Proton pump inhibitors (PPI) as a new strategy for therapy in sepsis: clinical trial to reduce severity of organ failure and in vitro experiments to search specific hallmarks in monocytes from septic patients and to characterize the mechanism of action of PPI [PPI-SEPSIS]

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1. Overall Summary

Sepsis is a severe disease with a high mortality rate and lack of efficacious therapies. Proton pump inhibitors (PPI) are drugs widely used to inhibit acid secretion by gastric cells and with a high safety profile. We have recently reported that PPI, such as esomeprazole, inhibit TNF-alfa and IL-1ß secretion. Moreover, we showed that a single administration of PPI protects mice from endotoxic shock with no adverse effects. We will design a randomized, double blind, controlled against placebo clinical trial with esomeprazole in septic patients to reduce the severity of organs failure. We will also investigate mortality and other clinically relevant outcomes. In parallel, we will evaluate ex vivo in monocytes from septic patients:

i. redox state and response to inflammatory stimuli;
ii. ATP release;
iii. metabolic changes and pH;
iv. cytokine production;
v. the effects of PPI on these parameters.

These studies will hopefully provide new insights into the pathophysiology of sepsis.

2. Background / State of Art

From data by the Intensive Care National Audit and Research Centre, the incidence of severe sepsis has increased by 68% over a nine-year period, such that the total number of severe sepsis cases in the UK is in excess of 45,000 per annum and with a hospital mortality rate of approximately 45%. Mortality rates increase with increasing number of organ failures. There are no registered drugs apart from antibiotics approved for sepsis treatment and in recent years many drugs failed to demonstrate efficacy in reducing mortality. Thus, sepsis represents a major healthcare and there is a huge need of drugs that can reduce morbidity and mortality in sepsis and septic shock.

Death in acute sepsis is attributed to hyperinflammatory responses but the underlying mechanisms are still unclear. Furthermore, increasing evidence indicates that mitochondrial damage associated to oxidative stress plays a relevant role. Finally, acidosis, a common treat of septic patients, especially non-survivors, has been implicated in the pathophysiology of sepsis. Acidosis is also a feature of all inflammatory microenvironments, due to metabolic shift toward aerobic glycolysis occurring upon activation. We have recently found that PPI are able to inhibit TNF-a and IL-1ß secretion in vitro by activated monocytes and in vivo in endotoxic shock in mice and identified the block of proton secretion (possibly by blocking surface bound v-ATPases) as a mechanism of inhibition (see preliminary data).

3. Hypothesis

a. Hypothesis and Significance

We hypothesize that PPI are promising new drugs against sepsis, in terms of SOFA score reduction. This hypothesis is based on the following evidence:

1. PPI block TNF-a and IL-1ß secretion in vitro and in vivo;
2. PPI were highly efficacious in the treatment of endotoxic shock and of other systemic inflammation conditions such as thioglicolate- induced peritonitis in mice without inducing toxic effects;
3. PPI-treated mice survived to sepsis are resistant to a second challenge with the same or a different sepsis-inducing agent.

The use of PPI as anti-sepsis drugs is of high significance: unlike other newly developed anti sepsis drugs, they are safe, low-priced and available in most countries.

b. Preliminary Data

We have shown that the simultaneous stimulation of human monocytes with different TLR agonists such as LPS (TLR4), R848 (TLR 7/8), Zymosan (TLR 6) causes a cytokine storm similar to that described in sepsis, with enhancement of IL-1b and TNF-a secretion and impaired secretion of cytokines downstream of IL-1 and TNF-a, such as the anti-inflammatory cytokine IL-1Ra. This loss of control of cytokine production is due to a strong increase of ROS production that induces a huge release of ATP, in turn responsible for the increased secretion of pro-inflammatory cytokines. Remarkably, the simultaneous intraperitoneal injection of the three agonists (LRZ) induces septic shock with a rate of mortality significantly higher than that observed after injection of individual agonists, even at 3-fold higher concentrations, indicating that LRZ stimulation mimics sepsis. Treatment with Apyrase (a drug blocking extracellular ATP) increase survival of LRZ injected mice, supporting the important role of the excessive secretion of ATP in sepsis.

We have investigated the molecular mechanisms underlying esomeprazole therapeutic effects in sepsis. Our data suggest that esomeprazole block cytokine production by blocking proton extrusion by activated monocytes. In fact:

1. Esomeprazole prevents the medium acidification induced by treatment of monocytes with LRZ and increases pHi;
2. Exposure of monocytes to esomeprazole associated to Amiloride (a drug that prevents proton externalization by the NHE proton pump) is synergic with esomeprazole, both on preventing medium acidification and cytokine secretion.

These preliminary data represent a basis for the in vitro investigation of:

1. The redox state in monocytes from septic patients and their redox response to stimulation;
2. The role of an exaggerated secretion of ATP in the pathogenesis of sepsis;
3. The mechanism of action of esomeprazole.

Lastly, esomeprazole in human has been used extensively and is considered a safe drug, even at very high dosage. There are no report of severe poisoning with drugs of the PPI category even at dosage higher than proposed in the present study. The proposed rationale for efficacy and the safety profile of the drug make esomeprazole a suitable objective for a clinical study.

4. Specific Aims

a. **Specific Aim 1**

To ascertain if high dose of esomeprazole safely reduces the incidence and severity of organ dysfunction as compared to placebo in adult patients with sepsis or septic shock, with reference to the mean SOFA score in the two groups.

b. **Specific Aim 2**

This aim is divided in two sub-aims:

a. Identifying new diagnostic or prognostic markers by analyzing functional traits in monocytes from critically ill patients with sepsis: definition of redox state and intra and extracellular pH, quantification of the levels of secreted ATP and cytokines (TNF, IL-1, HMGB1) at baseline and following in vitro TLR stimulation;
b. Understanding the molecular mechanism(s) underlying the therapeutic effects of PPI in acute sepsis: identification of the changes induced in the above parameters by treatment with esomeprazole vs placebo.

c. **Specific Aim 3**

Characterization of the presence of the following modifications in monocytes from survived patients, treated with esomeprazole or placebo:

i. epigenetic changes;
ii. changes of cellular metabolism;
iii. iii. different functional programs expressed in macrophage differentiation (M2 vs M1 polarization).

5. Experimental Design

The study will be a multicentric, randomized, double blind, controlled with placebo clinical trial. All the patients and the study personnel, including those involved in the postoperative management of the patients, will be blinded to treatment assignment for the duration of the study.
Patients will be randomized on a 1:1 basis. Randomization will be performed by a computer with the use of a permuted block design. The results of the randomization will be transferred in sealed envelopes, one for each patient. When the patient will be randomized in the study, the envelop will be open and the piece of information about the randomization present in the envelop will be followed by

the ICU staff.
Data will be collected during the drug administration (lasting 72 hours) and up to 90 days after randomization.

a. **Aim 1**

Study population

Inclusion criteria

1. Age ≥ 18 years old
2. Admitted to intensive care unit or emergency department
3. Sepsis or septic shock *(Sepsis defined as acute change in total SOFA score≥2 points consequent to the infection. The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction. Septic shock defined as sepsis plus persisting hypotension requiring vasopressors to maintain MAP ≥65mmHg and having a serum lactate level >2 mmol/L (18mg/dL) despite adequate volume resuscitation)*
4. Able to express informed consent as requested by Ethical Committee.

Exclusion criteria

1. Able to express informed consent and deny it
2. Known allergy or intolerance to study drug
3. Little chance of survival, as defined by a SAPS II score more than 65 point
4. Concomitant acquired immunodeficiency syndrome (AIDS, Stage 3 HIV infection according to CDC)
5. On immunosuppressant or long-term corticosteroid therapy (more than 0.5 mg/kg/day of prednisone or equivalent for over 30 days)
6. Receiving lifesaving drugs known to have a strong interference with esomeprazole
7. Sepsis or septic shock since over 36 hours
8. Severe hepatic dysfunction
9. Pregnant or breastfeeding

Primary outcome is severity of multiple organ failure measured by mean SOFA -Sequential organ failure assessment- scores between groups. The mean SOFA has been shown to be closely correlated to mortality.

Secondary outcomes

1. Death from any cause at day 28, 60 and 90 after randomization;
2. Antibiotic-free days at day 28;
3. Adjusted ICU-free day at day 28;
4. Single organ failure severity, evaluated by mean difference in each different category of SOFA score.

Study design

Patient will be randomized to two groups:

a. Experimental group will receive a bolus of 160 mg of esomeprazole and a IV perfusion of 12 mg/hr for 72 hours
b. Control group will receive a continuous a bolus and a continuous infusion only of sodium chloride without the experimental drug.

We will follow drug reconstruction guidelines reported in the relevant sections of the "Summary of product characteristic (SmPC) (Italian: "RCP: Riassunto caratteristiche del prodotto"). Section 6.6. of reference SmPCTo recommends to visually inspect the reconstituted solution for particulate matter and discoloration prior to administration. Only clear solution should be used. A solution for injection (8 mg/ml) will be prepared by adding 5 ml of 0.9% sodium chloride for intravenous use to the esomeprazole 40 mg vial.

The initial bolus will be prepared dissolving 160 mg of esomeprazole in 100 ml of 0.9% sodium chloride for intravenous use and administered over 60 minutes.

For continuous infusion, reconstituted esomeprazole at a concentration of 8 mg/ml will be injected at a rate of 1.5 ml/hr (12 mg/hr).

Clinical and physical stability of the study drug has been demonstrated up to 12 hours at 30°C. Therefore, solution will be prepared accordingly.

b. **Aim 2**

Peripheral blood samples will be obtained from healthy donors (20) and patients enrolled in the trial, 15 from experimental group and 15 from control group. Blood samples from healthy donors are necessary to validate the experimental method and to set baseline values. Data from healthy donors will not be collected and won't be part of the study. Monocytes isolated from blood samples will be assessed for basal production of ROS and antioxidants and stimulated with single or multiple TLR agonists. Redox response, secretion of ATP and of cytokines of interest (TNF-a, IL- 1ß, IL-6, HMGB1, IL-1Ra, IL- 10) will be measured. The serum of patients will be analyzed for total antioxidant capacity through TAC assay. The levels of ROS, ATP secreted and antioxidant systems will be compared in monocytes from the two groups of patients (and in the healthy donors) and will be correlated with the clinical outcome. The amplitude of the metabolic shift to aerobic glycolysis will be compared in vitro in monocytes by measuring the production of lactate and the acidification of the medium. The effects of esomeprazole on extracellular pH will be also assessed in vitro.

c. Aim 3

In monocytes from survived patients, treated with esomeprazole or placebo the presence of epigenetic modifications and changes in expression of miRNA that target specific genes involved in sepsis will be investigated. The changes of cellular metabolism at baseline and following TLR stimulation will also be analyzed by assessing the expression of key glycolytic enzymes, transporters for glucose and by the

levels of selected metabolites (lactate) and the pHi and pHe. Moreover, monocytes will be differentiated in macrophages and the effect of PPI treatment in the pro-inflammatory or anti-inflammatory polarization will be evaluated.

6. Methodologies and statistical analyses

a. Blinding

Each patient will receive both a bolus and continuous infusion. For the control group bolus and infusion will be done only of sodium chloride 0.9%, without study drug. For the experimental group, the solution will include esomeprazole. The solution of the experimental drug is colorless and this will maintain blinding. Every patient can receive an unblinded prescription of proton pump inhibitor for stress ulcer prophylaxis if clinically indicated.

b. Data sample

Following information will be recorded in day 0 before administration of first dose of study drugs

- Demographic, biometric and administrative data
- Ventilatory status and ventilatory settings
- Vital signs, Glasgow Coma Scale evaluation
- Arterial blood gas sample
- Serum electrolyte, hemochrome, clotting tests, liver and kidney profile, lactate
- Urine output
- C-reactive protein and procalcitonin
- Source of infection
- Inotropic / vasopressors drugs dosage
- Clostridium difficile antigen testing on stool

c. Sample size

Our planned sample size is 300 patients. This will provide more than 80% power to detect a 0.5-point difference in mean SOFA score assuming a SD of 1.5 and an overall mean SOFA of 3.55. In their validation study published on JAMA in 2001, Ferreira et al. analyzed data from 352 consecutive patients admitted to a general ICU in Belgium. They found that a 1-point rise in mean SOFA score was associated with a significant mortality increase (mean SOFA 2.1-3.0=20%, 3.1- 4.0=36.1% and 4.1-5.0=73.1% mortality; OR 3.06, 95%CI 2.36-3.97) and had a very good discriminative power (area under the ROC curve=0.88). However, the variable with the best discriminative power was the highest SOFA score (area under the ROC 0.90). Supporting previous work, the change in SOFA score during the patient's ICU stay was independently predictive of outcome. For those with an initial SOFA of >11, a mean SOFA that increased or stayed the same was associated with a 91% mortality rate. In summary, several studies have now described the use of the SOFA score. Increasing organ dysfunction as measured by the SOFA score consistently correlates with increasing mortality. SOFA is a reliable measure of organ dysfunction

at admission and during ICU stay.
We will recruit an additional 4% (14 patients) to account for potential loss to follow-up and withdrawal of consent.
For Aim 2 and 3 planned enrollment is 30 (15 experimental group, 15 control group). The number of subjects studied will be increased if necessary to reach statistical significance.

7. Method for in vitro studies

a. Isolation of serum from whole peripheral blood;
b. Isolation of monocytes from heparinized peripheral blood from patients and healthy individuals by Ficoll gradient and adherence on cell culture plates or by Monocyte Isolation Kit.
c. Cells stimulation with TLR agonists in the presence or absence of esomeprazole. Differentiation in macrophages M1 or M2 with GMCSF and IL-4 or LPS plus IFN a .
d. Fluorimetric methods or imaging for ROS and ATP determination.
e. RT-PCR and western blot analysis of antioxidant systems and glycolitic enzymes.
f. Colorimetric method for evaluate of cysteine release and the total antioxidant capacity (TAC) of the serum.
g. Acidosis analysis with fluorescent reagents (BCECF-AM, lysosensor) or pHmetre; kit assay for determination of lactate in medium.
h. ELISA for cytokine determination.
i. Analysis of DNA methylation: extraction of genomic DNA, bisulfite conversion, amplification of DNA using primers selected in the promoter region of the genes studied and sequencing of PCR products.
j. Purification of low molecular weight RNA, retro-transcription of miRNAs and RT-PCR for evaluated their expression.

8. Data Analysis

Demographic and baseline disease characteristics will be summarized with the use of descriptive statistics. Categorical variables will be reported as absolute numbers and percentages. Unadjusted univariate analyses, to compare the two treatment groups, will be based on Chi-square or Fisher exact test. Relative risks and 95% confidence intervals will be calculated by means of the two-by-two table method. Continuous variables will be reported as mean ± standard deviation (SD) or median and interquartile range (IQR). Here, between-group differences will be evaluated using the unpaired T test of Student or one-way Anova test, followed by Bonferroni post-test. Logistic regression models, adjusted for baseline values, will be used to estimate the treatment effect (and its 95% confidence intervals) with respect to primary and secondary endpoints. Statistical significance will be set at the two tailed 0.05 level for hypothesis testing.
Primary data analysis will be based on intention to treat analysis.

Data will be stored electronically via a web based CRF and analyzed by use of Stata (Stata Statistical Software: version 15, College Station, TX, USA).

Some pre-defined subgroup analysis will be performed

1. According to ICU/ER admittance
2. According to the baseline severity of sepsis and to SOFA score quartiles

9. Expected outcomes

For Aim 1: reduced severity of organ failure in experimental group, without safety issues.

For Aim 2 and 3:

a. identification of oxidative stress in monocytes from septic patients;
b. Correlation between redox stress, massive ATP release and altered cytokine production; amplitude of the metabolic shift to aerobic glycolysis in vitro in monocytes from the patients;
c. reduced redox stress and cytokines productions in monocytes from sepsis patient treated with esomeprazole; d. insight into the mechanisms of action of PPI on monocytes.

10. Risk analysis, possible problems and solutions

The proposed dose of esomeprazole is high: in treated patients, we will administer 448 mg of omeprazole on the first day (6.4 mg/kg in a 70 kg patient) and 288 mg (3.6 mg/kg in a 70 kg patient) for the next two days. Exclusion criteria will protect patients at theoretical risk of toxicity, even if poisoning from PPI has never been described despite more than 20 years of every day utilization. A strict monitoring of adverse events will be performed. A safety board, unblinded and independent, will be present.

Reports of overdosage with omeprazole in humans, with doses ranging up to 2,400 mg (120 times the usual recommended clinical dose) only produced minor clinical manifestations, including confusion, drowsiness, blurred vision, tachycardia, nausea, diaphoresis, flushing, headache, dry mouth, and other adverse reactions similar to those seen in normal clinical experience.

The symptoms described in connection with deliberate esomeprazole overdose (limited experience of doses in excess of 240 mg/day) are transient. Single doses of 80 mg of esomeprazole were uneventful.

In animals, a single oral dose of esomeprazole at 510 mg/kg (about 100 times the dose we will be administering in our trial), was lethal to rats. The major signs of acute toxicity were reduced motor activity, changes in respiratory frequency, tremor, ataxia, and intermittent clonic convulsions.

For non-clinical aims, the methodologies proposed in this study have been widely used in our laboratory. The analysis of cytokine production and redox state in monocytes from patients and healthy donors are adequately validated.

The acidosis analysis in vitro could be problematic because less routine. However, many different

reagents are available (BCECF, lysosensor) and in our laboratory is present a new pHmetre designed for measuring minor changes of pH in small volumes.

11. Significance and Innovation

Sepsis is an emergency worldwide, with no efficacious therapy available, and represents a major healthcare problem. The clinical trial with PPI proposed in this project will hopefully provide a new effective drug for severe sepsis and septic shock. PPI display other advantages over different newly developed anti-sepsis drugs: they are safe, display a very low risk of adverse events (almost absent in the case of a short-term use), are low-priced and available in most countries.
This project will also provide important information about susceptibility to develop sepsis, and about sepsis diagnosis and prognosis. This information will be translated into new diagnostic/prognostic tests.

12. Description of the complementary and synergy research team

The coordinating center, Scientific Institute San Raffaele is based in Milan and is the largest biomedical science park in Italy. It coordinates and carries out research into clinical and molecular medicine with dramatic impact on the National Health Service: new therapeutic approaches have been adopted for the treatment of hard-to-cure pathologies and procedures have become more efficient, faster, less-invasive as well as cheaper for the diagnosis and the treatment of more traditional diseases. The Department of Anesthesia and Intensive Care of San Raffaele is the one of the most prolific group in the field of anesthesia and intensive care in Italy. This is an assurance for the donor that the project will be finalized in due time and published in an international peer reviewed Journal within a short time.
The IRCCS AOU San Martino - IST is an highly specialized hospital of national importance. The group of Cell Biology has a strong background on the innate immune response mechanisms in healthy subjects or in auto-inflammatory diseases, as well as on the redox response mechanisms in various experimental systems. Moreover, recently this group has achieved promising results with PPI treatment in in a mouse model of endotoxic shock.
The collaboration between the two groups therefore offers a unique opportunity to understand and treat a disease that is now an emergency at the international level.

13. Training and tutorial activities

The study is pragmatical, randomized, double blind and controlled against placebo and will not require any specific training or tutorial for its implementation, especially for the clinical aim 1. Before study begin, however, will be organized some meetings for clinical staff working in ICU - ER for explaining and discussing inclusion and exclusion criteria and study procedures.
No further training is needed for aim 2 and 3.

14. Ethical considerations

The Investigators will conduct the study according to the procedures specified in the study protocol, and in accordance with ICH GCP notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments and NH&MRC National Statement on Ethical Conduct in Research Involving Humans.

The monitoring of the study will be performed according to international standards and GCP by an independent CRO.

It is recognized that patients with sepsis or septic shock and admitted in the ICU will experience a number of aberrations in laboratory values, signs and symptoms due to the severity of the undergoing disease and the impact of standard critical medicine therapies. For example, the data listed in the CRF are "expected" to occur and will be considered "disease progression". These will not necessarily constitute an adverse event or serious adverse event unless they are considered to be related to study treatment or a concern in the principal investigator's clinical judgement.

Due to pharmacological sedation or to the severity of sepsis, most patients will be partially or totally unconscious at the time of trial enrollment. For these patients, we will strictly adhere to the directives of the Italian Data Protection Authority and the Italian Law. A Legal Representor will be nominated by the Court if possible. When in emergency, if the Legal Representor is not available, the Principal Investigator will discuss the case with two colleagues and decide upon the inclusion of the patient in the trial in his/her best interest. Once the consciousness state of the patient has been restabilished, he/she will be informed on the administered therapy and he/she will be asked a consent to use his/her data.

For all patients, including those who will not regain a consciousness state (eg. exitus), all the most restrictive cares in terms of privacy will be used.

15. Bibliography

1. Mayr FB et al. 2013. Epidemiology of Severe Sepsis. Virulence 5 (1).
2. Singer M et al. 2016. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 315 (8): 801-10. doi:10.1001/jama.2016.0287.
3. Ferner RE et al. 1993. Omeprazole Overdose. Human & Experimental Toxicology 12 (6): 541-42.
4. Ferreira FL et al. Serial Evaluation of the SOFA Score to Predict Outcome in Critically Ill Patients. JAMA 286 (14): 1754- 58.
5. Forrester MB 2007. Pattern of Proton Pump Inhibitor Calls to Texas Poison Centers, 1998-2004. Journal of Toxicology and Environmental
6. Health. Part A 70 (8): 705-14. doi:10.1080/15287390601188045.
7. Neumann I et al. 2013. Comparison of Different Regimens of Proton Pump Inhibitors for Acute Peptic Ulcer Bleeding. The Cochrane Database of Systematic Reviews, no. 6: CD007999. doi:10.1002/14651858.CD007999.pub2.

8. Orme RM et al. 2014. An Efficacy and Mechanism Evaluation Study of Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis (LeoPARDS): Protocol for a Randomized Controlled Trial. Trials 15: 199. doi:10.1186/1745-6215-15- 199.
9. Angus DC et al. 2013 N Engl J Med. 29;369(9):840-51
10. Noritomi DT et al. 2009 Metabolic acidosis in patients with severe sepsis and septic shock: a longitudinal quantitative study. Crit Care Med. 37(10):2733-9.
11. Balza E et al. 2016 Proton pump inhibitors protect mice from acute systemic inflammation and induce long-term cross- tolerance. Cell Death and Disease ; 7:e2304; doi:10.1038/cddis.2016.218
12. Lavieri R et al. 2014. TLR costimulation causes oxidative stress with unbalance of proinflammatory and anti-inflammatory cytokine production. J Immunol. 1;192(11):5373-81.
13. Cui LH, Li C, Wang XH, Yan ZH, He X, Gong SD. The therapeutic effect of high-dose esomeprazole on stress ulcer bleeding in trauma patients. Chin J Traumatol. 2015;18(1):41-3

16. Timeline / Deliverables / Payable Milestones

As request, Ethical Approval will be obtained before the beginning of the project.

1. Buying the study drug (investigational drug and placebo)	Months 1-6
2. Activation of patients' insurance	Months 1-2
3. Identification of personnel (biostatistician, medical physician, research nurse, data manager)	Months 1-30
4. Identification and contract of CRO for the monitoring activities	Months 1-12
5. Preparation of data entry form	Months 1-2
6. Organizing the first investigator meeting	Months 12-18
7. Randomization and follow up	Months 2-36
8. Monitoring following national and international guidelines, declaration of Helsinki and GCP	Months 1-36
9. Statistical Analysis	Interim analysis and final reports till month 36
10. Dissemination: writing the paper with the study design	Months 12-18
Dissemination: writing the final paper with the results	Months 30-36
11. Organizing the final investigator meeting/congress to disseminate the results	Months 33-36

a. **Milestones 18 month**

i. Aim 1

Months 1-6	Buying study drug and identification and contract of CRO
Months 1-2	Activation of patients' insurance and preparation of data entry form
Months 3-5	Writing the paper with the study design

ii. Aim 2 and 3

Months 1-18	Analysis of ROS production, antioxidant response, ATP release and cytokines secretion in monocytes. Correlation of the redox state, ATP release and cytokines secretion with the clinical outcome. Analysis of serum of patients for total antioxidant capacity

b. **Milestones 36 month**

i. Aim 1

Months 2-36	Randomization and follow up
Months 34-36	Writing the final paper. Organizing final congress

ii. Aim 2 and 3

Months 18-24	Analysis of the production of lactate and acidification of the medium in monocytes from the two groups of patients and from healthy donors. Study of esomeprazole treatment on extracellular pH in vitro
Months 18-36	Study of epigenetic changes, modifications of cellular metabolism and macrophages polarization in monocytes from patients survived to sepsis

17. Equipment and resources available

IRCCS San Raffaele and IRCCS AOU San Martino IST are two large hospitals with coupled more than 50'000 ER access/year and 500 ICU admission / year. The Anesthesia and Intensive Care Unit directed by Prof. Paolo Pelosi (IRCCS San Martino-IST, Genoa) and The Anesthesia and Intensive Care Unit directed by Prof. Alberto Zangrillo (IRCCS San Raffaele, Milano), will randomize septic patients for Aim 1 and will provide blood samples for Aim 2 and 3.

Other centers could be invited to participate to the trial, without any further cost for the donor. Both the Institutions have a great network of skilled centers that could help to enroll patients.

According to data published by GIVITI (Italian Group of Vigilance on ICU) in 2015, the San Raffaele Hospital ICU admitted over 70 patients with sepsis or septic shock. Patients will be enrolled also in the ER, reaching the expected amount of patients in a timely fashion.

Both the Institution have extensive practice in clinical research. Dr. Giacomo Monti works in the San Raffaele ICU, that is a mixed ICU with 9 beds fully equipped. All physicians and nurses are expert in conducting clinical research. Dr. Carta Sonia will play her research activity in the laboratory of Cell Biology (IRCCS San Martino-IST, Genoa) directed by Dr. Anna Rubartelli. This unit is composed of 3 assistant researchers and 2 technicians. The unit occupies about 200m2 of fully equipped laboratories. Namely, the unit is supplied with cell culture, biochemistry and molecular biology laboratories, fully equipped for the necessity: vertical laminal flow hoods, CO2 incubators, direct and reverse

microscopes, refrigerated centrifuges and microfuges; gel apparatus for electrophoresis, gel dryer, refrigerators, -20° and-80° freezers, liquid nitrogen storage etc. Moreover, BL2 laboratories, confocal microscopy, ultracentrifuges, thermal cyclers, FACS, Mass spectrometer, animal facilities, sequencer, are available at the same Institute. The research team will be led by the principal investigators by means of a coordinating management committee and will include the physicians and the nurses involved in the postoperative management. Trained and skilled independent research coordinators; data manager and biostatisticians will collect and analyze study data. Expert biostatistician will assist the principal investigator throughout all the phases of the trial and will analyze the study findings. The study will be supervised by a Data Safety Monitoring Committee.

18. Translational relevance and impact for the National Health System (SSN)

The project will provide important information for the diagnosis, prognosis and treatment of sepsis. The disease is now an emergency not only locally but also transnationally. Therefore, the results obtained will allow not only the acquisition of new knowledge but will give the opportunity to develop new diagnostic/prognostic tests and new therapeutic strategies for patients with sepsis.
Researchers on the field of sepsis will benefit of the increased knowledge. In addition, the new results will benefit the national health systems, helping to make choices based on scientific criteria, which will allow invest in a strategic way the limited resources to achieve the best results in the prevention, diagnosis and treatment of sepsis.

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Dott. Giacomo Monti