



## *Research Protocol*

Project acronym **GenOSept**

Project full title

**Genetics of Sepsis and septic shock in Europe**

Operative commencement date of project: February 2005 (*Month o*)

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## 1. Project summary

Project full title                      Genetics of Sepsis in Europe  
Project acronym                        GenOSept

### PROPOSAL ABSTRACT

GenOSept is a specific targeted research project which uses a multidisciplinary fundamental genomics approach (gene expression, structural genomics, population genetics) to examine genetic predisposition to sepsis.

Sepsis (life-threatening infection) is a major public health problem throughout Europe. In the USA in 1995 it cost \$17 billion to treat 751,000 patients with severe sepsis, of whom 28.6% died. The Center for Disease Control suggests that sepsis-attributable mortality rates are rising. We hypothesize that susceptibility to expensive new treatments, and fatal outcomes from severe sepsis, are in part, genetically determined.

GenOSept work packages will test this hypothesis using the following approach. We will use gene expression studies to define novel candidate genes including those controlling programmed cell death. The novel candidate genes so identified will then be analysed in subsequent epidemiologic studies of genetic predisposition to sepsis-related mortality and morbidity in European intensive care units.

GenOSept will provide important data on gender-related mortality and morbidity. It will have a major impact on diagnosis and treatment of European sepsis patients in subsequent therapeutic trials by targeting risk subpopulations and focussing expensive new treatments. GenOSept will standardise protocols for genotyping, facilitate application of new knowledge in functional and structural genomics, harmonize high-throughput genotyping and quality control between major European centres, and contribute to reducing sepsis-related mortality in European health care.

## 2. Project objectives

The objectives of this project are to link fundamental genomics with several of the leading research groups within EU working in the field of sepsis genomics to obtain a significant and critical mass of research material to enable realistic genotype/phenotype association studies as indicated in detail below.

There are numerous pragmatic advantages from the establishment of a network such as this in addition to those expected from closer and wider intellectual collaboration. The groups forming the core of this project met with unanimous and directed application with a determination to establish this European network, enabling a rationalisation of effort, resources and skills and a reduction in research wastage.

GenOSept is a specific targeted research project which focuses on a multidisciplinary fundamental genomics approach (gene expression, structural genomics, population genetics) to unravel genetic predisposition of Sepsis. Novel findings derived from gene expression studies and genome scan analyses will provide data and set the requirements for a genetic epidemiologic study applying this knowledge directly to a major health care problem in intensive care.

The genetic epidemiologic study as part of this project has been designed utilising the considerable experience that the ESICM has accrued over several years in linking intensive care units across the European Union in a variety of short duration but powerful research and audit projects. Using a cohesive and uniform platform of practice in intensive care units, together with a tried and tested format for data recording, the

research material collection process will be carried out over a twelve month period and this will be followed by a co-ordinated analytical and evaluating process by the genome centres.

One of the objectives of this project will be to demonstrate the ability of the hitherto relatively competing research groups and genome centres to work closely and efficiently in such a large scale network in order to directly apply novel findings from fundamental genomics (gene expression, structural genomics) to a medical problem of high relevance to European citizens in a timely manner. The establishment of this network will enable the development of further projects within the field of functional genomics and related areas of research. It is widely recognised that the study of functional genomics and associated pharmacogenomics, proteomics and metabolomics will provide valuable insights into the pathophysiological mechanisms of complex diseases in the arena of critical care. These are expected to lead to targeted therapy for a number of conditions encountered but particularly in the treatment of sepsis which has such a high mortality rate. Included in the partners involved in this project are groups developing and evaluating technologies that will be applicable to these newer techniques that are as yet unfeasible in this network and that will become so, partly resultant upon the scientific leads expected from GenOSept.

A further pragmatic object will be to discern, clarify and hopefully unify the ethical issues that pertain to this type of research. It is recognised that there are variations in the protocols and in national public and institutional attitudes towards both clinical research and to the collection of genomic material from patients in intensive care, the majority of who are incapable at that stage of providing informed consent. The project has defined the mechanism that can be utilised across European Union for this essential process but the object and subsequent value is expected from unifying this approach across European Union.

### **3. Potential impact**

#### **STRATEGIC IMPACT**

GenOSept is a project supported by the ESICM European Critical Care Research Network (ECCRN) that is designed to provide a forum for facilitating, integrating and supporting research in critical care medicine conducted by members or research groups. The Executive Committee of the ESICM have identified this project as one that matches the most pressing clinical need of the moment to intensive care practice as well as providing an ideal model for the ECCRN. GenOSept will also link to COBaTrICE, an EC funded programme run by ESICM which intends to train European intensive care physicians with regard to sepsis diagnostics and therapeutic options aiming at harmonizing European intensive care of patients with sepsis on the highest level of evidence available to date. A more standardized medical care of these patients would facilitate GenOSept evaluation.

One of the potential strategic impacts of this project will be the integration of a powerful collection of research scientists working at the forefront of molecular medicine in the European clinical research arena. To date the scale and scope of basic science research in critical care has, as is the case for most other similar developments in science, been restricted to the local or occasional national level. Even though there are significant examples of collaboration at national level in several European countries within the framework of ESICM none has had the opportunity to obtain large databases on the scale envisaged by GenOSept. Those working across the world in the field of functional genomics recognise the essential value from wider collaboration and open access to scientific developments and networks of a similar nature are developing in the United States ('GenPSS') and in Japan ('JapanGenPSS'). The groups in the US recognise that Europe has a significant advantage in establishing such a network as the quality of scientific research and expertise is high and that there is a structured clinical environment within intensive care clinical practice increasingly influenced by training programmes run by the ESICM. The steering group has received advice and encouragement in the development of GenOSept from representatives from the US critical care genomics group (GenPSS) and the developing co-operation with this network will have a considerable impact on the pace and efficiency of this work.

#### **INNOVATION**

GenOSept will link fundamental genomics to a prominent medical problem in European intensive care in a timely manner. Application of gene expression studies and structural genome analysis detecting genomic variation will generate novel data on relevant genes as well as novel genomic variations involved in the genetic predisposition of incidence and outcome from sepsis.

The project will also be innovative in the context of its unique collection of genomic material from a large group of Europe-wide critically ill patients, in the scope of the functional genomic approach evaluating the potential genotype/phenotype associations in a large number of candidate genes, in the opportunity for population and evolutionary genomics, in the evaluation and use of novel techniques in the gene chip technology and in the establishment of an European network of clinical and laboratory groups working in the field of critical care medicine.

## INTERACTION WITH NATIONAL AND INTERNATIONAL RESEARCH ACTIVITIES

This has also been described but the steering group of GenOSept includes principal investigators from the leading national projects in this field. Some of these longer duration national projects will have different focus, targeting different disease groups within the umbrella of sepsis, collecting different phenotype data and carrying out complex expression profiling. There is anticipated a strong collaboration between these national groups and the GenOSept collection and analysis and the national groups see the potential benefit for their hypothesis testing projects. Close international collaboration is already established and GenOSept will provide and absorb benefit from this effort.

This project has developed in synchrony with the Surviving Sepsis Campaign (SSC – [www.survivingsepsis.org](http://www.survivingsepsis.org)) which is a global initiative launched in October 2002 under the auspices of the ESICM, the Society of Critical Care Medicine (SCCM) and International Sepsis Forum (ISF). This campaign has several targets including increasing awareness of sepsis, improved education in the diagnosis and management and encouraging research in order to achieve a reduction in mortality rates from sepsis by 25% within five years. The executive committee of SSC fully endorse the GenOSept project which is expected to enhance many of the aspirations of the campaign in addition to the addressing and attempting to provide answers to a key scientific hypothesis.

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## 4. Project management and exploitation / dissemination plan

### 4.1 PROJECT MANAGEMENT

#### Co-ordination of the project

The project will be co-ordinated by two of the partners: ESICM (Professor Julian Bion and Audrey Augier), and the University of Bonn (Professor Frank Stuber); ESICM has the experience of managing large projects (and budgets).

The University of Bonn also has experience in scientific management of projects, as scientific data managers of numerous national as well as international studies. Prof. Wienker is a leader in the field of genetic epidemiology in complex diseases, whereas Prof. Stuber conducts and chairs genomic studies in the field of cardiac and other diseases (McSpi: multi centre study of perioperative ischemia, SepNet: Genetics and Sepsis in Germany, Genetics and Major Trauma).

The means and procedures to accomplish all the tasks detailed above (e.g. reporting, financial accounting etc.) will be part of the Consortium Agreement.

#### Steering Committee

##### Composition

A Steering Committee has been created, consisting of the lead investigator from each of the 14 partners, and co-chaired by the representatives of the two co-ordinators, ESICM and the University of Bonn. The composition of the Steering committee is therefore as follows:

Participants ID	Organisation names	GenOSept Lead investigator
1	ESICM	Prof. Julian Bion (co-chair)/ Mrs Audrey Augier
2	University of Bonn	Prof. Frank Stüber (co-chair)
3	University of Oxford / Wellcome institute for human genetics	Prof Adrian Hill / Dr Paul Holloway
4	Institute Pasteur, Cochin Institute	Prof. Jean-Daniel Chiche
5	University of Torino, Ospedale S. Giovanni Battista	Prof. Marco Ranieri
6	University Rovira & Virgili of Tarragona, Hospital Joan XXIII	Prof. Jordi Rello
7	GSF	Prof. Thomas Meitinger
8	Hadassah Medical University Centre	Dr Yoram Weiss
9	SIRS-Lab	Dr Stefan Russwurm
10	University of Ulm	Prof. Marion Schneider
11	University of Jena	Prof. Konrad Reinhardt
12	Masaryk University Brno	Dr Vladimir Sramek
13	National medical Centre	Dr. Tibor Gondos
14	Tartu Univerity Clinics	Dr Silver Sarapuon

Each of the partners will contribute to the Steering Committee's decisions according to its competencies and resources.

#### Advisory Board

An Advisory Scientific Board will be constituted, composed of recognised experts in the field. The members are Dr. Timothy Buchman (Chairman GenPSS, President of the American Society of Critical Care Medicine, SCCM), Dr. Benoit Vallet (University of Lille), Dr. Derek Angus (University of Pittsburgh).

It will include representatives from major organisations in Intensive Care Medicine, such as the SCCM (Society of Critical Care Medicine - the American "sister society" of ESICM), the ISF (International Sepsis

Forum) and the American GenPSS programme which is integrated in the programme Impact (network of US intensive care units).

The task of this Group's members will be to advise the Steering Committee, with an external and neutral point of view, on any aspect of relevance to the project (organisational, scientific, ethical, financial, etc.).

#### **4.2 PLAN FOR USING AND DISSEMINATING KNOWLEDGE**

The general management of intellectual property rights will be arranged and approved by the Steering Committee. The management of these issues will be defined in further details in the Consortium Agreement.

##### **Ownership of data**

Investigators will own, and will be responsible for the integrity of, the data which they submit. This applies to projects concerning fundamental genomics (gene expression studies, genome scans) as well as individual ICUs involved in patient recruitment, and to national groups where these are formed as collaborating units. A copy of the whole dataset will be held by the GenOSept steering committee and by the ECCRN on behalf of the ESICM. We also consider that intellectual property rights are held by the ESICM on behalf of all clinical investigators in individual ICUs.

##### **Publications**

The major paper(s) reporting results from this study will be published in peer-reviewed journals under the collaborative name of the ESICM Critical Care Research Network GenOSept investigators, with responsible authors and attributions including participating ICUs in an appendix.

#### **4.3 RAISING PUBLIC PARTICIPATION AND AWARENESS**

The material collected in this project will be a valuable and unique resource. A special attention will be given to disseminating the results of the project, targeting a varied population: doctors, nurses, researchers, professors, universities, national and international societies of intensive care or related fields, and industry. In addition, major results will be made public to the European citizens by utilizing national media (newspapers: health care news, television: expert panels in health care reports, internet). Acknowledgement of the European Union – Framework Programme 6 Programme support will be included in all publications and presentations. This dissemination work will be done through the following media: Web site([www.esicm.org](http://www.esicm.org)), publications (articles in International, European or National scientific and educational peer-reviewed publications, as appropriate such as "*Intensive Care Medicine*" (4000 copies printed each month), ESICM Newsletter (3400 copies twice a year).

Results will be also presented during scientific congresses.

## 5. Implementation plan

### 5.1 INTRODUCTION - GENERAL DESCRIPTION AND MILESTONES

The present project is a systematic European study of genetic predisposition for mortality in sepsis related complex diseases occurring frequently in European intensive care units and causing high mortality rates: nosocomial pneumonia, peritonitis, pancreatitis and meningococcal disease.

The project will be divided into two different groups of work packages.

The first set of work packages (WP1-4) will develop consensus definitions, perform fundamental genomic studies on gene expression and structural genomics to detect novel candidate genes and their genomic variations. A European intensive care unit recruitment structure will be implemented as well as an adequate genetic epidemiologic study design. Analysis for optimal choice of genetic markers and data analysis will be performed.

The second set of work packages (WP5-12) will perform and evaluate a genetic epidemiologic study in European intensive care units to explore genetic predisposition for mortality and morbidity in sepsis based on the first part of the project and analyse the data. A harmonization and quality control of high throughput genotyping will be implemented. An SME will transform the obtained knowledge to a valuable diagnostic product.

The project will establish and perform genetic diagnostic tests to provide a greater understanding of one of the leading killers of hospitalised patients in Europe and to save the lives of patients with severe infectious disorders involved in nosocomial pneumonia, peritonitis, pancreatitis, and meningococcal disease.

### 5.2 WORK PACKAGE DESCRIPTIONS

#### WP 1. Consensus definitions

Workpackage number	1	Start date or starting event:							Month 1	
Participants involved	2	1	3	5	6	8	11	12	13	14

#### *Activity: Research*

#### Objectives

- To define phenotype of patients to be studied
- To develop consensus on inclusion and exclusion criteria

#### Description of work

Before starting any multicentre, empiric study, it is vital that consensus be obtained for inclusion and exclusion criteria and various prospective definitions. Experts in intensive care, infectious disease and microbiology, endocrinology and ethics, will come to a consensus on the various definitions that will be important for both genetic epidemiologic studies and for inclusion and exclusion criteria for the second part of the project – genetic predisposition for mortality and morbidity in sepsis. Consensus for definitions of severities of sepsis, septic shock, shock reversal, organ system dysfunction, in the context of four major complex diseases: nosocomial pneumonia, peritonitis, pancreatitis and meningococcal disease. It should be emphasised that several of the partners in this consortium have already established programmes that share common details in many aspects of protocol and many definitions and inclusion / exclusion criteria are in common usage.

This activity will take place from months 1 – 6. A meeting to develop the consensus criteria and definitions will occur at the beginning of month 4. The first several months will be used to develop a consensus for the inclusion and exclusion criteria that are presented in WP2 and the definitions. Because of the large number of people from different countries and different disciplines, it is our experience that it is important to hear

suggestions and have discussions over several weeks and go through several drafts before having a meeting. This will be done by e-mail, phone and fax. The criteria and definitions will be close to a final format by the time of the meeting. Another two months will be required to obtain Helsinki approval for each institution.

**Deliverables**

- Consensus definitions GenOSept
- Phenotype definition of patients
- Inclusion/Exclusion criteria
- Application for local ethics committees
- Consensus case report form, data items (CRF)

**Milestones and expected results**

- Phenotype definition of patients to be enrolled
- Consensus application for ethics committees
- List of data items to be included in case report form

**WP2. EVALUATION OF CANDIDATES GENES AND MARKERS**

<b>Workpackage number</b>	<b>2</b>	<b>Start date or starting event:</b>				<b>Month 4</b>	
<b>Participants involved</b>	4	3	2	9	10		

**Activity: Research****Objective**

To undertake a systematic chromosome-wide and then genome-wide review of candidate genes to identify candidate genes possibly affecting morbidity and mortality in sepsis. Newly available as well as well known single nucleotide polymorphisms for ultra-high-throughput genotyping technologies will be evaluated.

**Description of work**

This work package is complementary to WP7 in that it provides an approach to the identification of disease-associated genes. Instead of prioritising selected candidate genes, here a comprehensive chromosome-wide and then genome-wide approach will be taken to identifying genetic markers, and then genes, associated with poor outcome in sepsis. The major advantage of this approach is that it can identify novel genes and biochemical pathways not previously suspected to influence pathogenesis. Such novel pathways and target molecules are of even greater interest for the discovery of novel interventions that currently suspected target molecules and pathways.

Proof of principle of the feasibility of genome-wide genetic analysis in complex diseases has been provided by the identification of novel susceptibility genes for diseases such as Crohn's disease and asthma using genome-wide linkage scans. However linkage scans require many multi-case families and have lower power than association studies. We will undertake here a step-wise genome-wide association utilising the unique resource of patients recruited by GenOSept. The availability of about 6000 sepsis patients will provide an unprecedented opportunity to apply the power of modern genomics to a major cause of death in Europe. Currently over 4 million SNPs distributed across the human genome are available for association analysis. However use of all these markers would provide much redundant information. A major international consortium is currently applying linkage disequilibrium analysis to provide a more informative set of about 250,000 markers, a so-called haplotype map (or HapMap) that will allow efficient genome screening by association. The most advanced data are on chromosome 20 where 5000 preferred primers and markers are already available and provided in a set for ultra-high throughput genotyping (by Illumina Inc - see [www.illumina.com](http://www.illumina.com)). Similar sets for all 23 human chromosomes will be available by late 2004. Empirical Haplotyping will "condense" the information obtainable by SNP typing only. So called "tag" SNPs need to be identified for sepsis.

We will conduct a systematic evaluation of SNP's within the available databases suitable for haplotyping. Therefore, genotyping effort can be reduced to SNPs exclusively determining the haplotype of a given candidate gene.

***PROPOSED INCLUSION AND EXCLUSION CRITERIA FOR GENOSEPT***

As for WP7

***PROPOSED INCLUSION CRITERIA FOR SEPTIC SHOCK***

As for WP7

***PROPOSED EXCLUSION CRITERIA***

As for WP7

**Deliverables**

- Definition of "core" set of SNPs determining haplotypes for ultra-high throughput analysis
- Conduct of genome wide screen of SNP data

- Analysis of defined SNP for feasibility of typing

**Milestones and expected results**

- Completion of definition of core SNP set
- Completion of SNP testing for high-throughput screen

**WP3. GENE EXPRESSION: NOVEL CANDIDATE GENES**

<b>Work package number</b>	<b>3</b>	<b>Start date or starting event:</b>	<b>Month 4</b>			
<b>Participants involved</b>	9	10 2 3 4 7				

**Activity: Research, Innovation**

**Objectives**

- To identify differentially expressed genes in patients with SIRS vs. patients with sepsis, severe sepsis or septic shock (according the ACCP/SCCM consensus conference criteria) as well as survivors vs. non-survivors,
- To define potential targets for the future development of innovative sepsis therapies through the application of state-of-the-art methods in functional genomics (microarray technology).
- To provide superior defined clinical samples (according to ACCP/SCCM consensus criteria) collected in close collaboration with the University of Jena (Prof. K. Reinhart) and the University of Ulm (Prof. M. Schneider) from patients with SIRS, sepsis, severe sepsis and septic shock for the identification of differentially expressed genes in the abovementioned patients

**Description of work**

- Examination of the multitude of gene expression in patients with non-infectious (SIRS) vs. infectious causes of systemic inflammation (sepsis, severe sepsis or septic shock) as well as survivors vs. non-survivors using an unique, IP protected medium density polynucleotide microarray comprising about 5,000 inflammatory related genes.
- Delivery these data set to project partners in WP 2, 4, 8 to evaluate these gene loci for occurrence of SNPs with potentially high clinical value.
- Relevant samples from the abovementioned patients will be collected in close collaboration with the University of Jena (Prof. K. Reinhart) and the University of Ulm (Prof. M. Schneider) to build up a unique patient sample bank. The appropriate DNA from samples checked for differentially expressed genes will be provided to the project partners in WP 2,4,8 to identify so far unknown SNPs.
- Complete clinical description as well will be registered in a database with extensive data abstraction tools. Overall, patients' confidentiality is maintained by a stringent data coding system and the appointment of a trustee for patients' data.

**Deliverables**

- List of human genes differentially expressed in patients with non-infectious (SIRS) vs. infectious causes of systemic inflammation (sepsis, severe sepsis or septic shock) as well as survivors vs. non-survivors as base for the identification of SNPs with high clinical value.
- DNA samples of the above mentioned patient groups including full documentation (database) to the project partners 2,4 and 8.

**Milestones and expected result**

- month 3: first 100 complete documented DNA samples will be provided to the project partners
- month 6: next 100 DNA samples will be provided to the project partners
- month 12: delivery of gene profiling data (non-infectious vs. infectious) to the project partner
- month 24: delivery of gene profiling data (survivors vs. non survivors) to the project partner

**WP4. EUROPEAN RECRUITMENT PLATFORM AND CASE REPORT FORM**

<b>Workpackage number</b>	<b>4</b>			<b>Start date or starting event:</b>							<b>Month 3</b>			
<b>Participant id</b>	1	2	3	5	6	8	10	11	12	13	14	4		

*Activity: Innovation, Research*

**Objectives**

- To establish a European recruitment platform to enlist intensive care units
- To develop an electronic case report form
- To establish data handling process

**Description of work**

A genetic epidemiologic study of genetic predisposition for mortality in patients with sepsis will be performed throughout Europe. All intensive care patients over 18 years of age with 4 main complex diseases as basis for sepsis, severe sepsis and septic shock (nosocomial pneumonia, peritonitis, pancreatitis and meningococcal disease) meeting the inclusion and without exclusion criteria will be studied. Our hypothesis is that genetic predisposition contributes to adverse outcomes like sepsis, organ dysfunction and death in patients admitted to European intensive care units and has diagnostic value. This part of the study will take place from months 1-9.

**Deliverables**

- Recruitment structure for European intensive care units based on the Registry (database) of Intensive care units of the European Society of Intensive Care Medicine (ESICM), and via its platform for research and communication on research, ECCRN (European Critical Care Research Network)
- Electronic CRF (internet based)
- Monitoring structure for epidemiologic data input/sampling
- Implementation of data safety structure (hardware/software)
- Implementation of data trustee for anonymized data processing

**Milestones and expected results**

- ESICM recruitment centre
- Functioning electronic CRF
- Implemented data trustee

**WP5. GENETIC EPIDEMIOLOGIC STUDY DESIGN**

<b>Work package number</b>	<b>5</b>			<b>Start date or starting event:</b>							<b>3</b>			
<b>Participants involved</b>	2	3	4	5	6	8	7	9	10	11	12	13	14	

**Activity: Research, Innovation**

**Objectives**

To design an adequately powered genetic epidemiologic study: GenOSept

**Description of work**

An association study design based on the candidate gene approach will be developed. Genetic testing is to be associated with the severities of sepsis and septic shock, organ system dysfunction and mortality.

Development of the study design including genetic markers to be analysed will take place from months 1-9.

**Priority for genotyping:** We propose to examine multiple SNPs in a large number of genes. Criteria for selecting SNPs within a gene are possible relevance for gene function, previous positive association reported, and contribution to empirical haplotyping. We will assign priority for the candidate gene analysis based on the following. 1) We will give priority to genes that we expect to be significantly associated with sepsis phenotypes. We have preliminary data suggesting that variation in specific genes is significantly associated with outcome from sepsis in other samples. We will give priority to these genes (e.g. TNF, PAI-1, etc.) because we believe that this will offer the greatest opportunity to contribute to understanding the determinants sepsis mortality. This group has extensive and diverse expertise in the biology of inflammation, immunological processes and mediator systems pathophysiologically involved in sepsis. As new data are published suggesting a particularly crucial role for a particular gene in sepsis, inclusion in the list of candidate genes will be considered.

Special attention will be given to the prevention of false positive associations in genetic testing in complex diseases. The concept of genomic controls will be embedded in the study as well as other strategies to improve power of study analyses as, amongst others, discussed in a recent publication (The Lancet March 8th 2003 Vol 361 pp 865-72, Helen Colhoun et al. Problems of reporting genetic associations with complex outcomes).

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Table 1. Proposed candidate genes to be analyzed for sepsis phenotypes in patients with Nosocomial pneumonia, peritonitis, pancreatitis and meningococcal disease

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**1. Endocrine related**

\*Estrogen receptor  $\alpha$  (ERA)

\*Estrogen receptor  $\beta$  (ERB)

\*Androgen receptor (AR)

Glucocorticoid receptor (GR)

Catechol-O-methyl transferase (COMT)

Insulin-like growth factor-1 (IGF1)

IGF2

IGF receptor 1 (IGFR1)

IGFR2

IGF binding proteins 1-5 (IGFBP1-5)

Cytochrome P4502A (CYP2A)

Peripheral benzodiazepine receptor

17-beta-hydroxysteroid dehydrogenase 1 (HSD17B1)

3-beta-hydroxysteroid dehydrogenase 2 (HSD03B2)

Growth hormone (GH)

Growth hormone receptor (GHR)

Growth hormone releasing hormone (GHRH)

GHRH receptor (GHRHR)

Adrenergic receptors ( $\square$ ,  $\square$ )

**2. Apoptosis related**

BCL2

BAX

Fas

FasLigand

p53

Caspase 9

**3. Coagulation related**

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Plasminogen-activator-inhibitor-1(PAI1)

Factor V

Factor VII

#### **4. Signal transduction related**

Toll like receptors (TLR 2,4,5,6,9)

#### **5. Cytokine related**

\* Tumor necrosis factor

Nuclear factor-kappa B (NF $\kappa$ B)

Calcineurin B

\* Interleukin 6 (IL6)

IL6 receptor (IL6R) gp80 and gp130

IL1A

IL1B

IL1 receptor (IL1R)

IL1 receptor agonist (IL1RA)

Transforming growth factor- $\beta$  (TGFB)

TGF-receptors (TGFBR1 and TGFBR2)

Latent TGF binding protein

TNFSF11 receptor (TNFRSF11A)

IL-8

CRP

MBL

CD16

CD32

Mannose receptor

Il4

CD40 ligand

INF-gamma

INF-gamma receptor

Lymphotoxin alpha

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### **Sample size estimates**

The incidence of organ system dysfunction in patients with severe sepsis is 8% for adult respiratory distress syndrome (ARDS), 18% for disseminated intravascular coagulation (DIC) and 23% for acute renal failure (ARF); it is 18% for ARDS, 38% for DIC and 51% for ARF in patients with septic shock (41). There are few studies using ACCP/SCCM consensus criteria for sepsis and septic shock and evaluating organ system dysfunction in addition to mortality. Based on all of the above data, 1500 patients for each of the four diseases underlying sepsis should allow the correlation of the different severities of sepsis and septic shock and prognosis with genetic testing, according to current statistical power estimates.

### **Deliverables**

- Specific list of Candidate genes to be genotyped in a first round of genotyping
- Specific list of markers/candidate gene to be genotyped, new markers/genes to be included as they are published in the literature and discussed by the consortium
- Specification of haplotype contribution of individual markers
- Specific list of non-candidate markers suitable for genomic controls
- Design and implementation of quality control in genotyping/phenotyping

### **Milestones and expected results**

- Protocol of study design
- Transfer of gene and marker lists to genotyping centres
- Dissemination of study design protocol

**WP6. PROMOTION OF GENOMIC RESEARCH IN EASTERN EUROPEAN COUNTRIES**

Workpackage number	6			Start date or starting event:						Month 1	
Participants involved	1	2	4	9	10	12	13	14			
Person-months per participant	6	3	3	3	12	24	24	24			

**Activity: Innovation****Objectives**

- To establish a platform for genomic research in Sepsis and Intensive Care Medicine in general in Eastern European countries
- Inclusion of partners from Estonia, Czech Republic, Hungary
- To establish a formal Genomic Research Programme related to Intensive Care Medicine in this region

**Description of work**

Estonia, Czech Republic as well as Hungary show dedicated genomic projects initiated in single institutes which are about to open up to national research projects and efforts. Genomic Research in acute diseases like sepsis or other Intensive Care Medicine related diseases has not been conducted yet. With the help of ESICM (Partner 1) and the other well established partners in the field of genomic research within this consortium (Partners 2, 3, 4, 7, 9 and 10) the partners from Eastern European countries (12, 13, 14) will establish their national connection linking Intensive Care Medicine and fundamental Genomic Research. In addition to the status of patient recruitment centres for GenOSept, these partners will develop their collaboration with national genomic research centres of excellence in Estonia ([www.geneforum.ee](http://www.geneforum.ee), EUROCAT Programme), Czech Republic (Institute of Molecular Genetics, Academy of Sciences of the Czech Republic) and Hungary (Department of Genetics, Eötvös University, Múzeum krt 4/a, H-1088 Budapest, [www.caesar.elte.hu/ICSEB5/program.htm](http://www.caesar.elte.hu/ICSEB5/program.htm))

**Deliverables**

- Recruitment structure for European intensive care units based on the Registry (database) of Intensive care units of the European Society of Intensive Care Medicine (ESICM), and via its platform for research and communication on research, ECCRN (European Critical Care Research Network)
- Establishment of a formal collaboration with national Genomic Centres in Estonia, Czech Republic and Hungary
- Formal Genomic Research Programme in Intensive Care Medicine in Estonia, Czech Republic and Hungary
- Implementation of steady contact and cooperation with ESICM Genomic Research activities data safety structure (hardware/software)
- Implementation of national/European Ethics procedures in Intensive Care Medicine related genomic research

**Milestones and expected results**

- Collaboration with national Genomic Research Centres in Estonia, Czech Republic and Hungary
- Genomic Intensive Care Research Programme in Estonia, Czech Republic and Hungary

**WP7. GENETIC PREDISPOSITION FOR MORTALITY IN SEPSIS: GENOSEPT**

<b>Work package number</b>	7			<b>Start date or starting event:</b>				<b>Month 10</b>			
<b>Participants involved</b>	1	2	3	5	6	8	11	12	13	14	

*Activity: Research*

**Objective**

To enrol European intensive care patients for the genetic epidemiologic study GenOSept.

**Description of work**

An epidemiologic study of genetic predisposition for mortality in patients with 4 major diseases leading to sepsis, severe sepsis and/or septic shock will be performed throughout Europe. Patients meeting the inclusion and without exclusion criteria developed in the first part of the project (WP1-3) will be studied in the second part of the project. Our hypothesis is that the individual genetic background contributes significantly to the patients' outcome from sepsis related to the diseases pneumonia, pancreatitis, peritonitis and meningococemia. This part of the study will take place from months 10-27.

**4.1. Proposed inclusion criteria for septic shock – 1, 2, 3 and 4 required.**

*These criteria are adapted from the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria for sepsis (40).*

1. CLINICAL EVIDENCE OF INFECTION WITHIN THE PREVIOUS 72 HOURS (a, b or c)
  - (a) Presence of polymorphonuclear cells in a normally sterile body fluid.
  - (b) Culture or Gram stain of blood, sputum, urine or normally sterile body fluid positive for a pathogenic micro-organism.
  - (c) Focus of infection identified by visual inspection (e.g. ruptured bowel with the presence of free air or bowel contents in the abdomen found at the time of surgery, wound with purulent drainage)
  
2. EVIDENCE OF A SYSTEMIC RESPONSE TO INFECTION AS DEFINED BY THE PRESENCE OF THREE OR MORE OF THE FOLLOWING SIGNS WITHIN THE PREVIOUS 24 HOURS.
  - (a) Fever (rectal temperature  $>38.0^{\circ}\text{C}$  [ $>100.4^{\circ}\text{F}$ ]) or hypothermia (rectal temperature  $<36.0^{\circ}\text{C}$  [ $96.8^{\circ}\text{F}$ ])
  - (b) Tachycardia (heart rate of  $>90$  beat/min)
  - (c) Tachypnea (respiratory rate  $> 20$  breaths/min,  $\text{PaO}_2 < 32$  mmHg) or patient requires mechanical ventilation
  - (d) Leukocytosis ( $>12,000 \text{ mm}^3$ ) or leukopenia ( $<4,000 \text{ mm}^3$ ) or  $>10\%$  immature (band) forms.

3. EVIDENCE OF SEVERE SEPSIS WITH HYPOTENSION, HYPOPERFUSION OR ORGAN DYSFUNCTION WHICH ARE NOT THE RESULTS OF UNDERLYING DISEASES BUT ARE ATTRIBUTABLE TO SEPSIS WITHIN THE PREVIOUS 24 HOURS, INCLUDING ONE OF THE FOLLOWING:

- (a) Arterial hypoxemia ( $\text{PaO}_2 < 75$  mmHg or  $\text{PaO}_2/\text{FIO}_2 < 250$  in the absence of pneumonia)
- (b) Hypotension (systolic blood pressure of  $< 90$  mmHg or reduction of  $> 40$  mmHg from the baseline)
- (c) Metabolic acidosis (pH of  $\leq 7.3$ , or a base deficit of  $\geq 5.0$  mmol/L, or an increased lactic acid concentration)
- (d) Sustained oliguria (urine output  $\leq 0.5$  ml/kg/hr for a minimum of 1 hour in the presence of adequate fluid resuscitation)
- (e) Platelet count  $\leq 100,000/\text{mm}^3$
- (f) f. Coagulation abnormality -  $\geq 20\%$  increase in prothrombin time or a  $> 20\%$  increase in partial thromboplastin time
- (g) g. Altered mental status (Glasgow Coma Scale  $< 14$ ) in the absence of sedation or acute change from baseline in behaviour or cognitive function.

4. DIAGNOSIS OF ONE OF THE FOLLOWING DISEASES:

- (a) Nosocomial Pneumonia
- (b) Peritonitis
- (c) Pancreatitis
- (d) Meningococcal disease

**4.2. Proposed exclusion criteria**

- Pregnancy
- Age less than 18
- Underlying disease with a prognosis for survival of less than 2 months.
- Cardiopulmonary resuscitation within 72 hours before study.
- Immunosuppression, chemotherapy or radiation therapy within 4 weeks before the study.
- Presence of an advanced directive to withhold or withdraw life sustaining treatment (i.e. DNR).
- Lack of consent for GenOSept.

**4.3. Proposed measurements**

All patients enrolled will have demographic information including age, sex, diagnoses and operative status; clinical data including temperature, pulse, systolic and diastolic blood pressure, respirations, vasopressor and amount, mechanical ventilation, severity score, organ system dysfunction, performed at baseline before study entry and 24 hours later, 28 day mortality and a 90 day outcome follow up, in hospital mortality.

**4.5. Measurements**

To be discussed and determined by the Consortium (WP 1).

**4.6. Quality control**

Validity checks for entry variables will be incorporated within the study. Interrater reliability will be evaluated on the variables from a random sample of 10% of the patients.

**Deliverables**

- Inclusion of patient sample
- Generation of a phenotypic data set of patients
- Sampling of patients' blood sample
- Establishment of blood sample bank
- Transfer of blood samples to Genotyping Centres

- Transfer of phenotypic data to Biostatistics Centre

**Milestones and expected results**

- Enrolment of adequate numbers of patients
- Closing of database
- Closing of DNA sample bank

**WP8. GENETIC TESTING AND HARMONISATION OF GENOTYPING**

<b>Workpackage number</b>	<b>8</b>	<b>Start date or starting event:</b>					<b>Month</b>
<b>Participants involved</b>	3	4	7	2			<b>19</b>

*Activity: Research, Innovation*

**Objectives**

- To perform high throughput genotyping in DNA samples from patients enrolled in GenOSept.
- To validate the quality genotyping by comparing the performance to the gold standard of high throughput genotyping performed by three genotyping centres
- To harmonize genotyping protocols throughout the European genotyping centres
- To implement quality control of genotyping for GenOSept

**Description of work**

Genetic testing will be performed by six European partners of the consortium and their collaborators in high throughput genotyping:

1. Prof. Chiche (Paris, Cochin Institute, France)
2. Prof. Hill (Wellcome Trust Centre, Oxford, UK)
3. Prof. Meitinger (GSF, Munich)

Each centre will perform genotyping of approximately 300 genotypes in some 2000 patients (600.000 genotypes per centre). Some centres may need a complete set of samples in case of availability of a unique marker set for genotyping in a given institution. All centres take part in a quality control programme for genotyping embedded in GenOSept and developed in WP5. At least each of three single milestones needs to be achieved by genotyping centres in a timely fashion (quality control testing, testing of batch one and batch two of patients' samples) in order to proceed with funding and testing. Centres which can not deliver and pass those internal milestones may not complete the project.

- The three European genotyping centres develop a concept of quality control in cooperation with the biostatistics partners (Partner 2: Bonn, Partner 4: Wellcome Institute)
- The concept is to be discussed with national experts in good laboratory practice including national societies
- The consensus concept is to be implemented as a quality control genotyping procedure within GenOSept

**Deliverables**

- Standardized concept and protocol for quality control in high throughput genotyping
- Analysis of data quality (genotyping)
- Analysis of genomic control SNP
- List of genotypes for each patient enrolled in the study
- Technical evaluation and quality control of two genomic microarrays

**Milestones and expected results**

- Data set on genotypes of patients finalized by genotyping centres
- Validation and means of quality control using samples and data from high throughput genotyping centres as standard
- Protocol of a consensus on quality control of the three genotyping centres
- Consensus with national societies on good laboratory praxis

- Standard set of DNA samples to be genotyped for quality control

**Endpoints**

- Transfer of genotyping results to Biostatistics Centre

**WP9. STATISTICAL ANALYSIS**

<b>Workpackage number</b>	<b>9</b>	<b>Start date or starting event:</b>				<b>Month 28</b>		
<b>Participants involved</b>	2	3	4	7	10	12	13	14

*Activity: Research, Innovation*

**Objectives**

To analyse the dataset generated by GenOSept (WP4, WP7): Association of Phenotype and Genotype data

**Description of work**

- Phenotype and Genotype data under the authority of a data trustee will be transferred to Biostatistic partners (Bonn, representatives from the Wellcome Institute and the genotyping centres with embedded biostatisticians)
- An analysis plan according to study design (WP3) will be established
- Computational analyses will be performed
- Analyses and results will be discussed on a joint meeting of Biostatisticians involved in GenOSept
- A report to the study coordinator will be finalized

**Deliverables**

- Evaluation of sample size
- Association between phenotypes and genotypes
- Analysis of data quality (phenotyping)
- Analysis of data quality (genotyping)
- Analysis of genomic controls
- Estimation of patient stratification

**Milestones and expected results**

- Meeting of biostatisticians
- Consensus on analyses protocol
- Detailed data analysis

**Endpoints**

Report of Statistical Analysis to Study Coordinator

**WP10. DEFINITION OF A DIAGNOSTIC SET OF GENETIC MARKERS IN SEPSIS**

<b>Work package number</b>	<b>10</b>			<b>Start date or starting event:</b>								<b>Month 30</b>		
<b>Participant id</b>	2	1	3	4	5	6	8	7	9	10	11	12	13	14

*Activity: Innovation, Research*

**Objectives**

To define a relevant set of genetic markers to be used as a diagnostic tool in European intensive care patients

**Description of work**

- A consensus conference of the consortium will present the results of the analysis of GenOSept database to all partners
- A definition of a relevant genetic marker set will be established
- The results will be discussed
- Publication of results will be planned and organized
- A report of the consensus conference will be given to the study co-ordinator

**Deliverables**

- Evaluation of study results
- List of genomic markers to be used in future diagnostics and testing

**Milestones and expected results**

- Consensus on results
- Definition of a diagnostic genomic marker set

**WP11. FINAL REPORT**

<b>Workpackage number</b>	<b>11</b>	<b>Start date or starting event:</b>				<b>Month 33</b>	
<b>Participants involved</b>	1	2					

*Activity: Management*

**Objective**

To deliver the GenOSept study report to the European Commission, FP6

**Description of work**

During months 33-36, a final report will be developed and submitted.. Besides administrative and financial data, the final report will include the results of all the components of the study, the usefulness of GENETIC TESTING in categorising and prognosticating in sepsis, the diagnostic use of genomic microarrays in patients with sepsis, policy recommendations, and the dissemination of the results of this project. The final report will have approval of all partners (steering committee members).

**Deliverables**

- Summary of the project's results
- Distribution of results to partners and investigators
- List of tentative publications derived from the project

**Milestones and expected results**

- Approval of the report by consortium partners
- Delivery of the report to the European Commission, Brussels

## 6. Ethical issues

GenOSept is a genetic epidemiological study using anonymised data from patients. Therefore, no data can be used to track personal identification data of single patients by researchers. Nevertheless, recruitment of patients will require written informed consent of patients or their legal guardian in case a patient is unable to consent.

### ETHICAL ASPECTS OF THE PROJECT

The practice of genetic analysis for clinical research has been evolving for several decades, predominantly in the field of monogenic disorders, and there is well defined legislation in most countries, and particularly within the European Union, that determines that this process is not only ethically acceptable but documented and accountable. Studies of genetic influence in complex (polygenic) disease have been carried out with increasing prominence over the past decade and it is the promising evidence that has emerged from these that has stimulated this project in the most vulnerable and needy group of patients.

The clinical investigators in this project have all been involved in such studies in intensive care patients and all anticipated and sought careful local and/or national guidance and authority for the ethical implications. The overwhelming security that is enshrined within this project is the fact that there is complete anonymisation of the patient data and material under the control of a data trustee who is directly responsible to national legislation. In other words the protocol defines without any ambiguity that there can never be a link between the patient's identity with the results obtained from the genomics analysis. There are safeguards that have been well tested that prevent any breakdown in this secure process, notably at least three coded numbering systems for clinical data, blood samples and DNA samples prior to the genomic analysis and physical and electronic separation of clinical datasets from genomic results. Genetic studies in intensive care patients to date, approved by local and national ethical committees, have followed these principles of anonymity and have been accepted by patients and their relatives and/or legal representatives. Thus this study, which has no direct impact on the recruited patients' care and certainly negligible potential for deleterious influence on these patients' outcome, is considered safe and ethical by clinical research ethical committees. Legal rights of the patient are strictly adhered to as directed by the instructions from the relevant ethical committees.

#### Research design

Genetic epidemiologic study design employing data anonymisation will be used. Patient's identity and rights will be secured. A legal data trustee will control the data master file containing the list of primary and secondary identifier codes.

#### Use of human biological samples

Ethical issues raised by this project relate predominantly to the collection of material for genetic analysis (one single sample of 10 ml blood), and in particular those that emerge for the collection from patients who are unable at the time to provide informed consent.

A single sample of 10 ml of peripheral blood will be obtained from patients (venous or arterial blood, no extra puncture of a vessel is required as existing lines will be used for blood drawing). This sample is needed for genomic analysis as planned within GenOSept.

### RESEARCH INVOLVING PERSONS UNABLE TO GIVE CONSENT

#### *Justification for such research in terms of the potential benefits of the research in relation to the possible risks to persons*

In general, the patients targeted in this study fulfilling the entry criteria will be unable to consent as they are treated on intensive care units. The condition of these patients is critical. Therefore, consent needs to be obtained by a legal guardian of the patient.

Risk for participants are minimal to none, while the benefit would be for future patient cohorts receiving better focussed care because of better risk stratification.

A single blood drawing of 10 ml whole blood which will be performed in the context of routine blood drawing does not impose a specific risk for the patient.

The patients will not benefit directly from this study. Comparable cohorts of patients will possibly benefit from resulting risk stratification procedures. Thus, the study complies with the UNESCO guidelines.

#### ***Number of persons involved and selection criteria***

According to power calculation by genetic epidemiologists involved in GenOSept some 5.000 patients will be recruited for the study. The selection criteria comprise criteria of sepsis classification combined with specific underlying diseases (peritonitis, pancreatitis, pneumonia, meningococemia).

#### ***Details of the arrangements made for providing information to persons and for obtaining informed consent***

Genotyping of patients (peripheral blood will be used) will require consent of patients or their legal guardians in case patients are unable to consent. Information forms and consent forms accompany this document (: GenOSept Patient's information sheet, GenOSept patient's legal representative information sheet, GenOSept patient's consent form, GenOSept patient's legal representative consent's form).

Participants will receive study information and consent forms. In case a patient who fulfils entry criteria is unable to consent, a legal guardian of this patient will be involved and asked for consent.

Consent will only be obtained from a legal guardian in case the patient is unable to consent. This complies with national legislations in European countries as well as with international regulations. It is the task of local investigators to obtain consent from a legal guardian.

#### ***Data storage and handling***

Genotyping data as well as any data used for analysis and association studies obtained within GenOSept will undergo anonymisation. Data of locally recruited patients will receive an identifier code which will be transferred to a data trustee. The data trustee will transform the primary identifier code to a second identifier on a master list. The second identifier code will be used for further data evaluation. Researchers will be unable to track back the patient's identification as they use the second identifier code. All data will be evaluated in an anonymised fashion. Personal anonymized data analysed will be gender and age of an individual.

There are safeguards that have been well tested that prevent any breakdown in this secure process, notably at least three coded numbering systems for clinical data, blood samples and DNA samples prior to the genomic analysis and physical and electronic separation of clinical datasets from genomic results. Genetic studies in intensive care patients to date, approved by local and national ethical committees, have followed these principles of anonymity and have been accepted by patients and their relatives and legal representatives. Thus this study, which has no direct impact on patient care and certainly negligible potential for deleterious influence on its patients' outcome, is considered safe and ethical by clinical research ethical committees. Legal rights of the patient are strictly adhered to as directed by the instructions from the relevant ethical committees.

Anonymisation will take place guarded and controlled by a data trustee who also controls and stores the masterfile of codes for participants and data. 2 different steps of coding will take place as outlined above so that researchers working with the second identifier cannot track any participant / patient enrolled in the study. Patients' request for their personal data needs to be directed to the data trustee. Collected data will not be used for commercial purposes.

#### ***National and International regulations on ethics***

Participating investigators in this study will submit the project protocol for approval by local, regional and national research ethics committees according to the national regulations. In most cases it is anticipated that these will be accepted by multi-centre research ethics committees, themselves influenced by national and EC directives appropriate to this type of genetics research. The GenOSept organising committee, with support

from ESICM, will scrutinise each participating centre to ensure that the Helsinki Declaration and the Universal Declaration on the human genome and human rights adopted by UNESCO will be respected.

This project fits well in the ethos of the European Commission Life Sciences and Biotechnology Strategy for Europe (COM(2003) 96 final published 5.3.2003, enabling close interaction of European researchers in this field to encourage novel developments in the field of biotechnology and its clinical applications. Published disclosure of the results from this project will enhance public understanding of the science of functional genomics and the potential of this approach to improving clinical care off the critically ill.