1 Publishable final activity summary

1.1 Project execution

Project number: LSPH-CT-2004-503578
EC contribution: € 15,900,000
Duration: 66 months
Starting date: 1 April 2004
Website: www.biomalpar.org

Summary description of project objectives

Malaria is a mosquito-borne disease caused by a unicellular protozoan pathogen that is estimated to cause up to 500 million clinical cases worldwide and over 2 million deaths each year, most of them children under five in sub-Saharan Africa.
Due to the rapid spread of parasite strains resistant to common drugs, the emergence of insecticide-resistant mosquito vectors and the continued absence of affordable, long-lasting preventive measures, such as a safe and efficacious malaria vaccine and new drug treatment, the strategic objective of EU-funded research on Poverty Related Diseases is to contribute to confronting malaria at global level and with a multidisciplinary approach through the development of effective preventive and controlling strategies.

By funding the Network of Excellence BioMalPar, the European Commission aimed to **strengthen scientific and technological excellence** on malaria research by **integrating** at European level the **critical mass** of resources and expertise needed to provide European leadership and to be a world force in malaria research. This expertise was networked around a joint programme of activities aimed principally at creating a **progressive and durable integration** of the research capacities of the network partners while, of course, at the same time advancing knowledge on malaria.

BioMalPar (**Bio**logy and Pathology of the **Mal**aria **Par**asite) was the first large malaria research consortium on basic biology (European Network of Excellence (NoE)) funded by the European Commission under the sixth Framework Programme and coordinated by Institut Pasteur with Artur Scherf as Scientific Director. The NoE activity started out with forty-four partner laboratories in eighteen institutions from eight European countries and three malaria endemic countries. Created in April 2004, BioMalPar was established with the vision of forming a Europe-wide virtual institute, enhancing cohesion between its component research groups and building sustainable research capacity in Africa. A major goal of the BioMalPar consortium was to boost collaborative basic malaria research within the European Research Area (ERA).
The scientific aims of the network were to address, in an integrated, collaborative manner, fundamental questions of the biology of the malaria parasite, its vector, and the disease, allowing optimal exploitation of the resulting knowledge to impact on public health. The objectives have been accomplished through a joint program of activities organized around four major goals:

- a set of **integrating activities** aimed at creating an interwoven network that brought together leading malaria research teams across Europe and African countries;
- a programme of **jointly executed research** to support the network's goals;
- a set of activities designed to **spread excellence**, an essential element was the implementation of the BioMalPar PhD programme;
- All the network's activities were carried out within a coherent framework for the **management** of the consortium.

As part of the work, the consortium engaged with actors beyond the research community and with the public as a whole, to help spread awareness and knowledge and to explore the wider societal implications of the network activities.

Achievements made by the BioMalPar consortium since its implementation in 2004

Since its foundation, BioMalPar has jumped major hurdles:

1. It has created greater transparency in European malaria research activities
2. It has significantly increased the coordination of new collaborative projects between Institutional laboratories within Europe and with African partners. Many new grant applications have been catalyzed through BioMalPar meetings.
3. It has decreased redundancy in the research agenda and raised significantly complementary grants.
4. It has trained a new generation of students familiar with state-of-the-art malaria research.

o Network Management

Management of the Network included a strong intrinsic integration, since each management level involved multiple partners. Several structures with specific mandates were then involved in the management of the programme: Institutional Board, the Executive Committee, the BioMalPar Coordinator, the Cluster Coordinator, the Task Leaders, the Scientific Committee, the Liaison Managers, and the Advisory Committee.

Regarding the decision making process, it was distributed between two different bodies; the **Institutional Board** and the **Executive Committee** (see WP1.1 & 1.2).

Institutional Board: It was constituted of Top Level Executives mandated to speak on behalf of their participating institution. The IB was a body who had power on institutional decisions without interfering with science-related decisions. It just approved or rejected the decisions taken by the EC. Another task was to oversee, review and approve the annual implementation of BioMalPar JPA.

Executive Committee: The Executive Committee was composed of scientists with sound experience in the management of laboratories or institutions. This has proven to be important in order to deal with the multitude of different tasks that needed to be managed. Several EC members had actively lobbied for an extension of BioMalPar under the FP7 and promoted strategies to create independent funding and sustainable activities. The EC comprised the Scientific Director, the African representative, the cluster coordinators (see below Joint Research Activities) and their deputies.

Management office: The Scientific Director Artur Scherf has been assisted by a full-time project manager and a part time administrative assistant to coordinate the 'BioMalPar' office. Sylvie Gratepanche has been handling with dedication the various day-to-day administrative managerial aspects of the network (assisted in her task by a part-time secretary Ninon Chauvin). All the documents related to BioMalPar were centralised in the BioMalPar management office and the BioMalPar website has been managed by S. Gratepanche.

Cluster Management: Each Cluster had a **Cluster Coordinator** as its spokesperson, assisted by a Cluster deputy and Subcommittee composed of senior scientists (**Task Leaders**) who oversaw the technical and scientific issues of its Joint Activity programme. The Cluster Coordinator had a key role in overall integration. He/she oversaw the interfacing of the various groups and expertises within the cluster research and training activities, and had a pro-active role in promoting innovative approaches and associations or partnerships with additional groups as needed. He/she identified needs and proposed actions to achieve the Cluster goals, including workshops and training activities, establishment of common databases, and scientific sessions. The cluster coordinators have been identified in the preparative phase of the application. They have been confirmed in their function (after 2 years) at the cluster meeting in Heidelberg (2006). Cluster 4 has adopted a second deputy (J. Langhorne).

Liaison Managers:

The functions of Liaison Managers were, in their respective field, to :

- develop a long-term plan for the review, allocation and procurement of any requirement needed per Participant
- propose the best strategy to accomplish the goals : integration and harmonisation of the tools/contents of the activities

- facilitate and oversee inter-cluster efforts, and provide feedback to EC in order to monitor the perceived quality of the activities, developed a way to properly awards credit to each participant

The Liaison Managers were appointed for the following topics as follows:

- Shared resources and technological platform (K. Louis)
- Joint ethics advisory group (R. Sauerwein)
- Graduate Programme Directors for the different malaria PhD programmes (F. Kafatos, H. Vial, M. Lanzer, M. Blackman)
- Annual conference (A. Scherf/S. Gratepanche)
- Integration of new African participants (Coordinator & MIM/TDR representatives)

Evaluation and monitoring: Network priorities were regularly discussed and if necessary revised (including budget amendments) at both cluster and Executive Committee meetings. An overall review of the consortium activities was conducted every year by an external ***Scientific Advisory Board*** (SAB) at the BioMalPar annual meeting. The SAB provided an oral ad hoc summary report at the end of the meeting and a detailed written report with specific recommendations to the consortium.

Evaluation of the PIs' network activity: In order to monitor the network activities of each PI, a detailed *Questionnaire* has been created which covered the four major activities described in the Technical Annex: Scientific excellence, Integration, Spreading and Management. The analysis of this document by the Executive Committee revealed the overall network performance of individual partners. The presence of a very small number of non-performing PIs was observed in the network. As consequence, a first warning was given to these PIs and drastic budget cuts (no funds after repeated non-performance) were applied by the EC. The procedure including the number of points for each category was communicated to the PIs of the network to create maximum transparency of the evaluation procedure. The annual Joint Programme of Research budget allocation for each PI was based on this evaluation.

o Integrating activities

These activities were those that are directly targeted at the creation of a strong and lasting integration of the activities of the participants in the network. They included:

WP1.1 & 1.2: Co-ordinated programming and adaptation of the participants' activities in research in order to strengthen their complementarities and develop mutual specialisation

Several bodies were implemented in order to achieve the ambitious goals of BioMalPar:

- ***Executive Committee*** *:* The Coordination of research activities was an ongoing challenge due to the existing disparate European institutions programmes. This could be only achieved by a strong and efficient management that was based on experienced scientific leaders in the field of basic malaria research. All along of the project the Executive Committee identified gaps in malaria research, avoided duplication of efforts and developed consistent mechanisms for malaria research collaboration. During the meetings, the Executive Committee set and adjusted goals, evaluated progress in relation to objectives and goals and approved the work plan.
- **An *institutional Board*** was also implemented to represent a Top Level Executive of each core partner. It was responsible for effective management of the co-ordinated inter-institutional Network programme. It ensured that the BioMalPar JPA and the needs in terms of infrastructure and resources were presented to, discussed and decided upon by the institution decision representatives. The institution Board assessed and approved the decisions made by the Executive Committee.
- ***A scientific Advisory Committee***: An overall review of the consortium activities was conducted every year by an external Scientific Advisory Board (SAB) at the BioMalPar annual meeting. The SAB provided an oral ad hoc summary report at the end of each meeting and a detailed written report with specific recommendations to the consortium.

WP1.3 & 2.5: Launch of several competitive calls for integrating additional partners

At its beginning, the network federates eighteen European research institutes, and three research institutes, of established international renown in the field of malaria biology.

It was important that BioMalPar does not act as "closed clubs", concentrating only on strengthening the excellence of the partners inside the network. One of missions was to integrate new partners in order to keep a dynamic structure, with new partners moving in and others eventually leaving the network. The continual integration of new members, either as full or as affiliated partners was achieved from the third year on. After four years of activity, the consortium has raised its members from forty-four to fifty-nine malaria research laboratories, which supported the network's goal to strengthen existing excellence in the European Malaria Research. Twelve affiliated partners and three new full participants from endemic malaria countries were integrated into the network.
The participant 47 was integrated following a specific BioMalPar call for new African participants and two additional participants from endemic malaria regions (participants 49 and 50) joined the network under a TTC call launched by the Commission.

This table below describes the list of full members of the network at the end of the project.

Partic. Number	PI's Name	Participant name	Participant short name	Country	Starting date (Month)	Ending date (Month)
Participant 1	A. Scherf P. Druilhe O. Puijalon J. Gysin R. Ménard	Institut Pasteur	IP	France	1	66
Participant 6	C. Braun-Breton	University Montpellier 2	UM2	France	1	66
Participant 7	M. Blackman A. Holder J. Langhorne	National Institute for Medical Research	MRC-NIMR	United Kingdom	1	66
Participant 10	A. Waters	University of Leiden	LUMC	The Netherlands	1	66
Participant 11	H. Stunnenberg R. Sauerwein	University of Nijmegen	KUN	The Netherlands	1	66
Participant 12	A. Thomas	Biomedical Primate Research Centre	BPRC	The Netherlands	1	66
Participant 14	D. Roberts C. Newbold A. Hill D. Kwiatkowski K. Marsh B. Urban	University of Oxford	UOXF + H4	United Kingdom	1	66
Participant 21	H. Vial J. Hoffman E. Levashina	Centre National de la Recherche Scientifique	CNRS	France	1	66
Participant 22	M. Lanzer K. Matuschewski	University of Heidelberg	UKHD	Germany	1	66
Participant 24	M. Wahlgren Q. Chen	Karolinska Institute	KI	Sweden	1	66
Participant 26	M. Troye-Blomberg K. Berzins	University of Stockholm	SU	Sweden	1	66
Participant 28	M. Ponzi P. Alano	Istituto Superiore di Sanita Italy	ISS	Italy	1	66

Participant 30	D. Modiano B. Arca	University of Roma	UR	Italy	1	66
Participant 32	B. Sinden A. Crisanti O. Billker	Imperial College of Science, Technology & Medicine	IC	United Kingdom	1	66
Participant 36	M. Berriman	The Welcome Trust Sanger Institute	Sanger	United Kingdom	1	66
Participant 37	F. Kafatos	European Molecular Biology Laboratory	EMBL	Internat Org.	1	66
Participant 40	T. Ioukeris K. Louis	Institute of Molecular Biology and Biotechnology/Foundation for Research and Technology Hellas	FORTH-IMBB	Greece	1	66
Participant 42	F. Kironde	Makerere University	MUK	Uganda	1	66
Participant 43	O. Doumbo	University of Mali	MRTC/DEAP	Mali	1	66
Participant 44	M. Ibrahim	University of Khartoum	IEDK	Sudan	1	66
Participant 45	D. Soldati	University of Geneva	Unige	Switzerland	13	66
Participant 47	O. Amodu	Univeristy of Ibadan	COMUI	Nigeria	36	66
Participant 49	C. Chitnis	International centre for Genetic Engineering & Biotechnology	ICGEB	India	36	66
Participant 50	W. Mbacham	Biotechnology centre, University of Yaounde I	BTC	Cameroon	36	66

Applications of several open calls in October 2005 and 2006 resulted in the selection of 12 new affiliated BioMalPar participants (out of 30 applications). These PIs joined the network in 2006 (8 members) and 2007 (4 members) as described in the following table:

Partic. Number	PI's name	Participant short name	Country	Starting date (Month)	Ending date (Month)
1'	Marcus Meissner	UKHD	Germany	25	66
2'	Freddy Frischknecht	UKHD	Germany	25	66
3'	Christian Doerig	INSERM	Switzerland	25	66
4'	Klaus Lingelbach	Un. Marburg	Germany	25	66
5'	Paolo Arese	Un. Torino	Italy	25	66
6'	Lars Hviid	CMP	Danemark	25	66
7'	Didier Fontenille	IRD	France	25	66
8'	Eleanor Riley	London School	UK	25	66
9'	Maria Mota	Un. of Lisbon	Portugal	37	66
10'	Sylke Muller	Un. of Glasgow	UK	37	66
11'	Volker Heussler	Berhardt Nocht Institute	Germany	37	66
12'	Alistair Craig	Liverpool School of Tropical Medicine	UK	37	66

WP 1.4: Shared resources and technological platform

One of the major objectives of BioMalPar towards the spreading of excellence in malaria research has been achieved through the *Sharing of Resources and Platforms*. This included not only the sharing of various technologies and platforms identified at the initiation of BioMalPar, but most importantly, the development of additional ones that encompassed new facilities and repositories of biological material, bioinformatics tools, including databases, and standardization and availability of protocols covering all aspects of malariology. During the project several SOPs have been worked on and developed to various degrees. A questionnaire has been filled in by participating partners to summarize the shared resources activities.

The major achievements toward this objective are illustrated by several distinct milestones:

- Standardization of consortium protocols has been largely improved by updating the fourth edition "Methods in Malaria Research" book (M. Wahlgren et al., Karolinska Institute, released in 2008). This activity was a collaborative work between BioMalPar, Karolinska Institute and MR4 and was financially supported by the BioMalPar consortium. Most of the BioMalPar members have been invited to contribute with the submission of updated, new or alternative methods. The main objective of this work was to provide the malaria community with an extremely valuable resource in disseminating freely this book. Indeed, the fourth edition was largely distributed at the 4th annual BioMalPar conference and is also available in electronic version on the BioMalPar and MR4 websites.

- The genome of the *P. falciparum* FCR3/IT strain has been completely sequenced and this effort has been fully financed by the consortium. The sequencing was done at the Sanger Center with major input of network participants from the Sanger Center and Oxford groups.

- ChIP on chip platforms have been developed in two partner laboratories (Pasteur and Nijmegen).

- A comprehensive website has been developed for the *P. berghei* model by the Leiden's group

- Keep up-to-date the "Malaria Parasite Metabolic Pathways" website created by Hagaï Ginsburg. Since the beginning of BioMalPar support, more than 55,000 people have visited the website and more than 100,000 since the inauguration of the website. The rate of entrances into the site is 1,000 to 1,500 per month

WP 1.5: Joint fundraising

A major effort has been made to obtain external funding to support different actions of the consortium. Applications for Marie Curie Action Host fellowship for Early Stage Research Training (EST) coordinated by H. Vial, University of Montpellier (9 PhD sandwich students) and by Mike Blackman MRC London (14 PhD sandwich students) were successful. In total 23 students have been enrolled in the PhD programme under the aegis of BioMalPar. Each early stage researcher conducted their work under the supervision of at two BioMalPar PIs. They also had the opportunity to attend all the training courses/workshops organized by the BioMalPar partners as well as the annual conference.

Additionally extra funds have been obtained by a specific call of the European Commission in 2006, aiming to integrate DC partners into existing networks.

WP 1.6: Joint ethics

BioMalPar recognized the need to continually update the network on issues that concern ethical questions. Robert Sauerwein has taken over the position as Liaison Manager for Ethical questions together with Dominic Kwiatkowsky, Marita Troye-Blomberg, Ogobara Doumbo and other participants. Questions on Bioethics have been discussed and updated during meetings (such as "Bioethics Symposium" at the MIM conference 2005). To put further effort into ethical issues related to the network's malaria research, lectures on ethical questions were organized for the students' course held in Kampala, Uganda in 2008.

o Joint Research Activities

The scientific problems that BioMalPar has addressed during the lifetime of this network have been grouped into four main themes, each representing a topic in which Europe has proven excellence, which is documented extensively by regular publications in top-rated journals. The consortium releases annually around 400 publications, of which the half arises from the BioMalPar project. This is also illustrated by the fact that two participants of BMP got nominated EMBO members in 2006 (Scherf, A.) and 2009 (Waters, A.).

Four main topics:

- Cluster 1: Development and Gene Regulation
  Coordinator : A. Waters, 1st Deputy :B. Sinden
- Cluster 2: Functional cell biology and metabolism
  Coordinator : M. Lanzer , 1st Deputy :H. Vial / 2nd Deputy : M. Blackman
- Cluster 3: Molecular and Cellular Aspects of the Pathogenesis of Malaria
  Coordinator : D. Roberts, 1st Deputy : D. Modiano
- Cluster 4: Immune responses and defence mechanisms in the host and the vector
  Coordinator : M. Troye-Blomberg, 1st Deputy : F. Kafatos 2nd Deputy : J. Langhorne (since April 2006)

Cluster 1: Introduction

With the publication of the *P. falciparum* (PF) genome in 2002 malaria research entered the post-genome era. This achievement created the imperative to exploit the information by supplementing the predicted gene models of the genome with observations of transcripts and proteins from all life cycle stages coupled with comparative information of the genomes and gene products of other PF strains and species of *Plasmodium*. This data must be assembled, curated, analysed and warehoused in forms accessible to the public. Furthermore, the availability of limited genetic transformation technologies for both human and some model *Plasmodium* species allowed functional analysis of gene products through reverse genetics. The modalities and species range of such technologies required improvement, furthermore, reverse genetics had yet to be applied to practical issues such as improvement of vaccine design and drug (resistance) development. The study of the molecular biology of the control of gene expression was in its infancy. Cluster 1 broadly addressed these requirements and deficiencies.

Achievements

1. Genome and post-genome data acquisition, (comparative) analysis and warehousing. Curation and completion of the (PF) 3D7 genome has been an ongoing process. In addition to manual inspection of the initially predicted gene models, integration with comprehensive and ever improving stage specific transcriptome and proteome datasets has allowed the annotation of the genome to evolve. Thus 100's of new genes have been confirmed and 1000's of initial errors in sequence and model structure have been corrected overlaid with a more complete impression of the quantitative nature of stage specific gene expression. These activities have been complemented by similar work on other human (species and strains) and model *Plasmodium* genomes and post-genome datasets allowing a full description of *Plasmodium* gene repertoire, orthology, genome plasticity, gene expression. Computational methods and tools that are generally useful for all of these processes (e.g. genome assembly, finishing, promoter identification) have been developed. The data are publicly available on member group web-sites, required public databases (e.g. GEO) and on PlasmoDB and GeneDB (a product of a NoE member). Databases of parasite mutants and analytical methods have also been produced (WTSI, IC1, LUMC, BPRC, ISS1, ISS2, KUN1, KUN2, UKHD2, UOXF2).

2. Regulation of gene expression and parasite development.

A. Transcriptional control, epigenetics and genes associated with antigenic variation (AV).

Epigenetic modification of histone composition has been shown to be important in control of gene expression in *Plasmodium* as in other eukaryotes. The histone code has been analysed on a genome wide scale and is dynamic varying in a stage specific fashion. Furthermore it may control gene localisation in the nuclear architecture and hence expression. For example actively expressed genes associated AV reside in a privileged site in the parasite nucleolus and silenced genes on the nuclear periphery. Epigenetic control is likely to be complex but AV relies upon formation of silencing complexes in situ on the nuclear periphery which can be specifically disrupted by disturbing the epigenetic balance such as deletion of SIR2A a histone deacetylase leading to aberrant gene

expression. Chromatin modelling complexes have been extensively studied revealing generally conserved features (IP1, KUN, ISS1, ISS2).
B. Post-transcriptional control of gene expression.
The existence of translational repression (TR) as a major post-transcriptional control of gene expression in *Plasmodium* female gametocytes has been demonstrated. The master regulator of TR is a highly conserved DDX6 helicase (DOZI) that is essential to post-fertilisation development of the parasite in the midgut. DOZI apparently controls the stability of >350 repressed mRNA species generally identifiable by a conserved RNA motif in the UTR of the mRNA. Cohorts of message have been described that are expressed in all blood stage parasites but only translated in gametocytes have been discovered (LUMC, ISS1)
C. Parasite development.
Many gene deletions have been carried out on human and model species and the phenotypic consequences monitored. Proteins that play a role in parasite signalling pathways, blood stage development, gametocyte development, gametogenesis, gamete fertility, zygote development, ookinete motility, ookinete infectivity, oocyst development, sporogony, sporozoite motility, sporozoite infectivity and exoerythrocytic development have been identified (ISS1, ISS2, IC1, IC3, UKHD2, IP5, KUN2, KUN1, UOXF2, FORTH2, UNIGE).

3. Production and exploitation of transgenic parasites for vaccine and drug development.
The production of genetically attenuated model parasites that give rise to long-lasting protective immunity whilst failing to infect their rodent hosts has spawned the possibility that such therapies might be applied to man. Such approaches would replace irradiated parasites as a reproducible source of immunogen assuming difficulties such as production and delivery can be overcome. Some of the difficulties of working with human parasites in vaccine evaluation have been overcome through the production of transgenic model parasites that contain orthologous replacements and give reliable parasitological assays as readouts. Numerous constitutively fluorescent parasite lines of various parasite species have been produced that allow the monitoring of vaccine efficacy and immune responses. (UKHD2, LUMC, BPRC, IC1, IC3)

4. The improvement and dissemination of transfection technology. Massive strides have been made in the improvements of gene transfection technologies in addition to those reported above. Many vectors for the transfection of model and human parasites have been developed and disseminated. Modalities of transfection include transgenesis, allele replacement, allele modification, allele interruption, allele deletion. The efficiencies of the processes have been significantly improved and novel methodologies to achieve genetic modification (e.g. flp/frt, Tc inducible gene expression/knock down) or produce genetic modification tools have been developed (recombineering) and applied. There has been very active training in these technologies to both NoE members and DEC citizens. Reagent databases have been substantially enriched by the products of these activities (IP1, IP5, LUMC, KUN, BPRC, UOXF2, UKHD2, ISS, ISS2, IC, IC3, WTSI, UNIGE).

Conclusions:
There have been numerous joint and individual publications, many of the highest impact. Public databases have been enhanced and created. There has been a quantum leap in technology development and its application. The progress of work in this Cluster has been rapid and substantial. The basis for continued discovery has been clearly defined and the collaborators are excited about its potential.

Cluster 2: Introduction
Malarial parasites possess an extended set of organelles to maintain their parasitic life cycle, alternating between vertebrate and invertebrate hosts. This includes i) organelles specifically involved in host cell entry, such as the rhoptries, micronemes and dense granules; ii) the apicoplast, a plastid remnant acquired as a result of a secondary endosymbiotic event; iii) parasite derived membrane profiles within the host erythrocyte cytoplasm, termed Maurer's that are involved in protein trafficking to destinations within the infected erythrocyte; an acidic food vacuole that is involved in degradation of the erythrocyte's hemoglobin and which is the site of action of several antimalarial drugs and whose membrane harbors transporters implicated in drug resistance.

Cluster 2 has investigated the structure and function of Plasmodial organelles. This includes the protein and lipid compositions of these organelles, their specific biochemical pathways, the mechanism by with they recruit residential proteins, organelle-specific ion homeostasis and pathways of intracellular signalling that affect organelle function. This work has provided novel insights into

fundamental biological processes and, at the same time, has revealed novel targets for rational intervention.

Achievements:
1.- Protocols have been developed for the isolation of several organelles, including Maurer's clefts, food vacuoles, lipid rafts, ookinetes, and micronemes. This has facilitated organelle-specific proteomic analyses. Candidate proteins identified have been validated, using immunolocalization studies, transfection and reverse genetic technology, and biochemical approaches. Putative transporters identified were expressed in heterologous systems, such as *Xenopus laevis* oocytes, to investigate their physiological function. Partners involved were: UM2, MRC-NIMR, BPRC, CNRS, UKHD, ISS, BTC.

2.- The process underpinning parasite invasion of host cells has been investigated in great detail. Novel parasite factors involved in invasion were discovered and their functional domains characterized, using nested deletion analyses and phage display technology. Moreover, the role of proteases during host cell invasion and egress was examined. (IP, MRC-NIMR, LUMC, BPRC, UKHD, UR, IC, EMBL, FORTH-IMBB, Unige).

3.- Host cell invasion by malaria parasites is an active process, requiring a functional actinomyosin motor. Several components of the motor complex were investigated, using genetic, biochemical and biophysical approaches. The phenotypic analysis of knock-out mutants, revealed novel insights into parasite motility during different life stages. (IP, MRC-NIMR, LUMC, BPRC, UKHD, UR, IC, EMBL, FORTH-IMBB, Unige).

4.-The extended set of organelles found in malaria parasites entails a sophisticated protein sorting and trafficking system. The mechanism trafficking of several proteins has been studied in detail, using biochemical, genetic and reverse genetic approaches, coupled with live cell imaging of GFP-tagged chimeric proteins. New protein trafficking motifs were identified. (UKHD, ISS, UM2).

5.- Phospholipids play an important role in parasite membrane biogenesis, vesicle trafficking and in signal transduction. Genetic manipulations of enzymes involved in lipid metabolism, together with biochemical studies, have identified essential metabolic pathways. For example, it was found that phosphatidylcholine and phosphatidylethanolamine, the main membrane phospholipids of the parasite, can be generated by different metabolic pathways, including a novel choline/ethanolamine pathway. These pathways have been analyzed in detail and the participating enzymes were cloned and biochemically characterized. (CNRS, BPRC, IC, Unige).

6.- It was found that lipid-mediated intracellular signaling, in the context of calcium and cyclic nucleotide dependent pathways, regulate gametocyte activation. Biochemical quantification of lipid precursors and metabolites revealed a complex interplay throughout gametocyte activation. (CNRS, BPRC, IC, Unige).

7.- The Plasmodial kinome was investigated, using reverse genetic and biochemical approaches. Moreover, the potential of several P. falciparum kinases as drug targets was explored.

8.- New technologies were developed, such as improved transfection techniques, conditional gene regulation technology, a conditional protein trafficking system, positive/negative selection strategies, novel methods to image live and cryo-preserved parasites and RNA_i technology. (IC, UKHD, EMBL, LUMC, BPRC).

Conclusions:
Beside many important discoveries, a respectable number of high impact publications have resulted from this cluster, of which many have joined authorships form different partner laboratories.

Cluster 3 : Introduction
The work of Cluster 3 has been transformed during the period of BioMalPar from the traditional work of different PIs and their individual projects to a series of coordinated projects using state of the art technologies. Given the previous history it is fair to say the group has made as much if not more progress than others towards integrated work. This is easily demonstrated by the many new collaborations that have been established.

Achievements

1.- A new project to understand the role of monocytes in malaria (Roberts and Druilhe) has found by in vitro cell culture and ex vivo immunophenotyping of monocytes form patients with malaria that discrete monocyte populations are induced during malaria and are associated with anti-parasitic activity. They have also demonstrated expansion of unusual monocytes with high phagocytic activity in a malaria endemic area, previously identified in chronic inflammatory diseases but not until now in malaria. Druilhe has begun to explore the mechanism of Antibody Induced Cytotoxicity and shown suicidal erythrocyte death in the presence of activated monocytes.

2.- Williams and Arese have established methods to determine phagocytosis of ring-stage parasites in patients with HbAS (sickle cell trait) and HbAA (normal) using fluorescently labeled cells and flow cytometry. Clinical studies are now underway.

3.-Langhorne and Roberts have explored the role dyserythropoiesis in the pathogenesis of anaemia. Langhorne has used murine models of malaria to show that, a novel IL 7 receptor alpha positive progenitor subset is found following infection. In human malaria, Roberts has shown haemozoin and cytokines independently reduce the growth of developing erythroid cells by distinct pathways using cellular, protein and whole genome transcriptional analysis. Clinical studies in Kenya have shown very high levels of erythropoietin are associated with protection from brain injury.

4.- Malarial anaemia is caused by the destruction of parasitized erythrocytes, the removal of non-parasitized erythrocytes and dyserythropoiesis. Gysin and Scherf have shown Rap-1, and 2 are transferred to the erythrocyte surface during aborted merozoite invasion and demonstrated substantial tagging of erythroid precursors in *P. falciparum*-infected patients and infected *Saimiri* monkeys. In vitro studies have shown RSP2 tagged erythrocytes have reduced membrane deformability consistent with reduced survival.

Chitnis and Roberts have examined the relationship between receptor-specific adhesion phenotypes. Both have shown platelet-mediated clumping was found to be associated with severe malaria. Further joint case control studies are planned in Kenya. The molecular basis of the platelet clumping phenotypes is being dissected for CD36 - and gC1qR- dependent platelet-iRBC clumping phenotypes.

5.- Newbold and Berriman have sequenced the IT strain of P. falciparum (8x coverage) using Illumina technology. Bioinformatic programs for sequencing correction and contig assembly have been developed. Sequence was released in the public domain at the end of 2009. A total of >180 field isolates have now been sequenced and genomic comparison between parasites of severe malaria and mild malaria has found potential virulence associated loci. This work is of tremendous benefit to the whole malaria research community. The falciparum transcriptome has been analysed by sequencing with Solexa to identify new genes, correct existing to gene models and predict alternative spice variants.

6.- Chen has identified a new mechanism of var gene transcription in the HB3 P. falciparum strain, where duplicated var2csa variants were simultaneously transcribed so challenging the dogma of mutually exclusive var gene transcription. Chitnis has found a candidate var gene mediating adhesion to gC1qR has but the domains mediating adhesion to gC1qR have not been located.

7.- Gysin and Scherf have studied the mechanism of Var2CSA binding to CSA with panels of domain-specific MAbs and the respective recombinant proteins. A small consortium of BioMalPar and non-BioMalPar partners is now funded under a new FP7 program and Bill & Melinda Gates Foundation.

8.- Modiano has investigated the association between ß-globin genotypes and Plasmodium falciparum transmission and both parasitological and entomological investigations provided solid evidence that the transmission of P. falciparum from humans to Anopheles is substantially enhanced in the presence of HbC.

9.- To give a general overview of progress within this work package over the past 4 years, the field of genetic susceptibility and resistance to common diseases has been transformed by advances in genome-wide association analysis and next generation sequencing. This has been matched by the recruitment of subjects into well-characterised clinical and epidemiological studies of genetic association led by Kwiatkowski. The MalariaGEN Consortium which includes the BioMalPar groups in Nigeria (Amodu), Sudan (Ibrahim) and Mali (Doumbo) as well as other partners in 15 malaria-endemic countries, has collected more than 60,000 DNA samples with detailed clinical data for large-scale studies of genetic association with severe malaria and with antimalarial antibody responses. This

represents a unique resource for trans-ethnic genome-wide association analysis that would provide a vast amount of data in the future.

10.- Progress has been made towards analysis of specific candidate genes. Troye-Blomberg and Berzins have been investigating specific polymorphisms in the Fc-gamma gene and in C-reactive protein. Williams is analysing 20 host candidate genes in a large sample set that his group has contributed to the MalariaGEN consortium. The MalariaGEN Consortium, coordinated by the Kwiatkowski Group in Oxford and at the Sanger Institute, has performed genome-wide association studies on over 10,000 individuals from the Gambia, Malawi and Ghana. This has revealed a number of novel candidate loci with multiple line variance in association with resistance to malaria, and has also led the way in defining methodological issues of genetic association studies in Africa, which are complicated by high levels of genetic diversity and population structure. An important outcome of this work has been proof of principle that studies in African populations offer greater opportunities for fine mapping of causal variants than is the case in European populations.

Conclusions:
This cluster has had a very strong effect on the community working on physiopathology of malaria. A number of common actions have had a strong federative effect leading to new grant applications between members of the consortium. In addition, a number of high profile papers have been published by members of this consortium.

Cluster 4: Introduction
The overall aim of this cluster is to analyse interactions between innate and adaptive immune responses to malaria parasites and to improve the understanding of how this interaction influences the development and regulation of defence mechanisms and how it is modulated by the parasite. The second major aim is to increase knowledge of the innate immune response of *Anopheles* mosquitoes against the malaria parasite and to identify molecules and pathways that play key roles in these responses.

1.- Interactions between innate and adaptive immunity to malaria parasites in human and mosquito. The research included analysis of cellular activation of human and murine memory B - and NK cells and dendritic cells and of their interactions, in naïve and *Plasmodium* exposed humans, in children with mild or severe malaria, in experimentally challenged humans and in experimental primate and rodent models systems.

2.- A human spleen system was developed to investigate to where cytoadherent parasites home. Investigations on the genetic regulation for malaria-induced innate and acquired immune responses were also included in this WP. Emphasis was made to develop quantitative cellular assays, like q RT-PCR, FACS and RNAi suitable for small blood volumes. The main participants in these objectives 1+2 were IP, MRC-NIMR, KUN, UOXF, SU, UR, MRTC/DEAP and LSTHM.

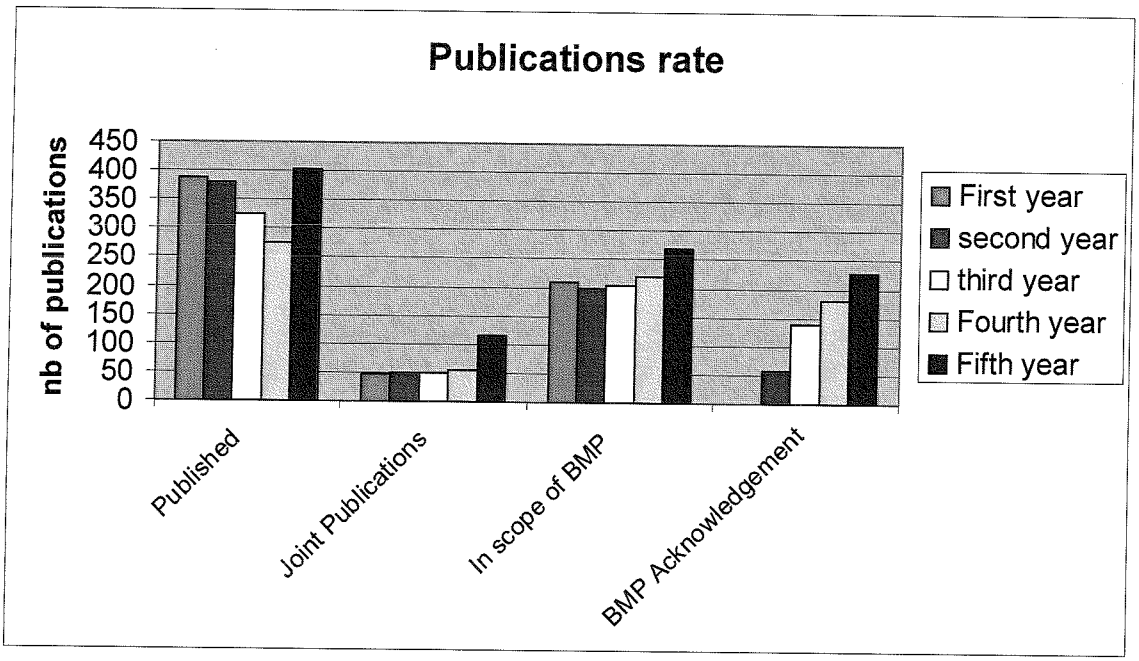
3.- The research performed in this WP included identification and function of novel gene products that play a role in the immune responses of the *Anopheles* mosquitoes (*An.gambiae, An.stephensi* and *Aedes aegypti)* as well as in *Drosophila*, which was used as a model for the malaria vectors. The traditional molecular/cellular line of work was supplemented by work in the areas of postgenomics and bioinformatics, which led to the development of a global database on insecticide resistance. Conventional molecular/cellular and immunological techniques were used, which were supplemented by microarray-based gene profiling and bioinformatics approaches. The main participants in this WP were CRNS, the EMBL, IC and IMBB.

4.-The research performed in this WP included identification of conserved signalling pathways involved in anti malaria immune responses both in mammals and in the mosquitoes and well as genetic regulation of the responses. A new *An.gambiae* rearing/*P.falciparum* facility was established at SU. An assay to determining mosquito exposure was established. A number of technologies and platforms such as breeding and typing of different TLR knock out mice, gene profiling for gene expression analysis was established by members of the consortium.The main participants in this WP were MRC, UOXF, CNRS, SU, EMBL

Conclusions:
This is the first coordinated action that brings immunologists from different areas to exchange ideas, results on human and mosquito host responses to the same pathogen. In summary, it was found to be an enriching experience. The research performed in this Cluster 4 was extremely successful as shown by the high numbers of publications in all sub-areas of the WP.

Annual Publications Rate

The histogram below shows the number of publications released annually by the consortium's members. It corresponds to around 400 publications, of which about half arises from projects covered by the network. These figures are good indicators of the scientific productivity which increasingly are based on collaborations between members. A large number of high profile publications (Nature, Cell, Science etc.) demonstrate the excellence of the malaria groups involved in the network.



Importantly, the funds provided by the Network of Excellence BioMalPar were more and more acknowledged by members as the consortium moved on.

In conclusion, one of the network's major objectives was to **reduce the fragmentation** and **avoid redundancy in malaria research**. It has been largely achieved with the development of new research tools and platforms for common use as described earlier in each cluster. As a result, collaborations within the network are now an integral part of the project execution and start to produce significant progress in malaria research, which is now perceptible with the increase of joint publications released in 2009.

o Activities designed to spread excellence (Dissemination and use)

An essential feature of the activities aiming at spreading excellence was the implementation of the BioMalPar PhD programme. Indeed the steady supply of skilled staff is indispensable to the sustainability of European excellence in malaria research.
Moreover BioMalPar aimed to ensure a broad dissemination of research results and enhance the public awareness of malaria research through the implementation of a public website (**http://www.biomalpar.org**) as well as the organization of annual conferences, workshops, student training courses,etc...

WP3.1 PhD Programme

BioMalPar placed a particular emphasis on its training role, and has established a high profile PhD scholarship coordinated by the European Molecular Biology Laboratory (EMBL), Heidelberg. The EMBL was a participating institution of BioMalPar and operated the BioMalPar PhD programme by procedures patterned on the EMBL International PhD programme. The Graduate Committee consisted of Fotis C. Kafatos (EMBL, Director of Graduate Studies), Michael Lanzer (UKHD), and Artur Scherf (IP).
The 16 PhD students who have been admitted to the BioMalPar International PhD Programme received theoretical and practical training for 3.5 years, and conducted a research project under the supervision of two or more PIs of the network, monitored by annual meetings of the Thesis Advisory Committee (TAC).The students received additional trainings, including inter-lab collaborations, seminars, took part in journal clubs and had training/practice in scientific writing and oral presentations. The students presented their Thesis work at their University in late summer 2008 or beginning 2009.
Given the success of this program, the Executive Committee decided to launch additional PhD programs. Since the network budget did not allow financing more fellowships, two Marie Curie fellowships (Early Stage Research Training) proposals were submitted by members of the EC. Both applications were successful adding 23 more PhD fellowships to the network.

In June 2006 9 students were recruited via an FP6 Marie Curie Action Host fellowship for Early Stage Research Training (EST) entitled "MalParTraining" coordinated by H. Vial at UM2, Montpellier, France.

In October 2008 14 additional students were recruited via FP7 ITN proposal "InterMalTraining", Marie Curie Action: "Initial Training Networks" coordinated by M. Blackman at MRC-NIMR, London.

All BioMalPar PhD students attended regularly the Annual BioMalPar Conferences and presented their data either in the posters sessions or in oral presentations (end of thesis).

WP3.2 Organization of training courses

BioMalPar aimed to provide cutting-edge training opportunities to ensure that the next generation of scientists will maintain a dominant role of Europe in malaria research. Another purpose of the organization of training courses in new technologies was to strengthen standardisation of procedures and facilities throughout the participant Institutions.

To these ends the BioMalPar members have organised training courses/workshops at their home institutions all along the lifetime of the network. These workshops were essentially addressed to the students, the post-doctorants and the staff scientists from the labs and institutes involved in the consortium including the affiliated members. Some methodology oriented workshops were organised in Africa institutions in order to facilitate the participation of African students and staff scientists who did not have generally the financial support to travel in Europe for training purposes. This kind of workshop was another means to strengthen the research capability in Africa.

In general the training courses were sponsored at 100% by BioMalPar, but few of them were co-organized and co-sponsored with other institutions such as the Karolinska Institute (KI) and the Makerere University (MUK) in Kampala and the American Society of Cell Biology in Ghana.

In total around 200 PhD students and post-docs received theoretical courses and practical training in attending the eleven BioMalPar workshops as described in the following table:

Title	Venue	Sponsors	Number of attendees	Dates
BioMalPar core Courses	UM2, Montpellier, France	BioMalPar	39	20 Feb to 11 March, 2005
Introduction to Plasmodium Bioinformatics	Wellcome Trust Conferen Center, Hinxton, United Kingdom	BioMalPar	18	24-26 Aug, 2005
Immunological techniques: From the bench to the field	AHRI, Addis Ababa, Ethiopia	BioMalPar and	20	12-21 Oct, 2005
Bioinformatics course	ISS, Rome, Italy	BioMalPar and AHRI	16	25-28 Sept, 2006
3rd Functional Genomics of Malaria Parasites	Bangkok, Thailand	BioMalPar, LUMC and WEHI	20	18-27 March, 2007
Bioinformatics course	EMBL, Heidelberg, Germany	BioMalPar	16	10 April, 2007
Perspectives on malaria research from the laboratory to t field	Kampala, Uganda	BioMalPar, KI & MUK	17	30 to May 18, 2007
Imaging Parasite infection	IP, Paris, France	BioMalPar	12	27-29 May, 2008
Malaria and Polymicrobial Interactions	Kampala, Uganda	BioMalPar, KI & MUK	17	28-May 16, 2008
Bioinformatics course	EMBL, Heidelberg, Germany	BioMalPar & Un. Of Pennsylvania	16	18 May, 2009
The first West African Regional Workshop on the Cell Biology of Protozoan Pathogens	Noguchi Institute at the University of Ghana, Lego	BioMalPar & American Society of Cell Biology	25	July 13 -23, 2009

WP3.4 Annual Conference

The annual meeting was initially launched as a communication platform for BMP scientists. Indeed the primary aims of this conference were to gather together the BioMalPar participants and to assess the consortium and its Joint programme of activities by the Scientific Advisory Board (SAB) and the evaluators appointed by the Commission. Under the impulse of the international SAB and in agreement with the EC, the following conferences were organized to present Cutting Edge Science in malaria research. In order to attract the non-BioMalPar participants, the annual BioMalPar conferences were broadly advertised via the BioMalPar, EMBL and the Commission websites, the MIM mailing list, a specific mailing list targeting mainly the American and Australian malaria scientists and the distribution of conference's posters to 500 major European institutions. Another goal of this annual conference was thus to make visible the excellence of the European malaria research amongst the worldwide malaria community.

From the second annual meeting on, contributions from non-BioMalPar members were equally considered for oral presentations. The scientific program of the annual conference was organized each year by different members of BioMalPar. Instrumental in the planning and execution of the meeting was the Conference Office at EMBL (Bettina Schaefer) and the BioMalPar coordination office, which managed poster advertisement, registration and logistics of the meeting.

Poster sessions were an important part of this conference and awards for the five best poster presentations were attributed from the third annual conference in order to maintain the high quality of these presentations. Prizes were sponsored by BioMalPar and the journal 'Cellular Microbiology'.
In the fourth year of the project, the BioMalPar PhD students were given the opportunity to present their thesis work in a plenary session. The quality of the student presentations was highly praised by the SAB.

In addition the Executive Committee had decided to attribute an award for the life achievements of an eminent scientist in the field of malaria. A special ceremony was thus held at each annual meeting to celebrate the award.
The five scientists awarded were:
- Life-time achievement Award 2005 - Prof. Hagaï Ginsburg
- Life-time achievement Award 2006 - Prof. Victor Nussensweig

- Life-time achievement Award 2007 - Prof. Mario Coluzzi
- Life-time achievement Award 2008 - Dr. Louis Miller
- Life-time achievement Award 2009 - Prof. Brian Greenwood

The feedback that the organizers received from participants after each annual conference was very positive. The consensus was that the annual meetings gave a good current overview of cutting edge Malaria Research and that the quality was at the level of the international malaria meetings. The most important general comment was that the network interactions had significantly improved and that more open discussions between participants were clearly visible, reaching one of the major goals, which was reducing fragmentation!

WP3.5 Communication with external stakeholders

Management of communication was critical to the success of the project.

Communication to the stakeholders: A communication plan was elaborated to convey activities, results and accomplishments to future EU Integrated Projects on malaria vaccines and drugs, to the other EU malaria-related programmes (EDCTP, EMVI, AMANET) and to international agencies (WHO, Wellcome Trust, Gates Foundation, NIH, JICA) as well as to national agencies.

This communication plan resulted in the implementation of a Memorandum of Understanding between BioMalPar and the ARC/NHMRC Research Network for Parasitology at the 2008 Molecular Approaches to Malaria Conference in Lorne, Australia. The objectives of this MoU are to develop common strategies between BioMalPar and the Australian Network for Parasitology to work on malaria. The first action was the participation of the Australian malaria groups as full partners in the NoE application under the FP7 program, which was successful and started in October 2009 under the name of EVIMalaR.

Communication to the scientific community:

The scientific community is informed of the progress and achievements of BioMalPar via:

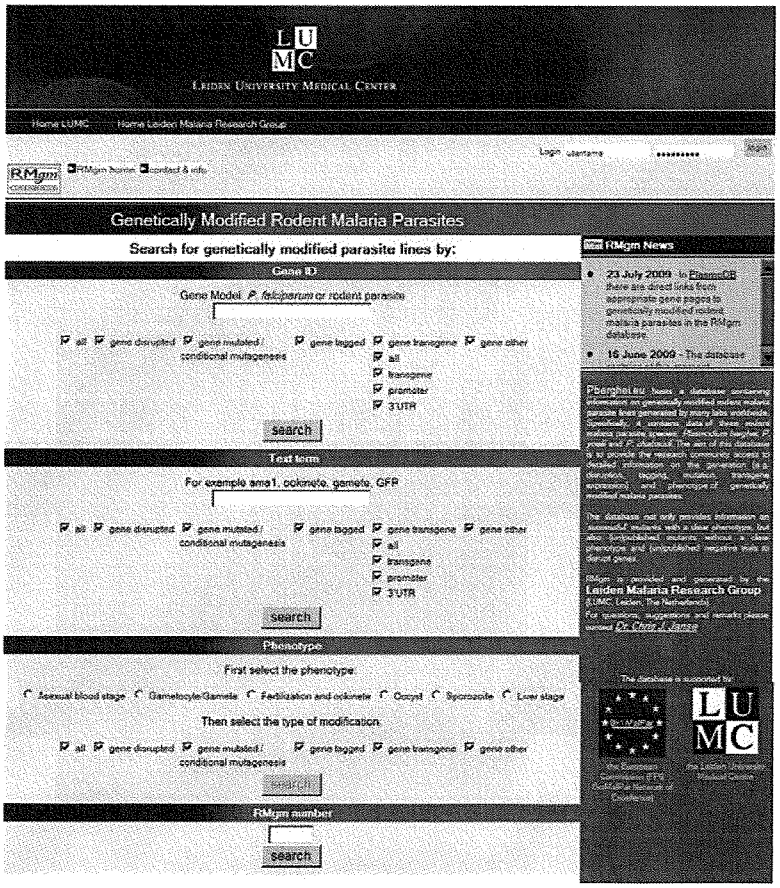
- the scientific publications released by the BMP members,
- the training courses organised by the network
- the presentation of data arising from the project in international malaria meetings (such as the MAM conference in Lorne, MIM conference, etc..)
- the organisation of the BioMalPar annual conference
- the contribution of BMP members in the update of the 4th edition "Methods in Malaria Research".

With the financial support of BioMalPar, two websites "Malaria Metabolic pathways" and "RM*gm* database" dedicated to the malaria community have been maintained and are regularly updated.

Homepage of the "Malaria Metabolic Pathways" created by Prof. Hagaï Ginsburg at the Hebrew University

Since the beginning of the BioMalPar support, more than 55,000 people have visited the "Malaria Metabolic Pathways" website and more than 100,000 since the inauguration of the website. The rate of entrances into the site is 1,000 to 1,500 per month.

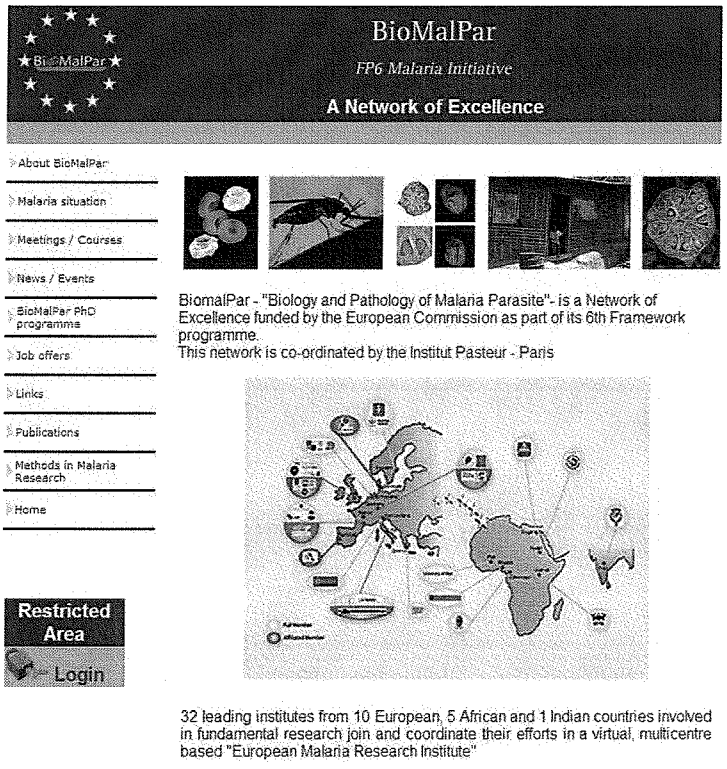
Homepage of RM*gm* database created by Chris Janse at the University of Leiden (LUMC)



Communication to the consortium and to the lay audience

A BioMalPar website has been set up in order to raise public awareness regarding malaria as a major public health problem, to promote BioMalPar activities and as a tool of communication between the network's members (secure part). The website was updated by the curator S. Gratepanche to announce new consortium activities (courses, calls for participants, annual meeting etc.). A secure BioMalPar website has been set up according to the specific needs of this consortium. The secure website with its reporting tool has significantly reduced the administrative burden of the coordinator's office during the reporting period.

Homepage of the BioMalPar website hosted at the Institut Pasteur



Achievement of the project as regards the state of the art

BioMalPar has become a cornerstone in European malaria research and is now renowned in the scientific community for its outstanding activities. Europe is now recognized as the world leader in the biology of the malaria parasite.

The members of the Executive Committee have built on the momentum created by BioMalPar to sustain its activity into the next FP7 programme. It is clear that additional support is needed to maintain the high profile of this network and to achieve the ultimate goal of the consortium, a European Malaria Graduate School and a virtual European Malaria Institute. The recent creation of a FP7 sponsored NoE on basic malaria research has paved the way for the malaria community to accomplish this objective.

Impact of the project on its industry or research sector

From basic biology to industrial application is a very long way and thus, no direct impact is tangible after five years of research activity. However, conceptually, great advances have been made at several levels. New parasite molecules have been discovered with a high potential for anti-malarial therapy. Some of them are now investigated as drug targets and are supported by international grants. Other molecules are now in the pipeline for vaccine development. The network's research activity can be considered as an important motor that continually fills the various pipelines of new intervention strategies against this deadly disease through cutting edge discovery.