

**FINAL EVALUATION REPORT OF COST ACTION B16**  
**REVERSAL OF ANTIBIOTIC RESISTANCE**  
**(BY INHIBITION OF TRANS-MEMBRANE TRANSPORT).**

**1. ACTION IDENTIFICATION DATA :**

Cost Action (*number*) B16

*Title "REVERSAL OF ANTIBIOTIC RESISTANCE"*

**(BY INHIBITION OF TRANS-MEMBRANE TRANSPORT):**

**TC Recommendation:** 10/05/2000

**CSO Approval:** 9/12/1999

**Start date:** (*day/month/year*) <sup>(1)</sup> 24/05/2000

**Duration:** *months* 5 years

**Extension:** *months*

**End Date:** (*day/month/year*) 9/05/2005

**First MC Meeting:** 24/05/2000

**Last MC Meeting:** 29/12/2003

**Final Report:** (*day/month/year*) <sup>(2)</sup>

**Evaluation Report:** (*day/month/year*) <sup>(2)</sup>

**Tc Evaluation:** (*day/month/year*)

**Number of signatories:** (*number*) 24

**Signatories and date of signature:** (*day/month/year*)

Austria 17.May.2000

Belgium 13.Sept.2000

Romania 28 June, 2002

Italy 14 June, 2001

Denmark 10 May 2000

Switzerland 16 May 2000

France 19 July 2000

Germany 5 July 2000

Greece 18 October, 2003

Hungary 23.May 2000

Ireland 2005

Czech Rep. 25. Nov 2000

Lithuania

Finland 12 July 2000

Netherlands 29 Nov 2003

Norway 15 Sept 2000

Poland 10 May 2000

Portugal 10 May 2000

Slovakia 12 May 2000

Spain 10 May 2000

Sweden 11 Oct 2000

Turkey 28 June 2002

United

Kingdom 10 May 2000

Israel 19 Dec 2000

**Institutes of non-COST countries:** (*list*)

**Area:** Medicine and Health

**Action Web site:** <http://www.ihmt.unl.pt/costb16>

**Chairperson:** Joseph Molnar MD, DSc

*Title name: Professor, MD, DSc*

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**PERIOD: FROM 24 MAY 2000 – 10 MARCH 2006**

CHAIRMAN OF THE MANAGENET COMMITTTE: PROF JOSEPH MOLNÁR

SCIENTIFIC SECRETARIATE:

AT COST-ESF DIRECTORATE: PROF. MIHAIL PASCU.

RAPPORTEUR:

PROF JANOS FEHER 2<sup>ND</sup> DEPT. OF MEDICINE, SEMMELWEIS UNIVERSITY, BUDAPEST, HUNGARY

## **2. OBJECTIVES**

The main objective of the Action is to investigate mechanisms of multidrug resistance in bacteria, fungi, cancer cells, viruses and parasites with a view toward developing new drugs capable of reversing this drug resistance.

The main reason for the concerted action is that many chemotherapeutic medicines and antibiotics have lost their effectiveness in the majority of European Countries during the last two decades due to the emerging multidrug resistance of micro-organisms, parasites, and cancer cells. Multi-drug resistant disease means that afflicted patients are ill for a longer period of time and are at greater risk of dying. Moreover, because therapy of these mdr diseases is problematic and often causes a reduced quality of life as a consequence of severe morbidity, much suffering is experienced by both the patient and his/her family.

The Action will be carried out in accordance with the provisions of the document COST 400/94 “Rules and Procedures for Implementing COST Actions”, the content of which are fully known to the signatories.

This comprises:

- The synthesis of novel derivatives capable to reverse resistance in vitro and in vivo.
- The investigation of molecular mechanism of resistance in cancer cells, parasites, bacteria, fungi and viruses.
- The evaluation of compounds prepared

## **3. TECHNICAL DESCRIPTION AND IMPLEMENTATION**

Four Working Groups were established by the MC as follows with the aim to achieve the technical scientific programme in the following areas:

- **WG1** on the field of Chemistry of the resistance reversal compounds, chaired by Pr. J. Barbe (Fr) untill 2004 and Pr. G. Hajos (Hun) from that time.

Compounds previously tested as chemosensitizers and listed in the literature belong to various chemical series. However, none of them can be used as a drug because of toxicity and/or a wide range of biological activities. Thus they have only to be considered as references in the field.

With the aim to obtain novel derivatives to be marketed and developed as drugs, attention was chiefly focused on synthesizing heterocyclic compounds.

This implied adjustment and perfecting of ways of synthesis, structural determination including X-ray crystallography, molecular physico-chemical parameter measurements (acid-base properties, logD, solubility, DNA/RNA interactions, etc.), and SAR description before selected compounds be investigated by other WGs.

WG1 is dealing with the chemical research chaired by Pr. Barbe and Pr. Hajós. This WG had as its main goal to elaborate new organic transformations, to prepare new, mostly heterocyclic (acridin, phenothiazine, pyridine, pyridazine, triazine, or benzthiazole) derivatives, and to provide samples for biological tests. The investigation – besides the synthesis – also implied research on physico-chemical properties of the new compounds, structure elucidation of the synthesized derivatives by spectroscopical methods and X-ray diffraction, theoretical calculation (quantum chemistry, quantitative structure-activity-relationship, etc.). The gradually growing number of participants in B16 had a significant impact to WG1: an extended network has been established with numerous research groups of the other three WGs.

- **WG2** consists of bacteriologists and mycologists whose collective aim is to elucidate the mechanisms by which mdr of bacteria and fungi develops, assess possibilities for the control and modulation of said mechanisms and evaluate compounds which can interfere or inhibit their activity.

WG2 deals with the reversal of multidrug resistance of bacteria and fungi, chaired by Pr. P. Henderson and Pr. K. Kuchler. The research activities of the **WG2** group were quite extensive and consisted of: purification and imaging of transporter proteins of bacteria; the characterisation of efflux pumps of bacteria and fungi at the level of specific genes; the physiological regulation of these pumps; evaluation of agents provided by WG1 members to members of WG2 for activity against well characterised efflux pumps of bacteria and fungi and for the ability of many of these agents to reverse multi-drug resistance; development of new methods for the assessment of efflux pump activity; and the formation of **WG2** collaborative associations for the presentation of symposia at international meetings; the editing of special issues of international scientific Journals; and the joint publications of many scientific studies.

- **WG3** consists of virologists and cancer cell biologists whose collective aim is to elucidate mechanisms that control the activity of transporter pumps which render parasites and cancer cells multi-drug-resistant as well as to evaluate a variety of new compounds provided by **WG1** for potential inhibitory activity against these transporter pumps.

**WG3** chaired by Prof Dr. Derek Sharples and co- chaired by Prof Dr Joseph Molnar deals with a variety of models that elucidate the mechanism by which cancer cells achieve mdr status and evaluates compounds that have the potential to render these mdr cells sensitive to antibiotics to which they were initially resistant. The sources from which agents for evaluation could be obtained was not to be limited those provided by WG1 alone but extended to plants, vegetables and existing medicinal compounds that had been employed for diseases other than cancer. The rationale for this extension was based on the knowledge that many plants and vegetables had reputations for reducing the cancer load and therefore, the responsible compounds would need to be isolated and evaluated for possible activity against mechanisms that render cancer cells immune to antibiotics.

- **WG4** consists of parasitologists whose main interest is to reduce the frequency of mdr parasitic infections in man and animals by first determining the mechanism by which parasites become mdr and secondly, evaluate compounds provided by **WG1** and Pharmaceutical companies allied with the group for abilities to reverse mdr of important clinically and veterinary important parasites.

**WG4** was formed in 2003 and has been chaired by Georg von Samson Himmlstjerna. The main activity to be conducted by this group was to standardise assays that evaluated eggs, parasites and adults of important parasites in order that studies which were to evaluate compounds for activity against mechanisms that rendered the organisms mdr could be conducted on a European wide basis. The **WG4** established close cooperation with a number of pharmaceutical companies that provided access to specialised expertise in the fields of molecular biology and molecular genetics.

#### **4. PARTICIPATION AND COORDINATION**

##### **4.1 Management Committee**

**Chairperson:** Prof. Joseph Molnar  
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**Vice Chairperson:** Prof. Jacques Barbe  
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**Secretary:** Anne Mandenoff till September 2001 then Prof. Mihail Pascu from that time

**Secretariat** (May-2000-Sept 2001): Dr. Anne Mandenoff  
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**Secretariat** (Sept 2001-present): Professor Mihail Pascu  
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### **Dr. Esin SENER**

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## **EUROPEAN COMMISSION**

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## **4.2 Participating Institutions**

### **AUSTRIA**

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Bio Center – Inst. Medical Biochemistry - Dpt Molecular Genetics, Vienna

### **BELGIUM**

Vet. & Agrochem. Res. Center - Dpt Bact. & Immunol., Brussels

## **CZECH REPUBLIC**

Dpt Anal. Chemistry, Charles University, Prague

## **DENMARK.**

Dpt Clinical Microbiology, Sønderborg,  
Dpt Clinical Microbiology, Hvidovre Hospital

## **FINLAND**

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Dpt Biology Abo Akademi University, Turku

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## **ISRAEL**

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## **NORWAY**

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Inst. Microbiology, National Hospital, Oslo

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## **TURKEY**

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Jozef Verduyck (B)

Christian Bauer (D)

Christian Epe (D)

Achim Harder (D)

Peter-Henning Clausen (D)

Nicole Wirtherle (D)

Bermejo Marival (E)

Dominique Kerboeuf (F)

Frank Jackson (F)

Elias Papadopoulos (G)

Fred H.M. Borgsteede (NL)

Grzegorz Bartosz (PL)

Janez Posedi (Slov)

Johan Höglund (S)

Marian Varady (SK)

Maeser Pascal (CH)

Hubertus Herzberg (CH)

Ronald Kaminsky (CH)

Veli Cirak (T)

Jon Cole (UK)

Gerald Coles (UK)

### **4.3 Meetings of the Management Committee**

1<sup>st</sup> MC, 24<sup>th</sup> May, 2000, Brussels

2<sup>nd</sup> MC, 30<sup>th</sup> November 2000, Lisbon

3<sup>rd</sup> MC, 30<sup>th</sup> June 2001, Prague

4<sup>th</sup> MC, 30<sup>th</sup> November 2001, Marseille

5<sup>th</sup> MC, 30<sup>th</sup> June 2002 Warsawa

6<sup>th</sup> MC, 29<sup>th</sup> November 2002, Berlin

7<sup>th</sup> MC, 29<sup>th</sup> October 2003, Rhodes

8<sup>th</sup> MC, 29<sup>th</sup> November 2003, Sonderborg

9<sup>th</sup> MC, 29<sup>th</sup> May 2004, Gent

10<sup>th</sup> MC, 29<sup>th</sup> October 2004, Corfu

11<sup>th</sup> MC, 12<sup>th</sup> May 2005, Antalya

12<sup>th</sup> MC, 12<sup>th</sup> October 2005, Novara

13<sup>th</sup> NC, 22<sup>nd</sup> April 2006 Closing Conference , Budapest

## **5. Results**

### **Preliminary comments from the Chairman**

The activities of Cost B16 during 2005 have realised the basic tenet of the Cost Action Programme of the European Commission and the European Science Foundation-namely, the establishing of cooperation and collaboration between European scientists for a common scientific goal. The plan and its goals defined at the inception of the Cost B16 in May 2000 were indeed ambitious, and consisted of a series of steps that would lead to the creation of compounds that would reverse the antibiotic resistance of bacteria, fungi, parasites and cancer cells. To a large extent, the defined goals have indeed been reached witness the listing of publications (138 and still counting) that present the compounds synthesised by the Chemical Partners of of Working Group 1, the distribution of compounds to Working Groups 2, 3 and 4, and the evaluation of these compounds by these Working Groups. Nevertheless, although the activity of many of the compounds at the level of the laboratory bench has been proven, it remains for animal studies and subsequent clinical trials for final demonstration of success.

The year 2005 was scheduled to be the last of the 5 year Cost B16 programme. However, due to the increase of membership from the new addition of 10 European countries to the European Union, the recent cooperation and collaboration between the latter and established members of Cost B16 required more time for these associations to reach full fruition. Hence, Cost B16 successfully applied for an extension of one year, and as is evident from the publication listing at the end o this Report, the extension did accomplish its intended mission. The individual reports from the Chairpersons of Cost B16 describe the many interactive cooperative and collaborative associations between members of the Working Group as well as those between members of different Working Groups.

The scientific activities of the members of Cost B16 during the first four years contributed to the visibility of Cost B16 within the scientific community. During 2005, the Working Groups of Cost B16 were invited to organise scientific programmes at International Scientific meetings and these are described by the various reports from the Working Groups contained in this Annual Report 2005.

The visibility of Cost B16 also includes the programmes that provide training at the level of the bench to many of the Graduate Students of the members of Cost B16. These students grouped into the programme “Short Term Missions” are identified by the STM report and their activities at the laboratories they had visited are described.

Although we can with confidence say that Cost B16 has been truly successful in its mission, there are still areas where certain activities could have made the programme even more successful. The area that is glaringly under-represented by the membership of Cost B16 was medicine. Unfortunately, Cost B16 consisted of only 5 medically trained members and although these interacted with each other prior to and during Cost B16, there was a real need for more medical representation. Consequently, aspects of medicine that are critical for the extension of basic science studies conducted by Cost B16 could not be exploited to a maximum. Nevertheless, the opportunities provided by medical organisations that recognised the contributions made by Cost B16 afforded Cost B16 members access to wide medically oriented audiences via the special issues of Medical Chemistry Journals that were devoted to reviews and original reports as well as the many programmes presented at medical meetings

## **6. DISSEMINATION of RESULTS**

The results of the activities of the COST Action B16 have been disseminated by:

- a) Publication in International Journals of high impact.
- b) Presentations at International and European scientific and medical meetings.
- c) Organisation of Symposia by MC members.
- d) Presentation of workshops devoted to technology resulting from the activities of members of the action.
- e) Extension of COST B16 website to other organs (cancer organisations).
- f) Involvement of Pharmaceutical companies in the activities of MC members.

### 6.1 Publications and Reports

**725** articles and reviews have been published or are in press (a detailed list is given in the Annex 2) .

The following Special Issues of International Journal were organised and edited by Cost B16:

International Journal of Antimicrobial Agents 2003;17:1-357

In Vivo 2005;19.

Current Drug Targets 2006;7:789-909.

Journal of Chemotherapy 2006 (in Press).

In Vivo 2006: Issue is being organised.

### 6.2 Conferences and Workshops

Non-Antibiotics and reversal of antibiotic resistance of Bacteria.

Danish Society of Chemotherapy 2001

South Danish University, School Med.

Odense, Denmark

Organiser: J. Kolmos

4th European Congress of Chemotherapy 2002

Paris, France

Inhibition of Efflux Pumps and Reversal of Resistance

Organiser: FESCI; Chairperson L. Amaral

"Efflux pumps and antibiotic resistance of micro-organisms"  
5th European Congress of Chemotherapy, 17-20 October 2003.  
Rhodes, Greece  
Organizer: Leonard Amaral and Nicos Legakis

"Non-Antibiotics and reversal of resistance of bacteria."  
Statens Serum Institute, 2 December 2003.  
Copenhagen, Denmark  
Organizer: Jette E Kristiansen

Cost B16 Symposia:  
International Conference of Cancer Research  
25-30 October 2004  
Corfu, Greece  
Organisers: Joseph Molnár, Mihail Pascu and John G. Delinassios

"Reversal of Antibiotic Resistance"  
6th European Congress Chemotherapy/RICAI  
1-3 December 2004  
Paris, France

Non-Antibiotics and efflux pumps of bacteria.  
7<sup>th</sup> European Congress of Chemotherapy  
20 October 2005  
Florence, Italy

International Seminar on Multidrug resistance in Cancer.  
5<sup>th</sup> December 2005,  
Szeged, Hungary  
Organizer: Joseph Molnár

**6.3 Website :** [www.ihmt.unl.pt/costb16](http://www.ihmt.unl.pt/costb16) (Enclosure, ref. Prof Leonard Amaral)

This website provides full description of the goals and objectives of COST B16, identifies each MC Member fully (CV, Address, phone, fax, e-mail) as well as interests. The website also provides full reports from each MC meeting, as well as a current listing of publications from the research activities of its MC members.

#### **6.4 SCIENTIFIC AND TECHNICAL COOPERATION**

Additional cooperations were initiated, based on the fruitful contacts established within the frame of the Action:  
- 2005/2006 French-Lusitanian joint research Programme „Efflux Pumps and MDR in Pathogen Bacteria (J.M. Pagès/ L. Amaral)  
- 2005/2006 Belgique- Hungarian joint research Programme „Reversal of multidrug resistance of cancer”  
- 2006/2007. Portuguese Hungarian Exchange Program (J. Molnar/L Amaral)

#### **6.5 TRANSFER OF RESULTS**

**European patent No: 1432717 „Substituted disiloxanes, methods for their manufacture and their use for reversing multidrug resistance (MDR)”.**

## 6.6 CONTACTS IN THE ERA

The following Programmes are in progress

- RTN Marie Curie MRTN-CT-2005-019335 „Translocation”(JM Pagès as a member)

## 7. ECONOMIC DIMENSION

<b>2000</b>	
Meetings	€ 38,400
STSM	€ 11,500
<b>TOTAL</b>	<b>€ 49,900</b>

<b>2001</b>	
Meetings	€ 35,088
<b>TOTAL</b>	<b>€ 35,088</b>

<b>2002</b>	
Meetings	€ 71,595
STSM	€ 11,500
<b>TOTAL</b>	<b>€ 83,095</b>

<b>2003</b>	
Meetings	€ 52,829
Publications	€ 7,080
<b>TOTAL</b>	<b>€ 59,909</b>

<b>2004</b>	
Meetings	€ 69,141
STSM	€ 5,106
<b>TOTAL</b>	<b>€ 74,247</b>

<b>2005</b>	
Meetings	€ 57,389
Workshop support	€ 2,570
<b>TOTAL</b>	<b>€ 59,959</b>

<b>2006</b>	
Meetings	€ 25,598
STSM	€ 1,711
Workshop support	€ 2,950
Publication	€ 2,048
Web site	€ 2,000
<b>TOTAL</b>	<b>€ 34,307</b>

**TOTAL ON  
ACTION'S LIFETIME € 386,507**



## 8. SELF EVALUATION

### 1. Results versus Objectives.

#### **European Interaction and Cooperation.**

This objective has been reached. Examples of this may be found in the listing of joint publications in the publication of the dedicated issue of the International Journal Antimicrobial Agents containing 24 articles from MC members; from the Special Issue of In Vivo 2005; 19 that contained 17 articles from members of Cost B16; from the and publication of a special issue of Current Drug Targets 2006;7: 789-910 containing 14 articles from Cost B16 members; from the special issue of Journal of Chemotherapy that will contain at least 11 articles from members of Cost B16 and which is to be published in 2006 and from publication record of Cost b16 Members that lists almost 400 publications during the period of Cost B16 as well as from the many symposia provided by Cost B16 at international meetings. All of this provides ample evidence that the goal of interaction and cooperation was successfully completed and involved almost all MC members.

#### **Outcome and achievements**

The noted close cooperation between MC members and expert associated groups has resulted in the synthesis of a large number of compounds have been and continue to be analysed for their properties with respect to efflux pumps of micro-organisms, parasites and cancer.

MC members in various countries have successfully obtained grants which supports the production of derivatives from biologically active drugs that promise to overcome antibiotic resistance in bacteria.

#### **Impact of COST Action B16.**

The activities of Action have been extended beyond the European continent for the purpose of evaluating results at the clinical level, a necessary requirement for the extension of these findings to the management of infectious diseases in Europe. Protocols involving the use of new and old compounds for the management of infectious disease are now in progress in Africa (malaria/thioridazine-Walter Reed Hospital), new variant CJD (Stanley Prussiner, San Francisco); Multi-drug resistant *Mycobacterium tuberculosis* (Buenos Aires, Argentina); patents have been obtained for new compounds designed by MC members. The derivatives of phenothiazines made by the Hungarian MC members are to be patented and assigned to Portugal as per grant agreement. Plans for Clinical Trials of phenothiazine compounds for the therapy of MDR tuberculosis have been drawn up by L Amaral and Martin Boeree of the Netherlands. The trials are to take place in Tanzania hopefully in 2007.

#### **European Added Value.**

In many European cities, the incidence of new infections of MDR, TB and nosocomial infections of methicillin resistant *Staphylococcus aureus* are costly in terms of human suffering and costly in terms of economics. The research conducted by the MC members has contributed to the identification of new compounds which are highly effective against intracellular infections by these pathogens. These compounds work where others fail and it is expected that when used at the needed level the incidence of these infections will be drastically reduced and the associated costs obviated. It should be stated that the early recognition by the MC members that there is a strong relationship in the biology of cancer and parasites with respect to the mechanisms that render resistance to antibiotics. Much of what is now being learned from the activities of COST Action B16 research can be applied to both areas.

#### **Coordination, Management Working Groups.**

The MCM and WG meetings have shown an active and effective cooperative work as illustrated by the listing of joint publications, joint organisation of international symposia and work-shops and joint organisation of special issues of international Journals. The interaction of the members of Cost B16 transcended from one Working Group into another (example-WG2 and WG4 (joint meetings in Paris 2003, Rhodes 2004 and Paris 2005) WG2 and WG3 (Corfu).

To sum up, strengths of the COST Action B16 are

- a) Strong Cooperation.
- b) No dominance by any member or country of the COST B16 action.
- c) High ethical and moral standards of MC of the COST B16 action.

while no outstanding weaknesses were noticed except for more representation from the medical community (only five physician trained are members of Cost B16).

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2000

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