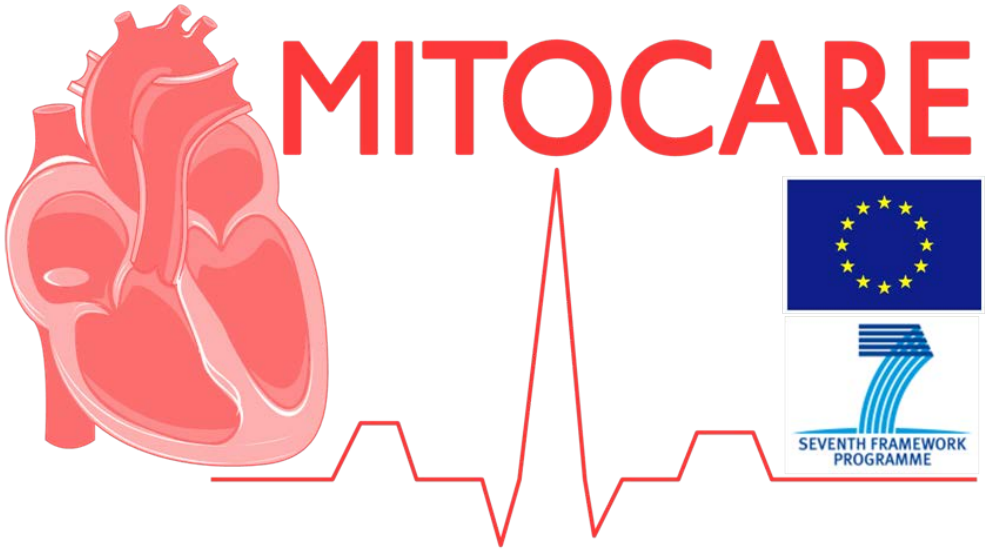


MITOCARE

Treatment of reperfusion injury using a mitochondrial targeted approach: towards a better understanding of the disease

Collaborative project – Small and medium scale research focused projects

HEALTH-F2-2010-261034

Period: Jan 2011 – Dec 2013
Project Coordinator: Rebecca Pruss
Project Coordinator Institution: Trophos S.A.

Executive summary

MitoCare's objectives & work plan:
The project “Treatment of reperfusion injury using a mitochondrial targeted approach: towards a better understanding of the disease” (MitoCare) lasted from January 2011 to December 2013 thanks to the collaborative effort of a consortium involving 17 institutional partners combining both expertise in clinical and preclinical research under the leadership of the research intensive SME Trophos S.A. (Marseille, France). The project involved four basic/preclinical research teams, ten clinical sites and four SMEs that worked together in a focused and integrated manner.

Cardiovascular diseases are currently the leading cause of death in industrialized countries and are expected to become so in emerging countries by 2020. Current treatment of acute myocardial infarction (AMI) by reperfusion using percutaneous coronary intervention (PCI) or thrombolysis has provided tremendous clinical benefits by reducing infarct size and mortality following cardiac ischemia. However the process of restoring blood flow to the ischemic myocardium can induce damages to the heart by itself. This phenomenon, termed myocardial reperfusion injury, has been identified in animal models and can paradoxically induce cardiomyocyte death and therefore reduce the beneficial effects of myocardial reperfusion [1-2]. This form of myocardial injury, may in part explain why, despite optimal myocardial reperfusion, the rate of death after an acute myocardial infarction approaches 10% and the incidence of heart failure after an acute myocardial infarction is almost 18% [3].

It was therefore a first main objective of the MitoCare project to evaluate a newly developed complementary therapy that would prevent or limit myocardial reperfusion injury, evolution to heart failure and the subsequent need for regenerative therapies.

A second main objective of the MitoCare project was to further explore patients’ related confounders through extensive sampling of biomarkers and clinical variables using a broad multidisciplinary approach combining both human and animal studies.

Those two objectives were reached by combining the expertise of both preclinical research scientists who are specialists in models of cardiac ischemia reperfusion injury, clinical cardiologists with a significant experience in the assessment of drug efficacy in reperfusion injury during PCI for the treatment of AMI and specialized SME experts in clinical biomarkers, Magnetic Resonance Imaging (MRI) and electronic case report forms (eCRF).

Indeed the preclinical research group explored similarities and differences of cardioprotection afforded by TRO40303 and mild hypothermia in various *in vitro*, *ex vivo* and *in vivo* models. In the meantime, the clinical research team assessed the ability of a newly discovered mitochondrial targeted compound named TRO40303 to reduce reperfusion injury in 163 patients treated by PCI for AMI enrolled in a Phase 2 clinical study conducted in 10 hospitals across 4 European countries.

Key results of the project are that while TRO40303 was confirmed to inhibit mPTP and reperfusion injury in some *in vitro* and *ex vivo* models, mild hypothermia was constantly active in all the tested models. Despite this, TRO40303 did not reduce infarct size in animal models nor in the human clinical trial, although the study proved that TRO40303 is safe and well tolerated in patients.

These results along with the results from other groups published over the last few years, strongly advocate for a review of the experimental protocols used to evaluate potential therapies in preclinical models and the relevance of the models themselves.

Although the MitoCare project confirmed that TRO40303 can inhibit the mPTP and some mechanisms implicated in reperfusion injury, whether reperfusion injury indeed occurs in humans remain an open question. The new information MitoCare provides will generate new hypotheses and research strategies to better understand cardiac ischemia reperfusion injury and discover useful approaches to improve long term outcome following an acute myocardial infarction.

MitoCare's project results:

The clinical trial was successfully completed, with high quality data, well balanced populations between the two treated groups (placebo or TRO40303) and a high standard of care at the participating centres.
There was no safety issue during the trial. The results showed no clinically relevant difference in infarct size assessed between the groups as evaluated by the co-primary endpoints (biomarkers) or the main secondary endpoint (MRI).

During the trial, a bio bank has been set-up with patients' plasma, serum, whole blood and RNA samples that is available to the scientific community for further analysis and for a maximum of 5 years. Some of these samples were used to validate the dosing of new biomarkers.

Additionally a portable eCRF to be used in emergency settings has been developed on iPads.

Confounding factors and innovative markers were analysed in the clinical trial. However there was no difference noted for any of them between the two groups. Thus, it does not seem that any of these confounding factors had an impacted on outcome as measured in this study. For the innovative markers, as the primary and secondary endpoints did not show any difference either, it is not possible to draw a conclusion on these markers in this trial.

In the preclinical models, the mode of protection and efficacy of TRO40303 and mild hypothermia were compared in various models. Both approaches conferred protection against mPTP opening and cell death following ischemia reperfusion in some of the models used. However in many other models, only hypothermia and not TRO40303 was able to provide protection leading to a final reduction of infarct size in the *ex vivo* and *in vivo* models.
Interestingly, TRO40303 was able to reduce mitochondrial dysfunction of the pig myocardium in the peri-infarct zone subjected to ischemia reperfusion; however that was not sufficient to translate into a reduction of infarct size in the pig model.
Because hypothermia was initiated prior to ischemia while TRO40303 treatment was provided a few minutes before reperfusion, the mechanisms underlying the effects of these two treatments in the animal models was different. It is proposed that mild hypothermia provided protection at least partly by slowing down the cellular metabolism during ischemia and this prevents reperfusion injury from occurring resulting in a reduced infarct. By contrast, TRO40303 appears to provide a specific protection of mitochondrial dysfunction following hypoxia and reoxygenation potentially by preserving the intactness of the outer mitochondrial membrane and preventing mitochondrial permeablization.

In the pre-clinical models standard biomarkers of cardiac injury, Troponin I and Creatine Kinases were measured; however, no innovative biomarkers were dosed due in part to incompatibility of the detection methods (antibody cross reactivity) between human and preclinical species variants. However, at the end of the MitoCare project, a Multiple Analysis of Variance (MANOVA) on all parameters assessed *in vivo* was made. The results confirmed that hypothermia provided a significant effect whereas TRO40303 did not show any effect either in the rabbit or in the pig models.

In isolated rat cardiomyocytes subjected to *in vitro* hypoxia/reoxygenation both male and female cardiomyocytes were used and TRO40303 provided a similar protection in both type of cells, excluding a potential gender effect.

During the project, a Safety & Ethics monitoring Committee (SEMC), composed of a data protection specialist and an ethics specialist, has been set-up to ensure the respect of ethics and personal data protection in the clinical trial and ethics in the preclinical work. The SEMC has provided an independent report to the EC concerning these aspects.

Finally, a *MitoCare Translational Advisory Council* has been set-up in order to prepare a publication on the translational questions raised during this project.

In conclusion, this project showed that the mPTP modulator TRO40303 although able to inhibit mitochondrial permeablization and cardiomyocyte death due to hypoxia-reoxygenation in *in vitro* studies and in peri-infarct zone of pig *ex vivo*, it was not able to reduce infarct size in the Langendorff isolated rat heart model or in rabbit and pig *in vivo* models nor did it have a benefit compared to placebo in the human clinical trial in ST elevated myocardial infarction (STEMI) patients. Further analysis of results of the clinical study with other recent studies suggest that improvements in mechanical revascularization principles and shortening time to treatment has provided a standard of care is so high, especially in the countries where the trial was conducted, that reperfusion injury is low and does not contribute significantly to the volume of infarcted myocardium. Another hypothesis is that we have yet to find a suitable treatment.

▪ Project's context & objectives

Key objectives

The MitoCare Consortium designed two main objectives:
The first main objective of the MitoCare project was to evaluate a newly developed complementary therapy that would prevent or limit myocardial reperfusion injury, evolution to heart failure and the subsequent need for regenerative therapies.
The second main objective of the MitoCare project was to further explore these patients' related confounders through extensive sampling of biomarkers and clinical variables using a broad multidisciplinary approach combining both human and animal studies.

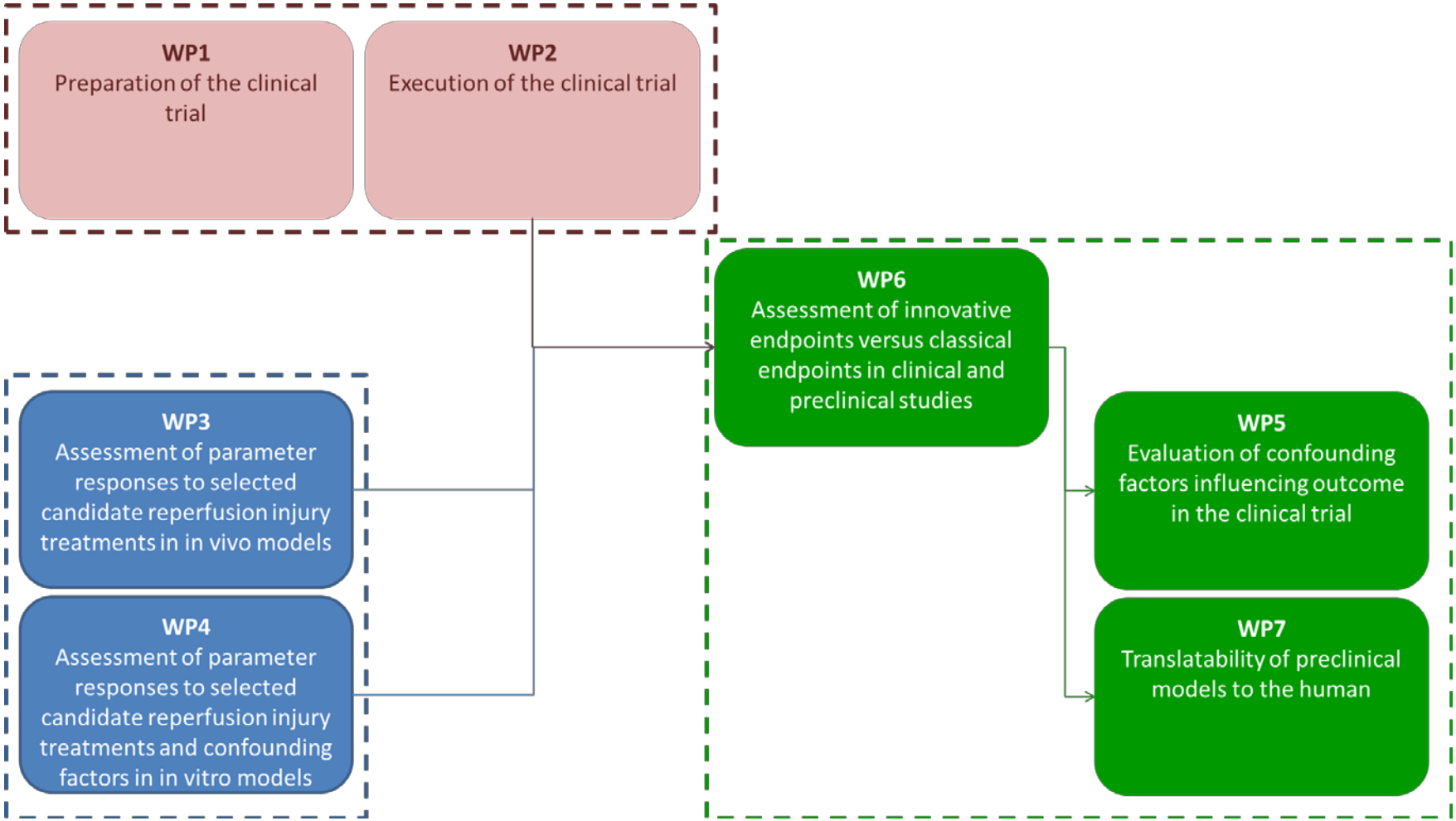
In order to reach the above cited main objectives, they were addressed through three interconnected sub-projects combining preclinical and clinical research into a translational research with the aim to address the key questions.

- The clinical subproject 1 (SP1) consisted of a medium-scaled patient study of a new mitochondrial permeability transition pore (mPTP) modulator TRO40303 developed by Partner 1 (Trophos). In addition SP1 also included the collection of samples and data needed to perform the translational subproject 3 (SP3) and to establish a sample repository available to the scientific community.

- The preclinical subproject 2 (SP2) consisted of the assessment of disease variables in relation to the preclinical models and the outcomes used as well as animal background. This subproject aimed at evaluating various models of the disease and the variability of the response depending on models and outcomes used. The SP2 allowed the collection of samples and data needed to perform the translational subproject 3 (SP3).

- The findings of the first two subprojects were directly fed into the translational subproject 3 (SP3). This subproject aimed to identify and/or validate patient related confounding factors; to identify and/or validate new biomarkers of reperfusion injury that may allow a better assessment of the prognosis for evolution to heart failure. Finally, by comparing clinical outcomes and endpoints assessed in preclinical models, it was the intention to evaluate the predictability of the animal models in order to identify the most relevant model for the disease.

Interdependency of components



Key Results

- A biobank of patients' samples has been set-up and is available for research purposes for a maximum of 5 years.
- A portable eCRF has been developed on iPads.
- A software program (Transfer) that blinds and handles secure transfer of imaging data has continuously been developed and refined into a new version based on the results and experiences of the MitoCare project. This resulted in a new Web-based image data transfer system with rapid quality feedback (IMITS).
- TRO40303 was able to inhibit the mPTP and cardiomyocyte death following hypoxia reoxygenation in *in vitro* studies as well as to protect from mitochondrial dysfunction in the myocardium from the peri-infarct zone obtained from the *in vivo* pig model of reperfusion injury.
- TRO40303 did not decrease the infarct size in other *ex vivo* and *in vivo* animal models (Langendorff isolated rat heart, *in vivo* rabbit and pig models) nor in STEMI patients in a clinical trial.
- TRO40303 proved to be safe and well tolerated in a medium scale clinical study.
- Mild hypothermia was protective in the *in vitro* experiments as well as in the reduction of infarct size in isolated rat heart and in pigs *in vivo* when mild hypothermia was initiated before occlusion.
- In the clinical study, the standard of care is now very high in the countries where the study was performed; reperfusion injury may be so low that there is little room for improvement to limit potential reperfusion injury. Another hypothesis is that we have yet to find a suitable treatment.
- In the present trial, the confounding factors seem not to be responsible for the lack of efficacy.

The Consortium

The MitoCare project is a collaborative project funded under the Small and medium scale research focused projects funding scheme of the 7th Framework Programme for Research and Technological Development of the European Commission. The project started on January 1st 2011 and ended on December 31st 2013.

The project was coordinated by Dr Rebecca Pruss, Chief Scientific Officer from the company Trophos S.A. (Marseille, France) supported in her task by Dr Sophie Schaller (Trophos S.A., co-coordinator for the preclinical part of the project, SP2), Dr Wilfried Hauke (Trophos S.A., co-coordinator for the clinical part of the project, SP1) and Mr Julien Veys (Trophos S.A.) as Project Manager. The coordinating team was backed by two steering committees, one for the preclinical research part of the project, the other for the clinical research. Finally a Safety and ethics monitoring committee was set-up to ensure the patient's safety, data privacy as well as all ethical aspects both in the clinical and preclinical parts of the project.

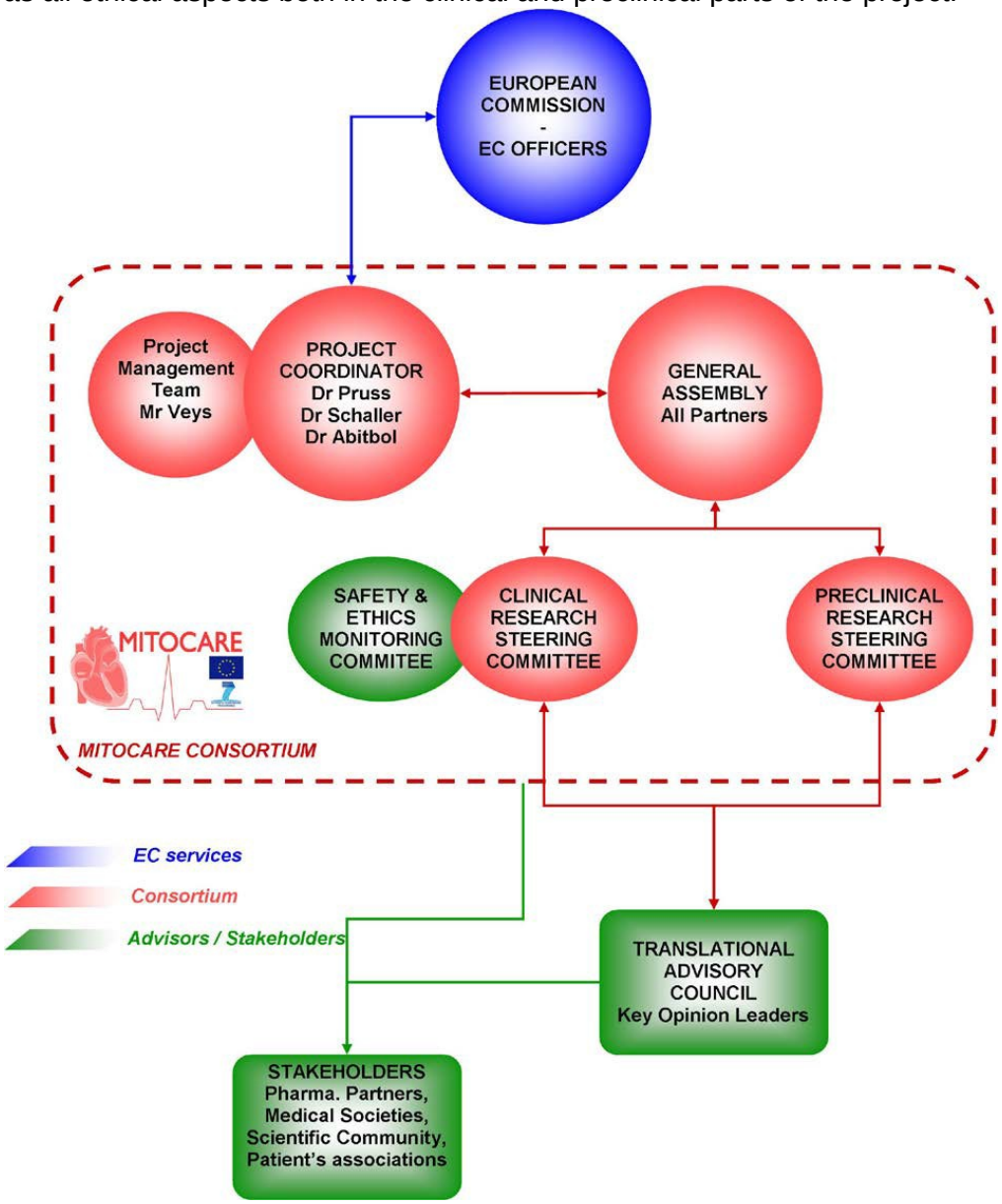
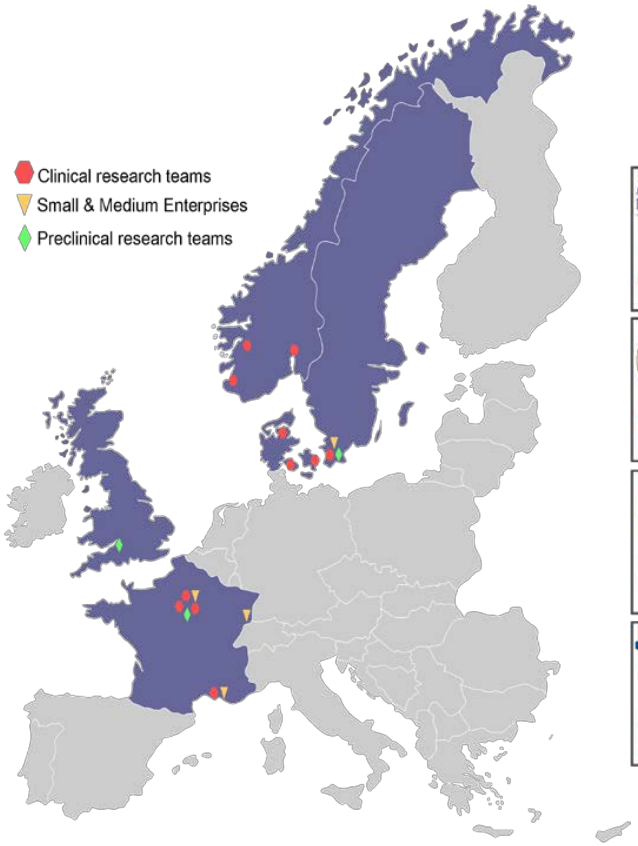


Figure 1: MitoCare Consortium's organization

The Partners



	TROPHOS Coordinator Partner 1		
APHP Partner 2	INSERM Partner 3	UIB Partner 4	RN Partner 5
ULUND Partner 6	RH Partner 7	UNIVBRIS Partner 9	FIRALIS Partner 10
IMACOR Partner 12	MH Partner 13	OUS Partner 14	OUH Partner 15
SUH Partner 16	APHM Partner 17		

Preclinical activities *Clinical activities*

SMEs

▪ Scientific & Technological results - foreground

Proof of concept clinical study using a new mitochondrial targeted compound for reduction of reperfusion injury in patients undergoing Percutaneous Coronary Intervention for Acute Myocardial Infarction .

Clinical study design

The clinical trial was a prospective, multinational, multicenter, 1:1 randomized, placebo-controlled study performed to evaluate the effect of TRO40303 on limiting reperfusion injury among patients undergoing revascularization for ST-elevation myocardial infarction (STEMI).

The primary objective of the trial was to assess safety and efficacy of a single dose of 6 mg/kg of TRO40303/Placebo by slow IV bolus (35 mL/min) administered just before balloon inflation during percutaneous coronary intervention (PCI) for limitation of infarct size in patients treated for Acute Myocardial Infarction (AMI).

Patients eligible for inclusion in the study were to above 18 years old, have signed informed consent form and with a first acute AMI (ST Elevated Myocardial Infarction). Occlusion should affect the Left Anterior Descending artery (LAD) or the dominant or balanced Right Coronary Artery (RCA) or the dominant or balanced left circumflex artery (LCx). Patients should be presenting within 6h of onset of chest pain with a clinical decision to treat with PCI and an occlusion of culprit artery with a TIMI flow grade 0-1 at time of admission and before PCI. Full definitions are available in study protocol.

Patients with cardiac arrest, ventricular fibrillation, cardiogenic shock, stent thrombosis, previous AMI, angina within 48h before infarction, previous CABG, treatment with intravenous fibrinolytic therapy within 72 hours prior to PCI, atrial fibrillation, pace-maker, concurrent inflammatory, infectious or malignant disease, biliary obstruction or hepatic insufficiency at the time of inclusion in the study were excluded. All female patients should be with non-childbearing potential (post-menopausal, ovariectomised or hysterectomised).

Patients enrolled in the study were then followed according to the flow chart hereunder:

Study Phase	TREATMENT PERIOD											FOLLOW-UP
Times (days)	Before balloon inflation (Admission)	D1						D2		D3	D3 to D5	D30[7]
Times (hours)		15'	1h	6h	12h	18h	24h	36h	48h	72h	72-120h	
Visits	V0 *(Hospitalization)*											V1
Clinical and Paraclinical Assessments												
PCI and angiography according to standard procedures	/----------/											
Informed Consent	X											
Physical examination and vital signs	X		X[2]				X[6]		X[6]	X[6]		X
Medical history		X										
Demographics (social status, life habits)		X										
Killip score		X										
Inclusion /exclusion criteria	X											
Randomization	X											
MRI											X	
Echocardiography											X	X
ECG	X		X[2]				X[6]		X[6]	X[6]		X
Laboratory tests												
Electrolytes (Na, K, Cl), creatinine, TG, urea, blood glucose	X						X[4]		X[5]	X[5]		X
Liver Function Tests (bilirubin, γGT, alkaline phosphatase, ASAT, ALAT)	X						X[4]					X
Hematology	X						X[4]					X
Haemostasis (PT, aPTT, INR)	X									X[5]		X
CK, Troponin I	X			X[3]	X[3]	X[3]	X[4]	X[5]	X[5]	X[5]		X
NT-Pro BNP, proADM, cystatin C, CRP, IL1b, 6, 10, Ig, TNFα cytochrome C, oxidized LDL, MPO, Troponin T, CK-MB	X				X[3]					X[5]		X
HDL/LDL Cholesterol	X									X[5]		X
HbA1c									X[5]			
Microalbuminuria								X				
Thromboxane (urine)								X				
TRO40303 or placebo administration (IV)	Within 5' to 15' before balloon inflation											
TRO40303 plasma exposition		X[1]		X[3]	X[3]							
AEs and concomitant medication	/----------											----------/
Biobank with patient consent												
Serum, plasma and RNA	X				X[3]					X[5]		X
Urine sample		X						X		X		

[1] ± 5min , [2] ±10min, [3] ± 1 hour, [4] ± 2 hours, [5] ± 4 hours, [6] ± 6 hours, [7] ± 3 days

Conduction of the study

Patients and treatments:
Ten centres from 4 countries (Denmark, France, Norway and Sweden) were enrolling 167 patients into the study. The study was performed according to international and national guidelines on good clinical practice. The monitoring of the study and the statistical analysis was performed by independent parties not involved in the documentation of data from the patients. Documentation and medical procedures were the sole task of the investigators after obtaining the written informed consent from the patients. The study protocol was presented to all concerned ethic committees and health authorities. A positive vote was granted by all bodies.

All patients had to present with STEMI within 6 hrs of onset of pain randomly received TRO40303 (n=83) or placebo (n=80) via intravenous bolus injection prior to balloon inflation during primary PCI in a double-blind manner. The patients were followed according to the provisions of the study protocol as well as the standard of care guidelines at the respective hospitals.

Outcome measures:
Blood was taken at predefined time points over a three-day period in order to calculate the infarct size expressed as area under the curve (AUC) for creatine kinase (CK) and for troponin I (TnI). Both parameters served as main outcome criteria for the primary endpoint and were analysed in a blinded manner at a central laboratory.

Secondary endpoints included measures of infarct size as Myocardial Salvage Index (MSI) assessed by Cardiac Magnetic Resonance (CMR) using the definition of MSI = (Myocardium at risk - Infarct Size) / Myocardium at risk. Further evaluations comprised infarct size assessed by late gadolinium enhancement (LGE) imaging and myocardium at risk assessed by either T2-weighted imaging or contrast enhanced steady state free precession (SSFP) imaging.
The size of the infarct was also expressed in absolute volumes (g) and percent of the left ventricular mass. Echocardiography- and electrocardiography-parameters relevant for the diagnosis and location of infarction as well as for possibly confounding and potentially predictive measures were analysed according to a predefined matrix.

Safety Assessment:
Safety parameters were analysed according to the principles as laid down in the protocol. An independent Data Monitoring Committee (DMC) followed the conduct of the study continuously and gave recommendations for the risk-benefit analysis performed at regular time points.

All clinical events were adjudicated by a Clinical Endpoint Committee (CEC) following a charter as part of the protocol and analysing all events in a blinded manner.

Statistical methods:
The statistical analysis was performed according to the protocol, the statistical analysis plan considering the results from the blind data review. The statistical model ANCOVA was used for analysing the co-primary endpoints with adjustment for multiple endpoints using the Horchberg step-up procedure with time to PCI and culprit artery serving as covariates for all parameters expect for Myocardial salvage index (MSI) for which culprit artery is no taken in the model.

Linear interpolation was used for imputation of missing value between two recorded values. Otherwise multiple imputation technique was used based on patient's characteristics (treatment, age, sex, infarct localization, post-PCI TIMI).
A mixed model of ANCOVA with no adjustment was used for the secondary endpoints also with time to PCI and culprit artery as covariates.
All analyses were performed on the Intention-to-treat (ITT) and Per-protocol (PP) collective with all tests being two sided at a 5% significance level.

Results

Analysis of data shows that the study was conducted with high quality and that all investigators were following the protocol as planned. The low number of major protocol violators (14%) as well as the equal distribution of demographic and baseline data throughout the groups support that finding.

Both treatment groups were comparable regarding age, gender, body mass index and patients were comparable amongst groups for social status, smoking habits (about 50% non-smoker), alcohol and drug consumption.

Median pain-to-balloon time was 3 hrs for both groups, median door-to-balloon time was 38 min for all sites, indicative of a high standard of care at the participating centers. Infarct size, as measured by CK and TnI AUCs at 3 days, was not significantly different between treatment groups (fig 1). The CMR-assessed myocardial salvage index (infarct size normalized to the myocardium at risk) was not significantly different between the groups (mean 0.58 vs. 0.52 with TRO40303, p=0.1000). There were no significant differences in CMR assessed infarct size (mean 21.9 g vs. 20.0g; or 17% vs. 15% of LV-mass) or left ventricular ejection fractions (LVEF) (mean 0.48 vs 0.46), or in the 30-day echocardiographic LVEF (mean 0.52 vs 0.51) between TRO40303 and placebo.

The safety analysis gave evidence that TRO40303 is a well-tolerated treatment. No signals could be detected that would lead to specific precautions or warnings. The pharmacokinetic data analysis showed that all patients receiving TRO40303 were well above a trough level set at 52.8 µg/ml (C_{5min} determined from preclinical efficacy study).

Although a greater number of adjudicated safety events occurred in the TRO40303 group, this is likely related to a higher rate of unsuccessful reperfusion post PCI (TIMI-flow 0-1 in 12.1% versus 6.3% in placebo), potentially reflected by the greater number of scheduled revascularization during the initial PCI procedure.

Subgroup analysis for time to PCI, infarct location, culprit artery, patients without CEC adjudicated events were performed and the data show the same result as for the main groups.

It can be concluded from the trial result did TRO40303 not show any difference compared to Placebo in cardiac ischemia reperfusion injury. Results of the co-primary endpoints are confirmed by the secondary endpoints. This study in STEMI patients treated with contemporary revascularization principles may leave little room for TRO40303 to limit reperfusion injury of the infarcted myocardium.

Establishment of a biobank.

Establishment of a biobank has been set-up including plasma, serum, whole blood and RNA collected before balloon inflation, and 12h, 72h and day 30 after balloon inflation and urine collected at day 1, day 2 and day 3 for all the patients who gave consent to this optional additional study. The samples of the bio bank are available at Firalis (Partner 10) for a maximum of 5 years.
Some of these samples were used to validate dosing of new biomarkers including CNN1, PGF and NGAL.

Establishment of a portable eCRF on iPad

An eCRF has been developed on iPads for the clinical trial. Although the development was largely more complicated than anticipated, the final tool was very useful and practical for entering and monitoring the data.

Establishment of a central MR imaging uploading platform.

A software program (Transfer) that blinds and handles secure transfer of imaging data has continuously been developed and refined into a new version based on the results and experiences of the MitoCare project. This resulted in a new Web-based image data transfer system with rapid quality feedback (IMITS).

Preclinical models.

In the preclinical models, hypothermia was selected as a positive comparator for infarct size reduction in myocardial infarction models and because this strategy has already been translated to humans. It was used in *in vivo* pig models, in *ex vivo* isolated rat heart model as well as in isolated rat cardiomyocyte experiments.

Both TRO40303 and mild hypothermia delayed mPTP opening, inhibited the generation of mitochondrial reactive oxygen species (superoxide anions) at reoxygenation and improved cell survival in isolated rat cardiomyocytes subjected to *in vitro* hypoxia/reoxygenation. Mild hypothermia provided protection in a starvation model in H9c2 cells, preserved the respiratory defects in isolated rat heart mitochondria submitted to anoxia/reoxygenation and reduced infarct size both in isolated rat heart and in the *in vivo* pig model, effects that were not observed with TRO40303. In biopsies of different areas in the pig myocardial tissue from the *in vivo* study, TRO40303, administered just prior to reperfusion, specifically preserved respiratory functions in the peri-infarct zone whereas mild hypothermia, initiated prior to occlusion, preserved both the ischemic core area and the peri-infarct zone.
In the *in vivo* rabbit model, TRO40303 was evaluated in anaesthetized rabbits with administration either before coronary artery reperfusion or before coronary artery occlusion. Studies were performed using either 20 min or 30 min occlusion. Regardless of the experimental conditions tested, TRO40303 did not reduce the infarct size.
It is proposed that mild hypothermia provides protection at least partly by slowing down the cellular metabolism during ischemia and when initiated during occlusion, prevents reperfusion injury to occur and results in a reduced infarct. By contrast, TRO40303 provides a specific protection of mitochondrial dysfunction following hypoxia and reoxygenation potentially by preserving the intactness of the outer mitochondrial membrane which however did not translate into a final reduction of infarct size in the *ex vivo* and *in vivo* models.

Confounding factors and innovative biomarkers.

In the sub-project 3 (SP3), confounding factors and innovative markers were analysed in the clinical trial. Among the confounding factors collected, there was no imbalanced observed in the groups apart from the number of CEC adjudicated events that were significantly higher in the TRO40303 group than in the placebo group; however, this is likely related to a higher rate of unsuccessful reperfusion post PCI in the TRO40303 group. In addition the number of programmed revascularizations, listed as a CEC adjudicated event, was higher in the TRO40303 group. Thus, it does not seem that any of these confounding factors could have impacted the results and explain TRO40303's lack of efficacy compared to placebo in the trial.
Among the innovative markers analysed (CK-MB, TroponinT, NTproBNP, Thromboxane, CRP, MPO, IgG, IgA, IgM, HbA1c), there was no notable difference for their mean, median, min and max values between the two groups in the clinical trial. However, as the primary and secondary endpoints did not show any difference either, it is not possible to draw a conclusion on these markers in this trial. Further correlation analysis by comparing infarct size and biomarkers could be performed.

In the preclinical models, no innovative biomarker was dosed due to incompatibility of the detection methods (antibody cross reactivity) between human and preclinical species variants. However a complete statistical analysis was performed both in the rabbit and pig models to take into account all the different parameters assessed to analyse treatment effect: TRO40303 or/and hypothermia versus Saline or/and placebo either independently or in combination into a Multiple Analysis of Variance (MANOVA) model. The results confirmed that hypothermia provided a significant effect whereas TRO40303 did not show any effect both in the rabbit and in the pig models.
In the preclinical studies, the only experiment that could assess one potential confounding factor was performed using isolated rat cardiomyocytes subjected to *in vitro* hypoxia/reoxygenation where experiments where performed using cells obtained from both male and female rats. TRO40303 had a similar protective effect for cardiomyocytes derived from rats of either gender.

Translational analysis.

Finally, a *MitoCare Translational Advisory Council* has been set-up in order to prepare a publication on the translational questions raised during this project. The predictability of the animal models to humans will be discussed, as previous studies showed that TRO40303 reduced infarct size in mice and rat models, whereas it was not effective here in larger animal models. This could then be proposed as a prerequisite before assessing new treatments in human clinical studies. However, it must be noted that efficacy of hypothermia, although very strong in pigs, was difficult to show in humans (CHILL MI, Erlinge et al. TCT 2013 presentation); whereas, cyclosporine A, a compound that has not shown reproducible efficacy in pig models, had a significant beneficial effect in a pilot study in man [4]. One key point is also to ensure that the animal models do really mimic what occurs in man: for example, treatment needs really to be given at a similar time (e.g. not before occlusion as it would not be close to the emergency clinical situation). Also, infarct characteristics in animal models need to be similar to that observed clinically. A main issue is that animal models produce large infarctions by occluding the Left Anterior Descending artery where this represents only a portion patients presenting with an acute MI. Furthermore, animal models are performed with a definite time of occlusion that is not too short and not too long so that

reperfusion injury can be demonstrated. These controls are needed in animal studies as otherwise the variability would be too high and groups of treated animals would have to be much larger, leading to tremendous costs and time for preclinical assessment as well as ethical questions. However, this control of time and infarct characteristic would not be feasible in a clinical setting and would not reflect reality. These differences could be an explanation for the lack of apparent and treatable reperfusion injury in man.

▪ Potential impact & dissemination

Impact & perspectives

Scientific impact and perspectives

The MitoCare project has provided interesting new information on cardiac ischemia reperfusion injury in animal models and in human.

This information will be useful for the development of new therapeutic strategies in this indication.

Overall the MitoCare consortium results highlight the need to fit the animal models and the experimental protocols used in such models with the real practice in the clinic.
Indeed, administration of therapeutics needs really to be given at a similar time and dose and using procedures that mimic clinical infarct characteristics sufficiently closely so that the results can potentially be translated to humans. Additionally, preclinical data should to be reproduced by multiple laboratories and in various species.

The MitoCare project results are in line with recently published trials on cardiac reperfusion injury where promising preclinical data could not be translated in man and for which the residual infarct in human was already very low [5-9]. The high standard of care might be an explanation and is potentially the most important factor in myocardium survival by simply preventing reperfusion injury that could be a syndrome occurring only after a rather long delay (>3h) between onset of nitrate positive pain until ballooning or in patients with large infarcts and no blood flow as seen with cyclosporine A [4].

The tools developed during the trial, such as the eCRF, the biobank, the Web-based image data transfer system with rapid quality feedback as well as the development of tests for potential new biomarkers will be exploited for future use and will generate further business opportunities for the 3 SMEs that developed them during the project.

Business impact and perspectives

Commercial opportunities arising from the results of the MitoCare project are driven by several factors.

In spite of the “null” result of the clinical trial using TRO40303 for reperfusion injury during PCI in AMI patients, TRO40303 has been shown to be safe and well tolerated in a medium multicenter trial leading to a total of 112 patients or healthy volunteers who were exposed to the drug at 6 mg/kg (27 in phase I and 85 in MitoCare phase II).

In addition, the results obtained in the preclinical work confirmed that TRO40303 can delay mPTP opening, reduce reperfusion injury as well as associated burst of reactive oxygen species and cell death as a consequence in some of the models. Thus the potential of the

drug towards the treatment of reperfusion injury or cell death associated with mPTP and mitochondrial stress in other disease states remains.

Hence it is Trophos' team belief that TRO40303 remains a drug of interest for the treatment of mitochondrial associated disease. Based on both the non-clinical pharmacology dataset generated during the Project as well as the reassuring safety data generated by the clinical trial, Trophos will further investigate the interest to test TRO40303 in other diseases such as acute hepatitis, acute pancreatitis or renal ischemia reperfusion injury. It should be kept in mind that many drugs that are now on the market were initially developed for another indication than the one it is now marketed for.

Additionally, several tools have been developed during the trial by SMEs Mobile Health, IMACOR and Firalis, such as an eCRF on iPads, a new Web-based image data transfer system with rapid quality feedback, and a panel of biomarkers including new assays for markers such as CNN1, PGF and NGAL were validated for use in clinical trials.

Dissemination activities

Publications

- One research article was published in Cardiology describing the design of the clinical trial: The MitoCare study group, Rationale and design of the “MITOCARE” study: A phase II, multicenter, randomized, double-blind, placebo controlled study to assess the safety and efficacy of TRO40303 for reduction of reperfusion injury in patients undergoing percutaneous coronary intervention for acute myocardial infarction. Cardiology 2012;123(4):201-7.

- At least two publications are currently under preparation: one describing the results of the clinical trial and one focussing on the comparative efficacy of hypothermia and TRO40303 in preclinical models. Translational aspects of the preclinical models to the clinical setting will be discussed in publications on TRO40303 as well as on cooling.

- A “clinical publication committee” and a “preclinical publication committee” have been set-up and a publication plan and strategy was made.

Congresses

- A poster was presented at the Gordon Conference on Cardiac Regulatory Mechanisms in June 2012: The cardioprotective effect of TRO40303 might be due to inhibiting respiration in stressed cells.

- An oral presentation was made at the ESC 2012, session 5238: Moderate hypothermia and TRO40303 reduce hypoxia-reoxygenation injury by affecting the kinetics of the mitochondrial permeability transition pore opening.

- A poster was presented at the ESC 2013, session 5530 and at the Mitochondrial Physiology Society summer School on Mitochondrial Physiology 2013: Reperfusion-induced mitochondrial dysfunction in the porcine heart is reduced by TRO40303 in the area at risk predominantly through preservation of outer mitochondrial membrane intactness

- The Consortium has submitted an abstract to present the results of the clinical trial at the European Society of Cardiology 2014.

Press releases

- Trophos announces the last-patient-out in its Phase 2 Proof-of-Concept study of TRO40303 in patients treated for an acute myocardial infarction (02/10/2013)

- Trophos initiates phase 2 proof of concept study for novel cardioprotective compound, TRO40303, in acute myocardial infarction (31/10/2011)

- Trophos awarded key US patent covering use of novel cardioprotective compound, TRO40303 (23/08/2011)

- Trophos successfully completes phase 1 study for novel cardioprotective compound, TRO40303 (24/02/2011)

- MITOCARE, a FP7 project, welcomes FIRALIS on board! (22/12/2010)

- Trophos to lead new MitoCare consortium awarded €6M by EU for POC study of novel cardioprotective compound, TRO40303 (14/12/2010)

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