7
Average				**19±10**	

TIMI 2/3 excluded patients with TIMI flow of 0/1 (ie no reflow on angio). IS = CMR measured infarct size (% of LV mass).

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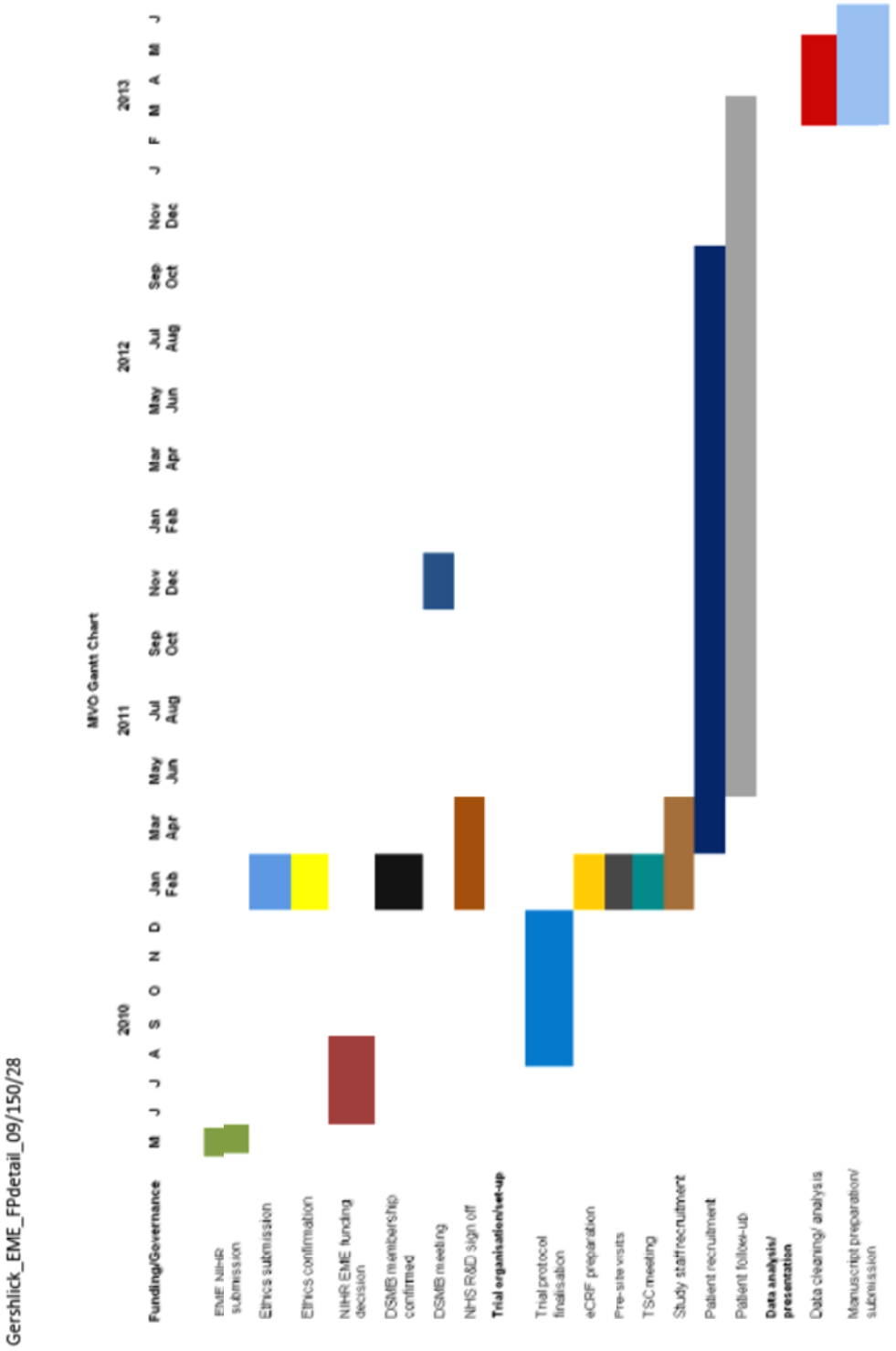
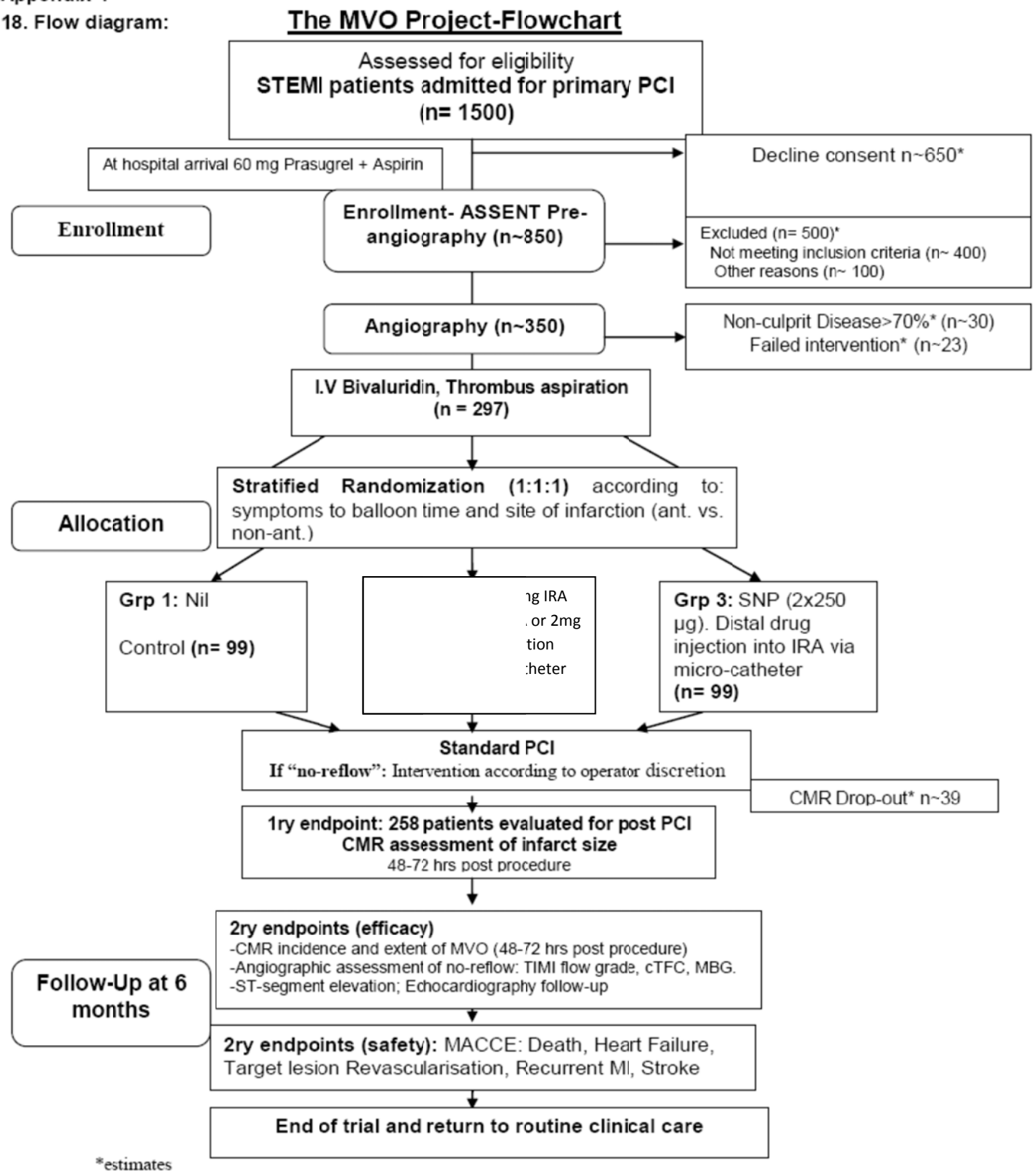
Appendix 3:


Gershlick_EME_FPdetail_09/150/28
MVO Gantt Chart
2010
2011
2012
2013
M J J A S O N D J F M A M J J A S O N D J F M A M J
Funding/Governance
EME NIHR submission
Ethics submission
Ethics confirmation
NIHR EME funding decision
DSMB membership confirmed
DSMB meeting
NHS R&D sign off
Trial organisation/set-up
Trial protocol finalisation
eCRF preparation
Pre-site visits
TSC meeting
Study start/recruitment
Patient recruitment
Patient follow-up
Data analysis/ presentation
Data cleaning/ analysis
Manuscript preparation/ submission

Appendix 4

18. Flow diagram:



*estimates