Take a deep (nitric oxide) breath and follow the reverse translational research pathway

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ABBREVIATIONS:

CMR: cardiac magnetic resonance; iNO: inhaled nitric oxide; IRI: ischemia/reperfusion injury; LGE: late gadolinium enhancement; LV: left ventricle; NOMI: Nitric Oxide for Inhalation in ST-Elevation Myocardial Infarction trial; Ppm: parts-per-million; RCT: randomized clinical trial; STEMI: ST-segment elevation myocardial infarction;

Timely blood flow restoration (reperfusion) is the cornerstone of therapeutic strategies for ST-segment elevation myocardial infarction (STEMI).[1] Reperfusion salvages the myocardium subtended by the occluded artery in a time dependent manner, such that the longer the duration of coronary occlusion, the smaller the amount of salvageable myocardium. Wide implementation of reperfusion strategies has massively reduced mortality: in-hospital mortality of MI patients was close to 25% in the 1970s and has since been reduced to ≈5% in less than three decades.[2] The development of reperfusion therapy for STEMI is thus one of the most successful stories in the history of medicine.[3] However, despite this progress, STEMI still frequently results in significant loss of myocardial mass, and infarct size has been confirmed as the main determinant of long-term post-STEMI mortality and morbidity.[4] There is therefore a need for complementary therapeutic strategies to further reduce infarct size and improve long-term outcomes.

Paradoxically, an important determinant of infarct size is reperfusion itself. This is because the reperfusion procedure, despite being essential for myocardial salvage, induces additional myocardial damage. Final infarct size is thus the combined result of injury caused by ischemia and injury caused by reperfusion to restore blood flow, and is therefore termed ischemia/reperfusion injury (IRI).[5] The dominant current view is that ischemia-related damage can be reduced only by shortening the duration of coronary occlusion, whereas reperfusion-related damage, occurring at the time of blood-flow restoration, could in principle be reduced by interventions at any time before reperfusion, including in the catheterization lab immediately before opening the occluded vessel. This latter strategy is logistically very attractive and has attracted major interest. Many interventions tackling reperfusion-related injury have been shown to be beneficial in animal models; however, translation to the clinic has generally been disappointing.[6] The idea of using inhaled nitric oxide (iNO) to reduce infarct size unfortunately appears to have joined this list of failed translations of intervention strategies.

NO is synthesized within the myocardium mainly by isoforms of NO synthase. Evidence from several laboratories has clearly demonstrated that endogenous NO protects against IRI,[7] and several interventions that reduce IRI have been shown to act via NO pathways.[8] The clear ability of NO production to increase myocardial tolerance to ischemia and reduce IRI led several investigators to test the benefits of delivering exogenous NO.[9] The wide range of mechanisms through which NO limits IRI include cardiomyocyte mitochondrial protection, preservation endothelial function, and inhibition of platelet aggregation and neutrophil-endothelium interactions. Among the various proposed NO-based therapies, iNO is especially attractive because of its ease of administration. Several independent studies have reported the positive infarct-limiting effects of iNO in different experimental models, including mice, rats and pigs undergoing reperfused MI.[9]

Encouraged by this robust preclinical evidence, some generated in their own laboratory,[10] Stefan Janssens and colleagues launched the *Nitric Oxide for Inhalation in ST-Elevation Myocardial Infarction* (NOMI) trial, the first randomized clinical trial (RCT) testing a therapeutic iNO strategy to reduce MI size in STEMI patients.[11] In this pilot trial, 250 STEMI patients undergoing primary PCI were recruited at 4 hospitals in 3 countries and were randomized in a double-blind, placebo-controlled manner to inhale oxygen supplemented with NO (80 parts-per-million (ppm); active arm) or without supplementation (control). Inhalations were initiated upon the patient's arrival in the catheterization lab and were maintained for 4 hours. The primary efficacy endpoint was the extent of late gadolinium-enhancement cardiac magnetic resonance (LGE CMR, % LV mass), a surrogate for infarct size, 48-72 hours (day 2-3) after MI. Key secondary efficacy endpoints included LGE relative to the high signal-intensity area on T2W CMR (a surrogate for the extent of edema), microvascular obstruction, and intramyocardial hemorrhage on day 2-3 CMR. The main results of the trial are that NO inhalation in STEMI patients is safe but has no impact on infarct size or any other endpoint. A prespecified subgroup analysis revealed significant interaction between iNO and the use of parenteral nitroglycerin, a known pharmacological NO donor. In nitroglycerin-naive patients (55% of the population), iNO was associated with smaller infarcts. The effect of nitroglycerin in this trial is difficult to explain, since in the control arm (no iNO) nitroglycerin use was associated with an infarct size reduction similar to that produced with iNO in nitroglycerin-naive patients, an unexpected outcome according to current knowledge.

Janssens and colleagues are to be commended for their outstanding work in identifying a potential cardioprotective strategy in experimental models[10] and moving these findings to the clinical arena by leading the first human trial.[11] In face of the natural disappointment of the authors and the wider research and clinical community, it is important to consider potential factors that may have contributed to the failed translation. 1) Primary outcome selection: The extent of LGE CMR, the authors' choice of primary outcome measure, is the most accurate and reliable surrogate for MI size, and revealed 18% LV mass in the iNO group vs 19 % LV mass in controls (p=0.4). Interestingly, the authors did find a trend toward smaller MI size in the iNO group when normalized to the edematous region on T2W CMR (used as a surrogate for area at risk; AAR): 53% vs 60% (p=0.09). Recent evidence from experimental models and human patients shows that edema development after STEMI is a bimodal phenomenon: an initial reperfusion-related wave of edema is significantly attenuated after 24 hours and is followed by a deferred healing-related wave of edema peaking between day 4 and 7.[12, 13] In addition, edema formation has been shown to be greatly affected by duration of ischemia and by cardioprotective interventions such as conditioning maneuvers.[14-16] Janssens et al found that NO inhalation was associated with a significantly smaller extent of edema on CMR in the patient subgroup not receiving

nitroglycerin.[11] The recently gained knowledge about the bimodal nature of post-MI edema and its unpredictable response to therapies challenges the use of edema-based methodologies as surrogates for AAR. Given that the primary outcome measure of the NOMI trial was CMR-measured MI size (% of LV),[11] and not MI size relative to edema, primary outcome selection will have had no influence on the observed results. 2) iNO dose: Experimental studies have shown that not all iNO concentrations have the same cardioprotective effect. In a mouse model of IRI, NO inhalation at concentrations of 40 and 80 ppm resulted in significantly smaller MI sizes than control therapy, whereas 20 ppm iNO showed no infarct-limiting effect.[17] The NOMI investigators based their selection of an 80 ppm iNO dose on the body of published experimental data[9] and the use of this dose as the most frequent high-dose iNO concentration used in human pulmonary hypertension studies. However, several reports have shown the clinical safety of iNO concentrations up to 160 ppm.[18] The 80 ppm iNO dose used in the NOMI trial significantly increased plasma cGMP concentrations[11]; however, stronger protective effects might have been expected at higher concentrations. This question could first be addressed in a dose response study in a large animal model, potentially opening the window to a further RCT with a more appropriate iNO dose. 3) Timing of iNO initiation: The timing of therapy initiation deserves special attention. iNO in the NOMI trial was initiated soon before reperfusion for reasons of feasibility (the gas tanks were located in the catheterization laboratory). However, a previous study in a rat IRI model showed no benefit from iNO initiated at reperfusion, whereas earlier iNO initiation during the ischemia period was associated with significant reductions in AAR-normalized infarct size.[19] This observation chimes with clinical experience with two therapies associated with infarct size reduction in STEMI patients; remote ischemic conditioning (RIC)[20] and intravenous metoprolol[21] have been found to be more effective when initiated long before reperfusion than when initiated close to reperfusion.[22] In both cases, the link between therapy initiation time and cardioprotection observed in clinical trials has been confirmed experimentally in the pig model.[21, 23] It is thus possible that early initiation of iNO during the ischemia phase (e.g. during ambulance transit to the hospital) would have resulted in a stronger cardioprotective effect in the NOMI trial; however, while such an outcome is plausible, it remains a matter of speculation at present.

The NOMI trial should not be seen as the final nail in the coffin of NO-based therapies to reduce infarct size. Rather, the trial findings leave many open questions to be addressed in further studies. Inhaled NO seems a safe and easy-to-implement strategy, and given the strong mechanistic data supporting its potential efficacy, it is too early for the scientific community to give up on this therapy. The impact of iNO dose and timing should be re-evaluated in large animal models in an exercise of reverse translational research (from the bedside back to the bench), see **Figure**. Moreover, this exercise should not be restricted to this intervention, but should be extended to all infarct-limiting strategies. Given the growing evidence that the timing of initiation with therapies

to reduce IRI influences their cardioprotective strength,[20, 21, 23] it is appropriate to question the dominant view that these therapies will be equally effective when applied at any time before reperfusion.

Figure Legend

In the classic view, translational research is viewed as a one-way transit from bench to bedside (translating basic discoveries into clinical applications). However, the reverse translational pathway (from clinic back to basic/experimental research) is increasingly acknowledged as a critical requirement for successful translational research. The reasons for neutral or inconclusive results in any step of the translational research pathway can often be identified and explained by returning to an earlier step.

The Figure shows the steps in the translational research pathway, together with the most important considerations for studies searching for infarct-limiting therapies. The translational journey can start with a basic research discovery or a clinical observation. Taking shortcuts in this pathway is usually a high-risk endeavor (e.g. performing a RCT after a positive small-animal study without first performing a large-animal preclinical trial).

In the NOMI trial, a successful transition from basic research to preclinical models was followed by a pilot RCT, yielding a negative outcome: the hypothesis that iNO initiated soon before reperfusion would result in smaller infarctions was not confirmed. Important questions can be evaluated by returning to conduct new tests in the preclinical models, such as dose response studies and different timings of iNO initiation. An alternative approach is to reconsider the cardioprotective target in new basic research studies.

iNO, inhaled nitric oxide; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MVO, microvascular obstruction; RCT, randomized clinical trial.

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Disclosures

None.

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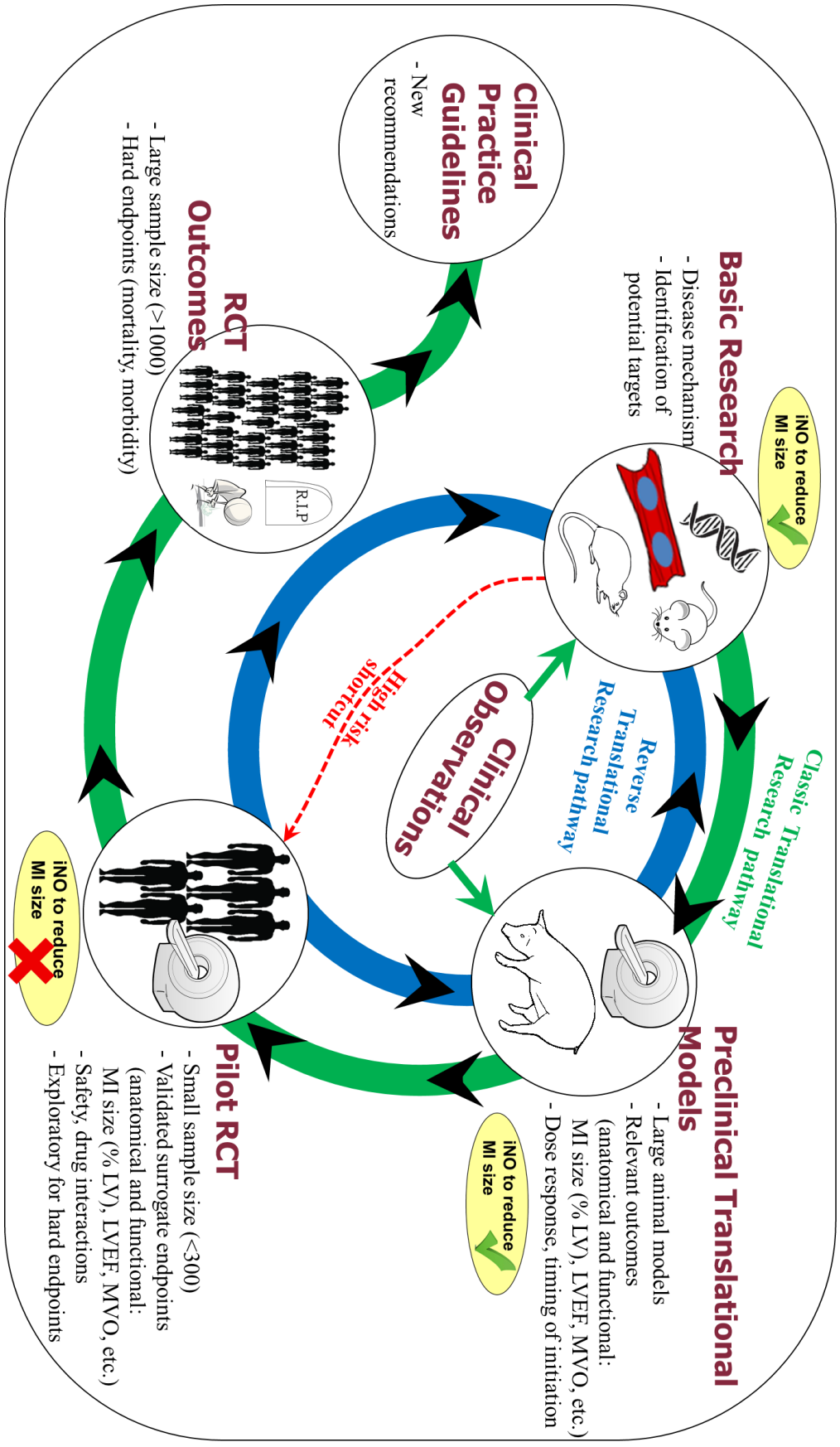
Figure


Basic Research
- Disease mechanism
- Identification of potential targets
iNO to reduce MI size
Preclinical Translational Models
- Large animal models
- Relevant outcomes (anatomical and functional: MI size (% LV), LVEF, MVO, etc.)
- Dose response, timing of initiation
iNO to reduce MI size
Pilot RCT
- Small sample size (<300)
- Validated surrogate endpoints (anatomical and functional: MI size (% LV), LVEF, MVO, etc.)
- Safety, drug interactions
- Exploratory for hard endpoints
iNO to reduce MI size
RCT Outcomes
- Large sample size (>1000)
- Hard endpoints (mortality, morbidity)
R.I.P
Clinical Practice Guidelines
- New recommendations
Classic Translational Research pathway
Reverse Translational Research pathway
Observations
Clinical
High risk shortcut